Hieff Clone™ Zero Blunt Cloning Kit

10909ES

INSTRUCTIONS FOR USE

Ver. EN20230918

CONTENT

Product description

This kit is further developed based on the principle of efficient and rapid connection of DNA fragments by topoisomerase. Compared with the traditional T4 ligase, it has the following advantages: 1) fast, and the connection reaction can be completed within only 1-5 min. 2) High efficiency, no self-connection, the positive cloning rate is close to 100%, and there is no need to set up blue and white spot screening; 3) The operation is simple, and it takes only 15-20 min from connecting to the coating plate. Ice bath, heat shock and 1 hour resuscitation are omitted in the operation process. 4) It can connect up to 5 kb of products.

For rapid cloning of blunt end PCR products; Rapid sequencing of PCR products after cloning (using M13F/M13R primers).

Specifications

Product type	Cloning Kit
Antibiotic-resistant Bacteria	Ampicillin (AmpR)
Methods of cloning	Blunt
Conditions of carriage	Dry ice

Components

Components No.	Name	10909ES20 (20T)
10909-A	pESI-Blunt vector (30 ng/μL)	20 μL
10909-B	1 kb control insert (40 ng/μL)	5 μL
10909-C	10× Enhancer	20 μL

Storage

The product should be stored at -25°C~-15°C for 1 year.

Instructions

1. Cloning experiment of control DNA fragment

1) Prepare the following DNA solution in sterile micro centrifuge tube. Take 10 μL as an example.

Component	Dosage
10 × Enhancer	1 μL
1 kb control insert (40 ng/μL)	1 μL
pESI-Blunt vector (30 ng/μL)	1 μL
ddH_2O	7 μL

2) Mix the above systems. React at room temperature (20-30 °C) for 5 min.

*The connection reaction cannot be carried out on ice. The reaction time should not exceed 5 minutes. Generally, the ligation reaction can be completed in 1-2 minutes to obtain enough recombinants.

3) The linked products can be transformed directly or stored at - 20 °C .

4) Total quantity 10 μL add 100 μL competent cells, gently mix and place at room temperature for 5 minutes.

*5 μL is also acceptable connecting liquid, add 50 μL in competent cells (the added volume shall not exceed 1/10 of the volume of

competent cells).

**Generally, commercial competent cells do not need ice bath and heat shock, and enough transformants can be obtained after being placed at room temperature for 5 minutes. If the efficiency of competent cells is low, it can be carried out according to the standard procedure of ice bath heat shock.

5) Add 300-500 μL LB or SOC medium (without antibiotics), shake at 37 °C 180 rpm for 10 min.

6) Take 200 μL bacterial solution coated plate (LB or SOC solid medium containing ampicillin resistance), cultured overnight (if there are few transformants expected, centrifuged at 4,000 rpm for 1 min to obtain more clones, absorbed and discarded part of the supernatant and retained 100 μL. Lightly flick the suspended bacteria and take all the bacterial liquid (coated plate).

2. Cloning experiment of general DNA fragments

The inserted fragment is a product containing A tail, which can be amplified by conventional THigh fidelity DNA polymerase(YEASEN, Cat#10164ES). If there is no non-specific band and primer dimer, it can be directly connected for ligation reaction. Otherwise, it is recommended to recycle the glue before use.

*PCR products cannot be phosphorylated.

**If the amplification template is plasmid, the template plasmid will cause false positive in subsequent experiments. Therefore, it is recommended to recover the PCR product and connect it.

1) Prepare the connection system according to the following table. Take 10 μL as an example.

Component	Dosage
10 × Enhancer	1 μL
pESI-Blunt vector(30 ng/μL)	1 μL
Insert clip	0.5-8 μL
ddH_2O	Up to 10 μL

*the reaction system can be adjusted according to the above proportion according to the specific experimental conditions.

**Refer to the following table for the amount of different fragments inserted:

Insert clip size	Recommended dosage
0.1-1 kb	20-50 ng
1-2 kb	50-100 ng
2-5 kb	100-200 ng

2) Mix the above systems. React at room temperature (20-30 °C) for 5 min.

*The connection reaction cannot be carried out on ice. The reaction time should not exceed 5 minutes. Generally, the ligation reaction can be completed in 1-2 minutes to obtain enough recombinants.

3) Total quantity 10 μL add 100 μL competent cells, gently mix and place at room temperature for 5 minutes.

*5 μL is also acceptable connecting liquid, add 50 μL in competent cells (the added volume shall not exceed 1/10 of the volume of competent cells).

