HE Swift Cloning Kit

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

Contents

Introduction

The TOOLS HE Swift Cloning Kit is an advanced positive selection system for the highest efficiency cloning of PCR products generated with *Pfu* DNA polymerase, *Taq* DNA polymerase, or other thermostable DNA polymerases. Additionally, any other DNA fragment, either blunt-end or sticky-end, can be successfully cloned using the kit. Cloning is fast and efficient (within 5 min) and yields more than 99% positive clones.

The kit features the novel positive selection cloning vector *p*HE. This vector contains a lethal gene that is disrupted by the ligation of a DNA insert at the cloning site. As a result, only cells with recombinant plasmids are able to propagate, eliminating the need for expensive blue/white screening.

The vector contains an expanded multiple cloning site, as well as a T7 promoter for in vitro transcription. Sequencing primers are included for the convenient sequencing of the insert.

Features

1. Quick and efficient: Blue/white screening is not necessary, and the entire ligation process can be finished within 5 min.
2. Sensitive and widely applied: Good for the ligation of both low-concentration fragments and long fragments and works for both blunt-end and sticky-end products.

Kit Contents

Contents	VTT-BB05
*p*HE Vector (35 ng/μl)	20 μL
T4 DNA Ligase (3 U/μl)	20 μL
2× Reaction Buffer	100 μL
DNA Blunting Enzyme	10 μL
Forward Sequencing Primer (10 μM)	200 μL
Reverse Sequencing Primer (10 μM)	200 μL
Control Insert DNA (700 bp, 50 ng/μl)	10 μL
ddH_2O	1 mL

Storage

All components of the Kit should be stored at –20°C. Repeated freeze–thaw cycles should be avoided.

Recommended DNA insert volume

1. The insert:vector molar ratios should be controlled to 1:3–1:10. For inserts shorter than 1 kb, a 1:3 molar ratio is recommended; for inserts longer than 1 kb, a 1:7 molar ratio is recommended.
2. Calculate the insert:vector molar ratio according to electrophoresis or UV spectrometer analysis result. The insert volume can be roughly calculated by the following:

Insert (ng) = (3–10) × vector (ng)

For a 35-ng vector, the $\frac{\text{insert length}}{\text{vector length}}$ optimal volume of a PCR product (insert) is recommended as below:

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

Protocol

A. Blunt-End Cloning Protocol

Note:

- Suitable for cloning blunt-end PCR products generated by proofreading DNA polymerases, such as *Pfu* 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 to clone blunt-end DNA fragments generated by restriction digestion. For this process, gel-purify the DNA fragments prior to ligation and use the molar ratio of the *p*HE vector/insert as recommended on page 1.

All procedures must be performed in an aseptic environment.

1. Set up the ligation reaction:

Component	Volume
PCR product	X µl
2× Reaction Buffer	5 µl
*p*HE 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 s.

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

proceed with the transformation.

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

3. Use the ligation mixture directly for bacterial transformation (see page 6).

B. Sticky-End Cloning Protocol

Note:

- Use to clone 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 to clone DNA fragments with 5'- or 3'-overhangs generated by restriction digestion. For this process, gel-purify the DNA fragments prior to ligation and use the molar ratio of the pHE vector/insert as recommended on page 1.
- The DNA blunting enzyme is a proprietary thermostable DNA polymerase with proofreading activity. It will remove 3'-overhangs and fill-in 5'-overhangs. Nucleotides for the blunting reaction are supplied in the reaction buffer.

All procedures must be performed in an aseptic environment.

1. Set up the ligation reaction:

Component	Volume
PCR product	X µL
2× Reaction Buffer	5 µL
DNA Blunting Enzyme	0.5 µL
ddH_2O	Up to 8 µL

Flick the tube to mix and centrifuge for 3–5 s.

2. 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
*p*HE vector (35 ng/µl)	1 µL
T4 DNA Ligase (3 u/µl)	1 µL

Flick the tube to mix and centrifuge for 3–5 s.

4. Incubate the ligation mixture at room temperature (22°C) for 5 min.

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

5. Use the ligation mixture directly for bacterial transformation (see page 6).

C. Control Experiment

Follow the sticky-end protocol

1. Set up the ligation reaction:

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

Flick the tube to mix and centrifuge for 3–5 s.

2. 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
*p*HE vector (35 ng/µl)	1 µl
T4 DNA Ligase (3 u/µl)	1 µl

Flick the tube to mix and centrifuge for 3–5 s.

4. Incubate the ligation mixture at room temperature (22°C) for 5 min.
5. Use the ligation mixture directly for bacterial transformation (see page 6).

