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pLB-T Fast Ligation Kit

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pLB-T Fast Ligation Kit

Cat. no. 4995011/4995012

Kit Contents

Category No.	Product name	Packaging
4995011	pLB-T Fast Ligation Kit	20 rxn
4995012		60 rxn

Contents	pLB-T Fast Ligation Kit	
	4995011	4995012
pLB-T Vector(50 ng/μl)	20 μl	60 μl
RapiLigation Mix(2×)	100 μl	3×100 μl
Control Insert DNA(688 bp) (50 ng/μl)	10 μl	10 μl
ddH$_2$O	1 ml	1 ml

Storage Conditions:

The the reagents were stored at -30~-15°C for one year. Avoid repeated freezing and thawing of all reagents. (vector and ligation reagent can be appropriately divided into small packages, to prevent repeated freeze-thaw, to ensure the quality).

Product introduction

The pLB-T Fast rapid cloning kit provides efficient cloning of a wide range of PCR products and any DNA fragment with a sticky end. The kit is effective for both phosphorylate and non-phosphorylated DNA fragments. More than 95% of the positive recombinant clones were obtained in only 5min by ligation of the positive selection vector and the insert. The kit is equipped with a new type of RapiLigation Mix as a high-efficiency reagent for T4 DNA ligase reaction, which contains a linking enhancer and an enzyme stabilizer, thus greatly shortening the linking time and improving the linking and cloning efficiency of PCR products. According to different needs, DH5α competent cells and the control supercoiled plasmid were equipped for convenient transformation.

Product features

- **Efficient and rapid:** rapid ligation within 5min, and the positive rate was nearly 100%.
- **Broad sensitivity:** Suitable for efficient ligation of fragments as low as 0.025pmol and fragments up to 3 kb.
- **It is easy to operate:** With the use of the new RapiLigation Mix, the ligation reaction can be performed simply by adding the vector and fragment.

Different fragment usage

The molar ratio of vector to fragment is controlled within the range of 1:3 to 1:8. Please calculate the molar ratio based on the concentration detected by gel electrophoresis or ultraviolet spectrophotometer and the fragment length. The amount of insert, which can be roughly calculated according to the following formula:

$$\text{The amount of the inserted fragment ng} = (3\text{–}10) \times \frac{\text{Insert segment length}}{\text{vector dosage ng}} \times \text{Vector length}$$

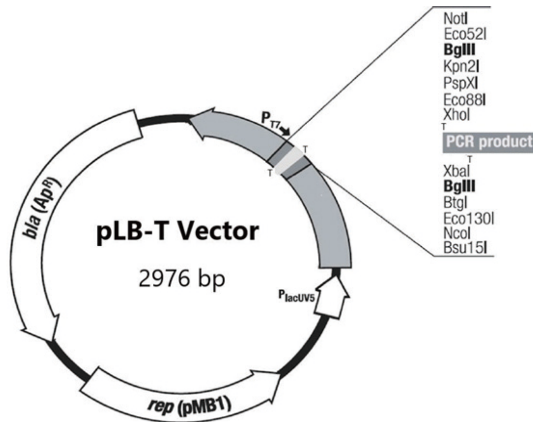
The optimal dosage of PCR products of different sizes using 50 ng vector in the ligation system is as follows:

PCR product length (bp)	Optimal usage (ng)
700 bp	35 ng
2000 bp	100 ng

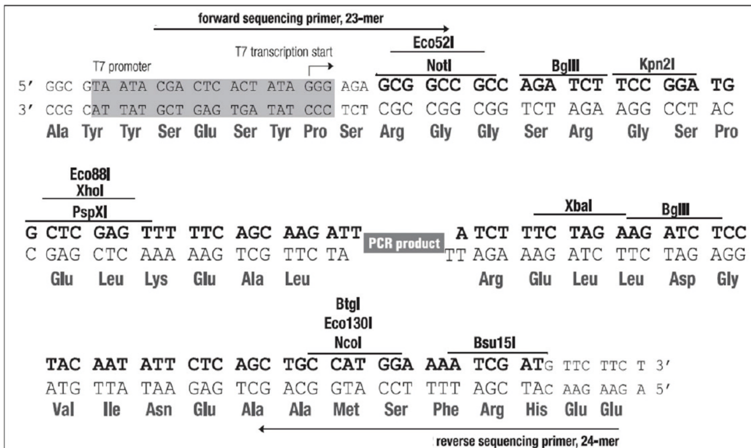
Reaction system

The standard system was 10 µl in volume, and a 5 µl reaction system could also achieve good results. The dosage of reagents was halved.