**Generally, commercial competent cells do not need ice bath and heat shock, and enough transformants can be obtained after being placed at room temperature for 5 minutes. If the efficiency of competent cells is low, it can be carried out according to the standard procedure of ice bath heat shock.

4) Add 300-500 μL LB or SOC medium (without antibiotics), shake at 37 °C 180 rpm for 10 min.

*Generally, when the commercial competent cells do not exceed 2 kb insertion fragments, enough transformants can be obtained after 10

min recovery. If the competent efficiency is low or the insertion fragments are long and there are few transformants, the recovery time can be increased to 30-60 min to obtain more transformants.

5) Take 200 μL bacterial solution coated plate (LB or SOC solid medium containing ampicillin resistance), cultured overnight (if there are few transformants expected, centrifuged at 4,000 rpm for 1 min to obtain more clones, absorbed and discarded part of the supernatant and retained 100 μL. Lightly flick the suspended bacteria and take all the bacterial liquid coated plate).

6) Screening and identification of transformants

a. Colony / bacterial liquid PCR identification, a single colony is selected into a colony PCR Mix using a sterile gun tip or toothpick (e.g. 2× Hieff™ Ultra-Rapid HotStart PCR Master Mix (with Dye) Cat#10157ES), adding primers for direct PCR reaction.

b. Plasmid size identification: select monoclonal and identify according to the plasmid size after extracting the plasmid.

c. Enzyme digestion identification: select appropriate restriction enzymes for identification according to the cloning experimental design.

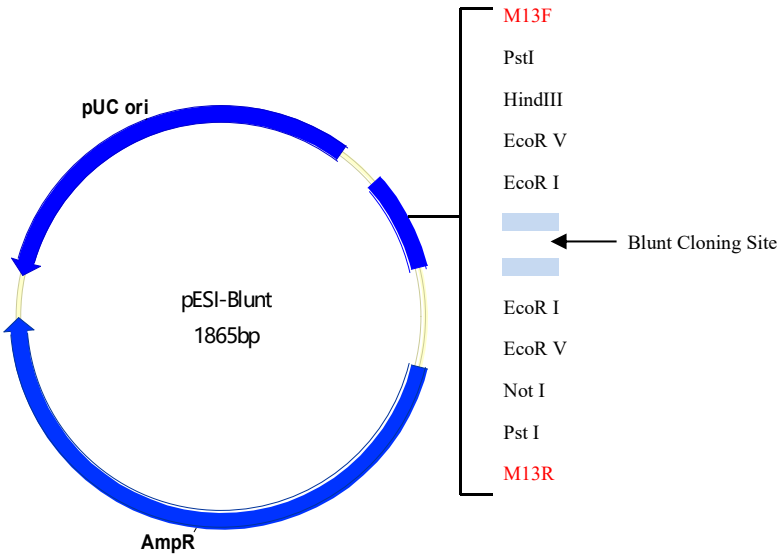
d. Sequencing analysis: optional sequencing primer sequences are as follows:

M13F：TGTAAAACGACGGCCAGT

M13R：CAGGAAACAGCTATGACC

*The positive rate of this product is quite high. Generally, the positive cloning rate is close to 100%. As long as the growing colonies are normal (not contaminated miscellaneous bacteria, and the number of transformants is not too small), they are basically positive clones. Therefore, when the inserted fragment does not exceed 2-3 kb, you can directly select 1-2 bacteria for sequencing without identification.

