pLB-T ligation kit

(Cat. No.: S2GNM03h30001)

Description:

The pLB-T Fast Rapid Cloning Kit facilitates the efficient cloning of a broad spectrum of PCR products and DNA fragments featuring sticky ends. It demonstrates effectiveness in handling both phosphorylated and non-phosphorylated DNA fragments. Achieving over 95% positive recombinant clones takes just 5 minutes through the ligation of the positive selection vector and the insert.

This kit incorporates an innovative RapiLigation Mix, serving as a high-efficiency reagent for T4 DNA ligase reactions. This mix includes a linking enhancer and an enzyme stabilizer, significantly reducing linking time while enhancing the efficiency of linking and cloning PCR products

Kit Contents:

Contents	S2GNM03h30001
pLB-T Vector (50 ng/μl)	20 μl
RapiLigation Mix (2×)	100 μl
Control Insert DNA (688 bp, 50 ng/μl)	10 μl
ddH_2O	1 ml

Storage:

All reagents were stable at -30~-15°C for one year. Avoid repeated freezing and thawing of all reagents.

It is recommended that vector and ligation reagents can be appropriately divided into small packages, to prevent repeated freeze-thaw, to ensure quality.

Different fragment usage:

The molar ratio between the vector and the fragment is maintained within the range of 1:3 to 1:8. It is advised to determine this ratio by calculating based on the concentration identified through gel electrophoresis or ultraviolet spectrophotometry and considering the fragment length. The quantity of the insert can be approximated using the following formula:

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

The recommended amounts of PCR products for optimal ligation using 50 ng of vector in the

system are outlined below for different sizes:

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.

Protocol:

Note: 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	
Objective PCR fragment	X µl	--
control Insert DNA	--	1 µl
pLB-T Vector(50 ng/µl)	1 µl	1 µl
RapiLigation Mix (2x)	5 µl	5 µl
ddH_2O	to a final volume of 10 µl	to a final volume of 10 µl

2. Gently flick the centrifuge tube to mix its contents, followed by a brief centrifugation for 3-5 seconds. Allow the mixed reaction solutions to undergo a reaction at 22°C for 5 minutes. Once the reaction is complete, place the centrifuge tube 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
   i. Prepare LB agarose plates with a final ampicillin concentration of 100 µg/mL. Incubate the plates at 37°C, preheating them for a minimum of 20 minutes.
   ii. Take 50-100 µl of DH5α competent cells (freshly retrieved from the -90°C to -65°C freezer and placed on ice). Add a portion of the ligation product when the cells have just thawed, ensuring that the added ligation product does not exceed one-tenth of the volume of the competent cells. Gently flick and mix the cells well, and then place them on ice for 30 minutes. Optionally, simultaneously transform the competent cells with

the supercoiled plasmid pUC19 as a control (adding 1 µl of Compcell Control Plasmid pUC19 to another competent cell tube). Carry out the subsequent steps in parallel with the ligand transformation.

iii. Place the centrifuge tube in a 42°C water bath for 90 seconds. After removal from the water bath, immediately transfer the tube to an ice bath for 2-3 minutes without agitating the centrifuge tube.

iv. Add 350 µl of SOC or LB medium (free of antibiotics) preheated to 37°C to the centrifuge tube. Incubate the tube with shaking at 180 rpm and 37°C for 45-60 minutes. This step aims to express the resistance marker gene on the plasmid and revive the bacterial cells.

v. Mix the bacterial liquid in the centrifuge tube evenly. Take 200 µl and spread it onto LB solid agar medium containing ampicillin. Gently coat the cells evenly using sterile elbow glass rods or glass beads. Once the plate surface is dry, invert it and culture at 37°C for 12-16 hours.

4. Testing

i. 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.

ii. Rapid detection: The selected colonies were directly subjected to PCR detection

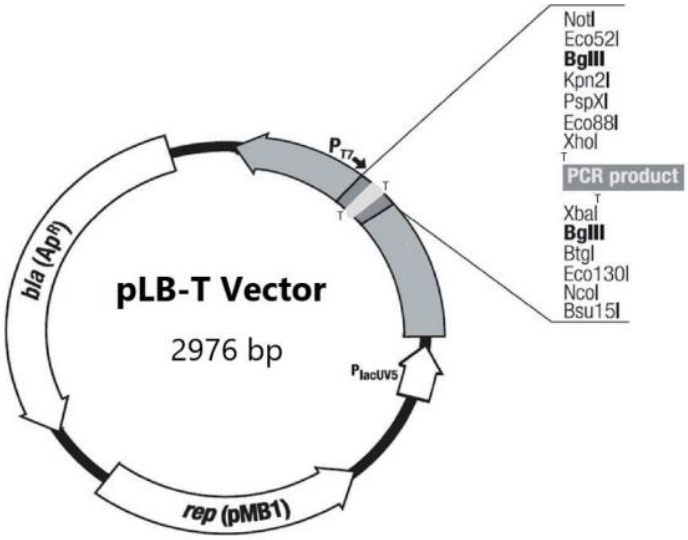
iii. Sequencing identification: The initial identification is followed by sequencing using conventional or rapid methods.

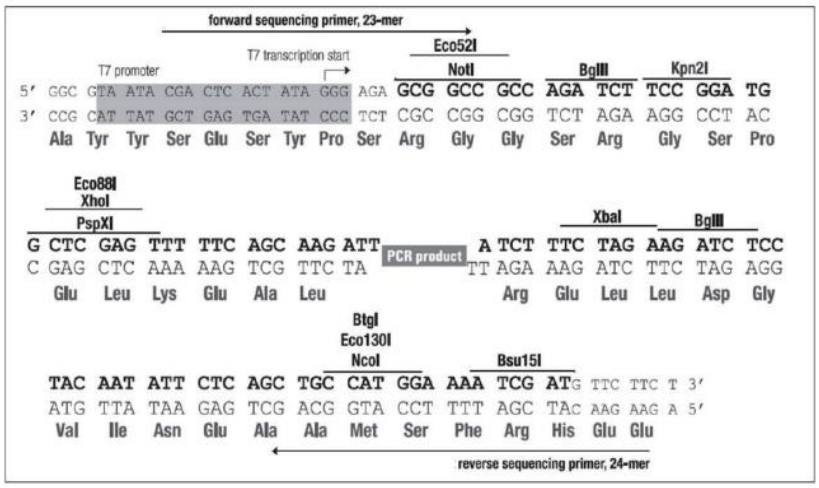
PLB-T Vector sequencing primers:

pLB Forward Sequencing Primer(23-mer): 5’-CGACTCACTATAGGGAGAGCGGC-3’

pLB Reverse Sequencing Primer(24-mer): 5’-AAGAACATCGATTTTCCATGGCAG-3’

pLB-T Vector map





◎ Revision History ◎

Description	Version	Date
Initial Release	S2GNM03h30001_Protocol_V1	Dec 2023