Hieff Clone™ Universal II One Step Cloning Kit

10923ES

INSTRUCTIONS FOR USE

Ver. EN20240717

CONTENT

Product description

Hieff Clone™ Universal II One Step Cloning Kit is a new generation homologous recombinant cloning kit. The carefully optimized 2nd generation 2× Hieff Clone™ Universal II Enzyme Premix combines the recombinant enzyme and the buffer required for the recombinant reaction with the addition of a unique recombinant enhancer to significantly improve the efficiency of recombinant cloning.

The kit can be directed to clone PCR products to any site of any vector, compatible with unpurified PCR products, directly recovered PCR products, low concentration of rubber recovered products, this product can reassemble the homologous arm GC content of 30%-70% of the joint fragment. The vector was completely linearized, and homologous sequences of the end of the linearized vector of 15-25 bp were introduced into the 5 'end of the positive and reverse PCR primers of the inserted fragment, so that the 5' and 3 'ends of the PCR products of the inserted fragment had exactly the same sequence corresponding to the two ends of the linearized vector, respectively. Under the action of recombinant enzyme, the recombinant reaction of PCR product and linearized vector can be completed in as little as 5 min at 50°C. The positive rate of cloning can reach more than 95%.

Components

Components No.	Name	10923ES05（5 T）	10923ES20（20 T）	10923ES50（50 T）
10923-A	2 × Hieff Clone™ Universal II Enzyme Premix	50 μL	200 μL	500 μL
10923-B	500 bp control insert (25 ng/μL)	5 μL	5 μL	5 μL
10923-C	pUC 19 control vector, linearized (50 ng/μL)	5 μL	5 μL	5 μL

Specifications

Cloning Process	Seamless cloning
Control	Positive control
Segments	Up to 6 fragments
Product type	Seamless Cloning and Assembly Kit

Product Applications

Rapid Cloning; Directed Cloning; Site-Directed Mutagenesis.

Storage

This product should be stored at -25~-15℃ for 1 years.

Instructions

1. Preparation of target vector and insert fragment

1) Preparation of linearized vector

Select the appropriate cloning site and linearize the vector. This product is not affected by GC content in the homologous arm region. Linearized vectors can be obtained by restriction enzyme digestion or reverse PCR amplification.

a. Enzyme digestion preparation of linearized vector *

Double enzyme digestion linearization: complete linearization, low conversion background. Recommended.

Single digestion linearization: the degree of linearization is poor. The time of enzyme digestion can be extended appropriately to reduce the conversion background.

* False-positive clones without inserted fragments may be formed by the transformation of incomplete linearized circular vectors. If the proportion of such false-positive clones is high, it is recommended to prepare the linearized vectors again and cut the glue for recovery.

b. Linearized vector was prepared by reverse PCR amplification

Vector amplification using a high-fidelity polymerase (such as 2× Hieff Canace™ AdvanceFast PCR Master Mix (With Dye) Cat#10164ES) is recommended to reduce the introduction of amplification mutations. The PCR amplification template should use pre-linearized plasmids as much as possible to prevent the influence of residual ring plasmids on the positive rate of cloning. Hieff Clone™ recombinant reaction system is compatible with almost all enzyme digestion reaction systems and conventional PCR reaction systems. When the purity of vector enzyme digestion products or reverse PCR amplified products is high, the recombinant reaction can be directly carried out without purification. However, when the purity is low and may contain unlinearized ring plasmids, it is recommended to use a high-quality kit for rubber recovery and purification of the linearized vector to improve the purity of the product and remove part of the unlinearized ring vector, which is conducive to improving the recombinant efficiency.

2) Primer design for a single insert fragment

Hieff Clone™ primer design method: The 5 'and 3' ends of the PCR products of the inserted fragment have identical sequences corresponding to the two ends of the linearized vector, respectively, by introducing 15-25 bp homologous sequences of the 5 'ends of the positive and reverse PCR primers of the inserted fragment.