pESI-Blunt vector Map



M13 Forward primer PstI

CTACCGAAGAAAGGCCCACCCGTGAAGGTGAGCCAGTGAGTTGATTGTGTAAAACGACGGCCAGTGTCTGAGGCTCGCTGCAGTCCTG

GATGGCTTCTTTCCGGGTGGGCACTTCCACTCGGTCACTCAACTAACACATTTTGCTGCCGGTCACAGACTCCGAGCGACGTCAGGAC

HindIII EcoRV EcoRI EcoRI EcoR V NotI PstI

AAGCTTGATATCGAATTCGCGTGTCGCCCTT 插入片段 AAGGGCGACACGCGAATTCGATATCGCGGCCGCCTGCAG

TTCGAACTATAGCTTAAGCGCACAGCGGGAA TTCCCGCTGTGCGCTTAAGCTATAGCGCCGGCGGACGTC

M13 Reverse primer

TCAATACTGACGATGGTCATAGCTGTTTCCTGTCCATAGCAGAAAGTCAAAAGCCTCCGACCGGAGGCTTTTGACTTGATCG

AGTTATGACTGCTACCAGTATCGACAAAGGACAGGTATCGTCTTTCAGTTTTCGGAGGCTGGCCTCCGAAAACTGAACTAGC

pESI-blunt vector sequence

ORIGIN

1 cttgaagtgg tggcctaact acggctacac tagaagaaca gtatttggta tctgcgctct

61 gctgaagcca gttacctcgg aaaaagagtt ggtagctctt gatccggcaa acaaaccacc

121 gctggtagcg gtggtttttt tgtttgcaag cagcagatta cgcgcagaaa aaaaggatct

181 caagaagatc ctttgatttt ctaccgaaga aaggcccacc cgtgaaggtg agccagtgag

241 ttgattgtgt aaaacgacgg ccagtgtctg aggctcgctg cagtcctgaa gcttgatatc

301 gaattcgcgt gtcgccctta agggcgacac gcgaattcga tatcgcggcc gcctgcagtc

361 aatactgacg atggtcatag ctgtttcctg tccatagcag aaagtcaaaa gcctccgacc

421 ggaggctttt gacttgatcg gcacgtaaga ggttccaact ttcaccataa tgaaataaga

481 tcactaccgg gcgtattttt tgagttatcg agattttcag gagctaagga agctaaaatg

541 agtattcaac atttccgtgt cgcccttatt ccctttttttg cggcattttg ccttcctgtt

601 tttgctcacc cagaaacgct ggtgaaagta aaagatgctg aagatcagtt gggtgcacga

661 gtgggttaca tcgaactgga tctcaacagc ggtaagatcc ttgagagttt tcgccccgaa

721 gaacgttttc caatgatgag cacttttaaa gttctgctat gtggcgcggt attatcccgt

781 attgacgccg ggcaagagca actcggtcgc cgcatacact attctcagaa tgacttggtt

841 gagtactcac cagtcacaga aaagcatctt acggatggca tgacagtaag agaattatgc

901 agtgctgcca taaccatgag tgataacact gcggccaact tacttctgac aacgatcgga

961 ggaccgaagg agctaaccgc ttttttgcac aacatggggg atcatgtaac tcgccttgat

1021 cgttgggaac cggagctgaa tgaagccata ccaaacgacg agcgtgacac cacgatgcct

1081 gtagcaatgg caacaacgtt gcgcaaacta ttaactggcg aactacttac tctagcttcc

1141 cggcaacaat taatagactg gatggaggcg gataaagttg caggaccact tctgcgctcg

1201 gcccttccgg ctggctggtt tattgctgat aaatctggag ccggtgagcg tgggtctcgc

1261 ggtatcattg cagcactggg gccagatggt aagccctccc gtatcgtagt tatctacacg

1321 acggggagtc aggcaactat ggatgaacga aatagacaga tcgctgagat aggtgcctca

1381 ctgattaagc attggtaatg agggcccaaa tgtaatcacc tggctcacct tcgggtgggc

1441 ctttctgcgt tgctggcgtt tttccatagg ctccgccccc ctgacgagca tcacaaaaat

1501 cgatgctcaa gtcagaggtg gcgaaacccg acaggactat aaagatacca ggcgtttccc

1561 cctggaagct ccctcgtgcg ctctcctgtt ccgaccctgc cgcttaccgg atacctgtcc

1621 gcctttctcc cttcgggaag cgtggcgctt tctcatagct cacgctgtag gtatctcagt

1681 tcggtgtagg tcgttcgctc caagctgggc tgtgtgcacg aacccccccgt tcagcccgac

1741 cgctgcgcct tatccggtaa ctatcgtctt gagtccaacc cggtaagaca cgacttatcg

1801 ccactggcag cagccactgg taacaggatt agcagagcga ggtatgtagg cggtgctaca

1861 gagtt

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*The yellow background is the sequence of polyclonal enzyme digestion sites.

Notes

1. Materials to be prepared:

1) Self-prepared reagents (only some are listed) :

a. High fidelity enzyme: 2 × Hieff Canace™ AdvanceFast PCR Master Mix (With Dye) (Cat#10164ES) or other equivalent.

b. Colony PCR mix: 2 × Hieff™ Ultra-Rapid HotStart PCR Master Mix (with Dye) (Cat#10157ES) or other equivalent product.

c. Nucleic Acid dye: YeaRed™ Nucleic Acid Gel Stain (10,000 × in Water) (Cat#10202ES) or other equivalent products.

2) Self-supplied instrument consumables (only listed part) : PCR instrument, horizontal electrophoresis tank, glue cutting instrument, EP tube, etc.

2. For your safety and health, please wear lab coats and disposable gloves for operation.

3. This product is for research use ONLY!

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