Ver. HB230112

Hieff Clone™ Universal One Step Cloning Kit

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.

Components

Components No.	Name	10907ES20 (20T)
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

Specifications

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

Storage

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

Instructions

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/μL)	1 μL
pESI-T vector (30 ng/μL)	1 μL
ddH2O	7 μL

1.2 Mix the above systems. React at room temperature (20-30 °C) 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 minutes to obtain enough recombinants.

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

1.4 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.

1.5 Add 300-500 μL LB or SOC medium (without antibiotics), shake at 37 °C 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 product containing A tail, which can be amplified by conventional Taq enzyme (YEASEN, Cat#10101-10106), hot start Taq enzyme (YEASEN, Cat#10110), or long fragment DNA polymerase (YEASEN, 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	Up 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 °C) 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 minutes to 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 °C 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 μ L 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.

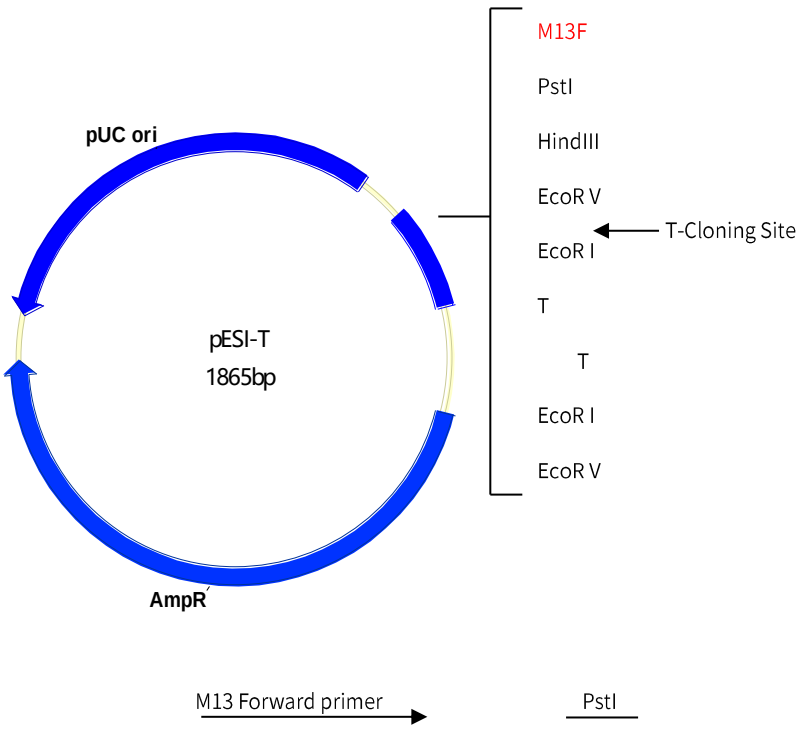
2.7.4 Sequencing analysis: optional sequencing primer sequences are as follows:

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-T vector Map



CTACCGAAGAAAGGCCCACCCGTGAAGGTGAGCCAGTGAGTTGATTGTGTAAAACGACGGCCAGTGTCTGAGGCTCGCTGCAGTCCTG
GATGGCTTCTTTCCGGGTGGGCACTTCCACTCGGTCACTCAACTAACACATTTTGCTGCCGGTCACAGACTCCGAGCGACGTCAGGAC

HindIII EcoRV EcoRI EcoRI EcoR V NotI PstI

AAGCTTGATATCGAATTCGCGTGTCGCCCTT Insert clip AGGGCGACACGCGAATTCGATATCGCGGCCGCCTGCAG
TTCGAACTATAGCTTAAGCGCACAGCGGGA TTCCCGCTGTGCGCTTAAGCTATAGCGCCGGCGGACGTC

M13 Reverse primer

TCAATACTGACGATGGTCATAGCTGTTTCCTGTCCATAGCAGAAAGTCAAAAGCCTCCGACCGGAGGCTTTTGACTTGATCG
AGTTATGACTGCTACCAGTATCGACAAAGGACAGGTATCGTCTTTCAGTTTTCGGAGGCTGGCCTCCGAAAACTGAACTAGC

pESI-T vector sequence

ORIGIN

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      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
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   1561 cctggaagct ccctcgtgcg ctctcctgtt ccgaccctgc cgcttaccgg atacctgtcc
   1621 gcctttctcc cttcgggaag cgtggcgctt tctcatagct cacgctgtag gtatctcagt
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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.

Notes

1. Please wear the necessary PPE, such lab coat and gloves, to ensure your health and safety!