Insertion fragment forward amplification primer design method:

5 '-upstream vector end homologous sequence + enzyme restriction site (can be retained or deleted) + gene specific forward amplification primer sequence -3'

Insertion fragment reverse amplification primer design method:

3 '- gene-specific reverse amplification primer sequence + cleavage site (can be retained or deleted) + downstream vector terminal homologous sequence -5'

It is recommended to use the following the seamless cloning primer design software (https://www.yeasen.com/calculator.html#/double-enzyme/), to automatically generate insert fragment amplification primer. The final primer length is more than 40 bp, and PAGE purification method is recommended for primer synthesis. When calculating the annealing temperature of the amplification primer, only the Tm value of the gene-specific amplification sequence should be calculated, and the homologous sequence at the end of the vector should not be involved in the calculation. In order to obtain high efficiency cloning, it is recommended that Tm≥48 ℃.

3) Primer design for multiple insertion fragments

Hieff Clone™ primer design method: The 5 'and 3' ends of the PCR products of the inserted fragment have identical sequences corresponding to the two ends of the linearized vector, respectively, by introducing 15-25 bp homologous sequences of the 5 'ends of the positive and reverse PCR primers of the inserted fragment.

It is recommended to use the following the seamless cloning primer design software (https://www.yeasen.com/calculator.html#/double-enzyme/), to automatically generate insert fragment amplification primer. For manual design, refer to the following example. Take the primer design of three gene segments inserted between the EcoR I and Hind III cleavage sites of pUC18 vector as an example, the specific design scheme of the primer is as follows:

a. First design the forward amplification primers of the first fragment and the reverse amplification primers of the third fragment (two inserted fragments adjacent to the vector).

First fragment forward amplification primer design:

5 '-upstream vector terminal homologous sequence + enzyme restriction site (can be retained or deleted) + first fragment gene specific forward amplification sequence -3'

Third fragment reverse amplification primer design method:

3 '-third fragment gene specific reverse amplification sequence + enzyme cut site (can be retained or deleted) + downstream vector terminal homologous sequence -5'

b. Secondly, the reverse amplification primers of the first fragment and the forward amplification primers of the second fragment were designed. Homologous sequences for recombination between fragments may be added to the reverse amplification primer of the anterior fragment, to the forward amplification primer of the posterior fragment, or to a part of each fragment. Take the example of adding a homologous sequence to a reverse amplification primer of the front fragment (the first fragment) :

First fragment reverse amplification primer design:

3 '-first fragment gene-specific reverse amplification sequence + second fragment 5' terminal homologous sequence -5 '

Second fragment forward amplification primer design method:

5 '-second fragment gene specific forward amplification sequence -3'

c. Finally, the reverse amplification primers of the second fragment and the forward amplification primers of the third fragment were designed. The design method is consistent with the reverse amplification primers of the first fragment and the forward amplification primers of the second fragment.

4) PCR amplification of the inserted fragment

The insertion fragment amplification can be amplified by any PCR enzyme, regardless of whether there is an A-tail at the end of the product (which will be removed during the recombination process and will not be present in the final vector). However, in order to reduce the introduction of amplification mutations, it is recommended to amplify With a high-fidelity polymerase (e.g., 2× Hieff Canace™ AdvanceFast PCR Master Mix (With Dye) Cat#10164ES). It is recommended to use the following the seamless cloning primer design software (https://www.yeasen.com/calculator.html#/pcr-amplification/), to automatically generate insert fragment amplification primer.

After PCR amplification, a small amount of the product was taken for agarose gel electrophoresis to test the amplification yield and specificity. The Hieff Clone™ recombinant reaction system is compatible with the conventional PCR reaction system. Therefore, if the amplification template is not a circular plasmid with the same resistance as the vector, and the electrophoresis band of the PCR product is single, the amplified product can be directly used in the recombination reaction without purification. However, when the purity of PCR amplification products is low, it is recommended to use high quality kit for rubber recovery and purification of PCR amplification products to improve the purity of products and improve the efficiency of recombinant.

2. Concentration measurement

The method of band brightness comparison by agarose gel electrophoresis is recommended for DNA quantification. The linearized vector and the inserted fragment amplification product were subjected to several equal volume dilution gradients. The original product and the diluted product were each taken 1 μL for agar-gel electrophoresis. The band brightness was compared with DNA Marker to determine the approximate concentration (especially in the case of unpurified linearized vector and inserted fragment amplification product). Nucleic acid concentration meter (Cat#80480ES) can also be used to determine the linearized vector and the inserted fragment, record the concentration value (ng/μL) and OD260/OD280 values, and then calculate the amount of input.