D. Transformation of competent *E. coli* cells

1. Prepare LB-ampicillin agar plates with finial ampicillin concentration of 100 µg/mL. Prewarm the plates at 37°C for at least 20 min.
2. Transformation

a. Take tube(s) of TOP10 competent cells from storage and place them in an ice bath until they are just thawed. Carefully add part of the ligation reaction mixture to 50–100 µl TOP10 competent cells. The added volume of the ligation reaction mixture should be less than one-tenth of the 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 cells to detect transformation efficiency. Add 0.1 ng of pUC19 to another tube with a proper amount of competent cells, and then follow the next steps and continue the step of transformation of the ligation product during the same period.)

b. Heat-shock the cells for 90 s in a water bath at exactly 42°C (do not shake). Immediately return the tubes to ice for 2–3 min (do not shake).

c. Add 250–500 µl room temperature SOC or LB culture medium per tube (not containing an antibiotic), and then incubate for 45 min at 37°C with shaking (approximately 150 rpm).

d. Mix bacteria in the tube completely. Then, plate 100 µl of the transformation cultures onto each

LB agar plate containing an antibiotic to ensure good separation of colonies for subsequent single-colony isolation. Smear bacteria completely with an aseptic elbow glass stick. After the surface of the plate is dry, leave the plate to rest at 37°C for 12–16 h.

3. Detection

a. General detection: pipet the transformation mixture into a 1–5 mL liquid LB culture medium (containing 50–100 μg/mL ampicillin) and culture at 37°C overnight with shaking. Save the bacterial strain and extract plasmids. To detect whether the fragment has been correctly inserted, use PCR or enzyme cutting.

b. For PCR detection of the control insert DNA, the follow program can be used:

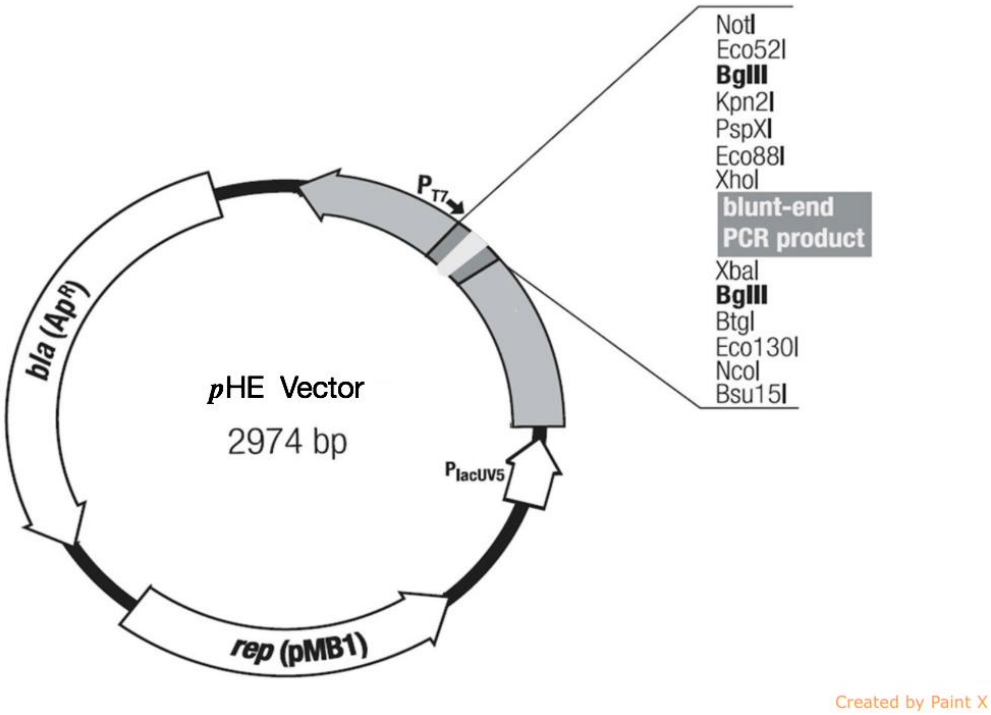
Step	Temperature	Time	Cycles
Initial Denaturation	94℃	3 min	1 cycle
Denaturation	94℃	30 sec	30 cycles
Annealing	55℃	30 sec	
Extension	72℃	1 min	
Final Extension	72℃	5 min	1 cycle
Soak	4℃	hold	1 cycle

c. Quick detection: detecting whether the fragment has been correctly inserted using bacterium PCR directly.

d. Sequencing: sequencing the fragment after a general or quick detection.

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

This product is for research only, not for diagnostic or clinical use.



*p*HE Vector Map

*p*HE Vector Cloning Sites