HB220505

Hieff Clone™ Zero TOPO-Blunt Cloning Kit

Product Information

Product name	Cat#	Size
Hieff Clone™ Zero TOPO-Blunt Cloning Kit	10909ES20	20 T

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 in 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.

Product use

Rapid cloning of flat end PCR products.

Rapid sequencing of PCR products after cloning (using M13F / M13R primers).

Product components

Component number	Component name	10907ES20（20 T）
10907-A	pESI-T vector (30 ng/μL)	20 μL
10907-B	1 kb control insert (40 ng/μL)	5 μL
10907-C	10× Enhancer	20 μL

Shipping and Storage

The product is shipped with dry ice and can be stored at -20℃ for 1 year.

Cautions

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

2. This product is for research use ONLY !

Protocol

1.Cloning experiment of control DNA fragment

1.1 Prepare the following DNA solution in sterile micro centrifuge tube. Take10 μL as an example.

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

1.2 Mix the above systems. React at room temperature (20-30 ℃) for 5 min.

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

1.3 The linked products can be transformed directly or stored at - 20 ℃.

1.4 Total quantity 10 μL add 100 μL competent cells, gently mix and place at room temperature for 5 min.

[Note]: a) 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).

b) 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 min. If the efficiency of competent cells is low, it can be carried out according to the standard procedure of ice bath heat shock.

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

1.6 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 4000 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 flat end product, which can be amplified by conventional Taq enzyme (Yesen, Cat#10101-10106), hot start Taq enzyme (Yesen, Cat#10110), or long fragment DNA polymerase (Yesen, Cat#10107ES62). 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.

[Note]: a) PCR products cannot be phosphorylated.

b) 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.

2.1 Prepare the connection system according to the following table. Take10 μL as an example.

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

[Note]: a) the reaction system can be adjusted according to the above proportion according to the specific experimental conditions.

2.2 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

2.3 Mix the above systems. React at room temperature (20-30 ℃) for 5 min.

[Note]: 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 minto obtain enough recombinants.

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

[Note]: a) 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).

b) 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.

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

[Note]: 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.

2.6 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 4000 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).

2.7 Screening and identification of transformants

2.7.1 Colony / bacterial liquid PCR identification;

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

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

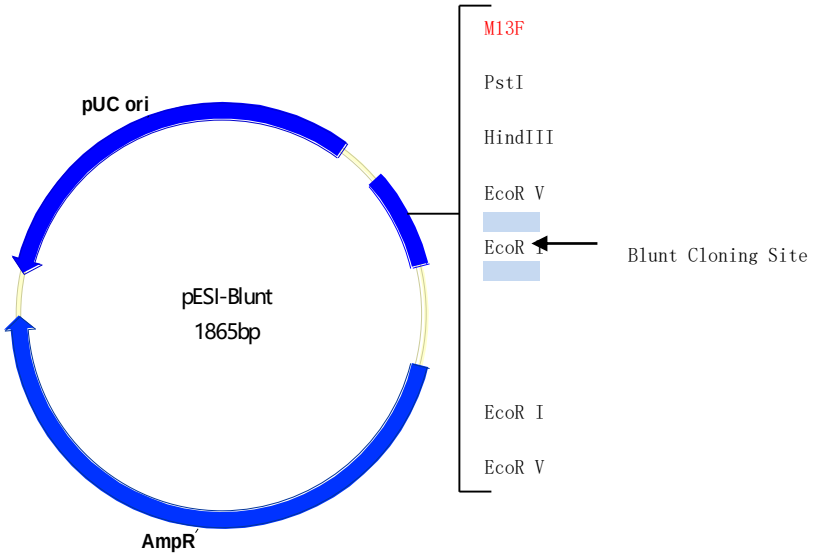
2.7.4 Sequencing analysis: optional sequencing primer sequences are as follows:

M13F：TGTAAAACGACGGCCAGT

M13R：CAGGAAACAGCTATGACC

[Note]: 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



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 ccctttttg 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//
```

[Note]: The yellow background is the sequence of polyclonal enzyme digestion sites