3.Recombination reaction

1) The amount of linearized vector and insert fragments

The total concentration of each fragment and vector inserted in Hieff Clone™ recombination reaction system was 0.02-1 pmol. The molar ratio of the vector to each inserted fragment was 1:2*. The corresponding DNA quality can be calculated by the following formula:

pmol = (insert segment usage ng) × 1,000 / (insert base pair number × 650 daltons)

A 50 ng 5,000 bp DNA fragment is about 0.015 pmol

A 50 ng 500 bp DNA fragment is about 0.15 pmol

* When the length of the inserted fragment is greater than the vector, the calculation method of the optimal vector and the amount of inserted fragment should be changed, that is, the inserted fragment is used as the vector, and the vector is calculated as the inserted fragment. The final reaction concentration of linearized vector and insert fragment can reach 1 ng/μL. When the amplification products of linearized vectors and inserted fragments are not purified and used directly, the total volume used should not exceed 1/5 of the volume of the reaction system, such as not more than 4 μL for the 20 μL system.

Simple input calculation formula: Insert fragment input =2× insert fragment length × insert vector input mass/vector length

2) Recombination reaction system (the reaction system can be expanded or reduced in equal proportions, prepared on ice, each component needs to be mixed before use)

component	Recombinant reaction
2× Hieff Clone™ Universal II Enzyme Premix	10 μL
Amount of input per insert fragment	X μL
Target vector input	Y μL
ddH_2O	To 20 μL

Table 1 Reaction system

3) Recombination reaction conditions

a. After the system is prepared, gently suck and mix each component with a pipette, and briefly centrifuge to collect the reaction liquid to the bottom of the tube.

b. When one fragment was inserted, the reaction condition was 50°C for 5 min; When the number of fragments inserted is 2-6, the recommended reaction condition is 50°C for 10-50 min (the reaction time increases with the number of fragments, as shown in Table 2). The recommended reaction time for ultra-long vector fragment connection is 40-60 min.

Number of connected fragments	Recombination reaction time
2	10 min
3	20 min
4	30 min
5	40 min
6	50 min

Table 2 Reaction time

c. The reaction products can be directly converted or stored at -20°C for thawing and conversion when needed.

4. Transformation

1) Preheat the screening plate to 37°C 15 min in advance.

2) Take out a F-DH5a (e.g., DH5α Fast Chemically Competent Cell, Cat#11803ES) from -80°C and quickly insert into the ice. After the bacteria melted, add the target DNA (plasmids or linking products) , stir gently and leave in the ice bath for 5 min.

*The volume of DNA added should not exceed one tenth of the suspended fluid volume of the competent cell.

3) The mixture from the previous step was transferred to LB medium already prewarmed at 37°C using a 200 μL pipetting gun and evenly coated.

4) Plates were placed in an incubator at 37°C and incubated upside down overnight. If blue and white spots were selected, the plates were incubated upside down at 37°C for at least 15 hours.

5. Clone Verification

The most convenient and rapid method is colony PCR. Pick individual colonies using a sterile tip or toothpick into a colony PCR mix (such as 2×Hieff™ Ultra-Rapid II HotStart PCR Master Mix, Cat#10167ES), and perform PCR directly with added primers. It is recommended to use at least one universal sequencing primer for colony PCR to avoid false positives. Subsequent verification can also be done through restriction digestion or sequencing.

Note

1. Materials to be prepared by oneself:

1) Sample: Prepare the linearized vector and insert fragments by oneself.

2) Self-prepared reagents (only some of them are listed) :

a. super receptive cells: conversion efficiency > 10^8 cfu/μg, such as YEASEN DH5α Fast Chemically Competent Cell (Cat#11803ES).

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

c. Colony PCR mix: 2×Hieff™ Ultra-Rapid II HotStart PCR Master Mix (Cat#10167ES) or other equivalent product.

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

3) 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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