PLB-T Vector map



PLB-T Vector polyclonal site



Method of use (please operate under sterile conditions for the following steps)

1. Add each ingredient to a sterile centrifuge tube as shown in the table below.

Component	volume
RapiLigation Mix (2×)	5 µl
pLB-T Vector(50 ng/µl)	1 µl
Objective PCR fragment/control Insert DNA	X µl/1 µl
ddH_2O	Make up to 10 µl

2. Flick the centrifuge tube gently to mix the contents and briefly centrifuge for 3-5 sec. The mixed reaction solutions were placed at 22°C for reaction for 5min. After the reaction was completed, the centrifuge tube was placed on ice for the subsequent conversion reaction.

   Note: If the length of the inserted fragment is less than 1 kb, the reaction time may be 5-10 min.

   If the length of the inserted fragment is 1-2 kb, the reaction time may be 10-20 min.

   If the insert length is greater than 2 kb, the reaction time may be from 30 min to overnight.

3. Transformation

1) Prepare LB agarose plates containing a final ampicillin concentration of 100 g/mL. The plates were placed at 37°C and preheated for at least 20min.
2) Add some of the ligation product to 50-100 µl of DH5α competent cells (the competent cells should be freshly removed from the -90~-65°C refrigerator and placed on the ice bath, add the ligand product when just thawed, the amount of ligand product added should not exceed one tenth of the volume of the competent cells), flick and mix well, ice bath for 30min (if necessary, please use the superhelical plasmid pUC19 to transform the competent cells simultaneously). As a control, add 1 µl of Compcell Control Plasmid pUC19 to another competent cell tube as a control, and perform the rest of the procedure in parallel with the transformation of the ligand).
3) The centrifuge tube was placed in a 42°C water bath for 90 sec. After the tube was taken out, it was immediately placed in an ice bath for 2-3 min without shaking the centrifuge tube.
4) Add 350 µl 37°C-preheated SOC or LB (antibiotic-free) medium to the centrifuge tube and incubate with shaking at 180 rpm and 37°C for 45 min–60 min. The purpose is to express the related resistance marker gene on the plasmid and resuscitate the thallus.

5) The bacterial liquid in the centrifuge tube was mixed evenly, 200 μl was sucked and added to LB solid agar medium containing ampicillin, and the cells were evenly coated gently with sterile elbow glass rods or glass beads. After the surface of the plate was dried, the plate was inverted and cultured at 37°C for 12–16 h.

4. Testing

1) Routine test: The obtained colonies were inoculated with 1–5 mL LB (containing ampicillin at a final concentration of 50–100 g/mL) medium, shaken and cultured at 37°C overnight, the strains were stored, and the plasmids were extracted. PCR or enzyme digestion was used to identify whether the inserted fragments were correct.

2) Rapid detection: The selected colonies were directly subjected to PCR detection (for specific methods, see Molecular Cloning Version 3) or the rapid bacterial liquid identification was performed using our pGM-T recombinant colony PCR identification kit .

3) Sequencing identification: The initial identification is followed by sequencing using conventional or rapid methods.

PLB-T Vector sequencing primers:

pLB Forward Sequencing Primer(23-mer):

5’-CGACTCACTATAGGGAGAGCGGC-3’

pLB Reverse Sequencing Primer(24-mer):

5’-AAGAACATCGATTTTCCATGGCAG-3’

Important Note

1. The 3' end of the PCR fragment to be used for ligation should have an A-terminal. In the case of a flat-ended fragment without an A-terminal that is amplified using a high-fidelity polymerase such as pfu, the pLB zero-background rapid cloning kit or the pGM-T flat-ended ligation kit can be selected for the ligation reaction.
2. It is very necessary to use Control Insert DNA as the control during the transformation, and the cause can be determined when there is any problem in the experiment.
3. It is recommended that a portion of the linker be left behind for rapid resolution of the problem and reduction of unnecessary repeat experiments.
4. The coating amount can be adjusted according to the specific experiment. If the total amount of transformed DNA is greater, a smaller amount of the transformed product may be coated on the plate; Conversely, if the total amount of DNA converted is small, 200 to 300 L of the conversion product may be coated on the plate. If fewer clones were expected, part of the medium could be removed by centrifugation (4000 rpm, 2 min) and a suitable amount of medium was left to suspend the cells and applied to a plate (the remaining applied liquid could be stored at 4 C; if the number of transformed colonies on the next day was too small, the remaining liquid could be applied to a new plate).

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- Cell biology products
- Protein molecular weight standard
- Protein staining, detection and quantitative related products