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LiClone™ Ultra One Step Cloning Kit

Cat. #: M0011 Size: 20 rxns

Introduction

LiClone™ Ultra One Step Cloning Kit is simple, fast, and highly efficient DNA seamless cloning technology. It enables rapid directional cloning of inserts into any site in any vector. Use any method to linearize the vector, and introduce the end sequence of the linearized vector at the 5' end of the insert forward/reverse amplification primer, so that the 5' and 3' ends of the PCR product have the same ends sequence (15 - 20 bp) as the linearized vector, respectively. The PCR product with the same sequence as the end of the vector and the linearized vector are mixed in a certain proportion. Under the catalysis of recombinase, the transformation can be performed at 50°C for 5 - 30 min to complete the directional cloning.

The kit is a new generation of recombinant cloning kits, compatible with 1 - 5 fragments homologous recombination. Highly optimized 2 × CE Mix further significantly improves the recombination efficiency of multiple fragments. This product has broader compatibility with the GC content of homology arms, which better guarantees the success rate of cloning in more difficult application scenarios.

Package Information

Component	M0011
2× CE Mix	100 µl
500bp control insert (20 ng/µl)	5 µl
pUC19 control vector (50 ng/µl, Amp+)	5 µl

Storage

At -20°C and avoid repeated free-thaw.

Additional Materials Required

1. PCR templates, primers, linearized vectors
2. Chemically competent cells prepared by cloning strains.
3. High-fidelity DNA polymerase (Cat # M0031)
4. Other materials: ddH_2O, PCR tubes, PCR instrument, *etc.*

Preparation before the experiment and precautions

Preparation of Linearized Vectors

1. Select appropriate cloning site to linearize the vector. It is recommended to select the cloning site from regions with no repetitive sequence and the GC content of the certain region is 40% - 60% both in the upstream and the downstream 20 bp regions flanking the cloning site.

2. Vector linearization: The linearized vector can be obtained by digesting the circular vector with restriction enzymes or by Inverse PCR.

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Protocol

1. Recombination

1.1 The concentration determination of linearized vectors and inserts

Suppose the high-quality gel DNA recovery kit has purified the linearized vectors and inserts, and there is no obvious nonspecific band or smear residue after gel electrophoresis. In that case, instruments based on absorbance, such as Onedrop, can be used to determine the DNA concentration, but the results of concentration are only reliable when A260/A280 value is 1.8 - 2.0. It is recommended to determine concentration by Nanodrop, Onedrop, Qubit, PicoGreen, etc. When the sample concentration is lower than 10 ng/µl, the concentration values obtained by different models of instruments based on A260 may have large differences.

1.2. The calculation of vectors and inserts usage

For single-fragment homologous recombination, the optimal amount of vector required is 0.03 pmol, the optimal amount of insert required is 0.06 pmol (the molar radio of vector to insert is 1:2). For multi-fragment homologous recombination, the optimal amount of inserts and linearized vectors are both 0.03 pmol (the molar radio of vector to insert is 1:1).

These mass can be roughly calculated according to the following formula:

Single-fragment homologous recombination

The optimal mass of vector required = [0.02 × number of base pairs] ng (0.03 pmol)

The optimal mass of insert required = [0.04 × number of base pairs] ng (0.06 pmol)

Multi-fragment homologous recombination

The optimal mass of vector required = [0.02 × number of base pairs] ng (0.03 pmol)

The optimal mass of each insert required = [0.02 × number of base pairs] ng (0.03 pmol)

Note: For single-fragment homologous recombination: The mass of amplified insert should be more than 20 ng. When the length of the insert is larger than that of the vector, the calculation method of the optimal mass of vector and insert should be inverted.

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For multi-fragment homologous recombination: The mass of each insert should be more than 10 ng. When the optimal mass calculated by the above formula is below 10 ng, just use 10 ng directly.

For single-fragment homologous recombination: If there are no obvious non-specific bands or smear shows in gel electrophoresis, the DNA can be directly used without purification and the total volume of vectors and inserts should be ≤2 μl (1/5 of the total volume of recombination reaction system), which will reduce the recombination efficacy (Purification is recommended before recombination).

1.3 Prepare the following reaction on ice:

Components	Recombination	Negative control-1[b]	Negative control-2[c]	Positive control[d]
Linearized Vector[a]	X μl	X μl	0 μl	1 μl
Insert[a] (n≤5)	$Y_1+Y_2...+Y_n$ μl	0 μl	$Y_1+Y_2...+Y_n$ μl	1 μl
2 × CE Mix	5 μl	0 μl	0 μl	5 μl
ddH_2O	to 10 μl	to 10 μl	to 10 μl	to 10 μl

Note: X/Y is the amount of vector/insert calculated by formula. For ensuring the accuracy of pipetting, dilute the vector and the insert at an appropriate ratio before preparing the recombination reaction system, and the amount of each component is not less than 1 μl.

It is recommended to use negative control-1, which can confirm whether there is the residue of circular plasmids in linearized cloning vectors. recommended before recombination).

It is recommended to use negative control-2, when the templates are circular plasmids and share the same antibiotic resistance with the cloning vector.

1.4 Gently pipette up and down for several times to mix thoroughly (DO NOT VORTEX!). Briefly centrifuge to collect the reaction solution to the bottom of the tube.

1.5 Single-fragment homologous recombination: Incubate at 50°C for 5 min and immediately chill the tube at 4°C or on ice.

2 - 3 fragments homologous recombination: Incubate at 50°C for 15 min and immediately chill the tube at 4°C or on ice.

4 - 5 fragments homologous recombination: Incubate at 50°C for 30 min and immediately chill the tube at 4°C or on ice.

Note: It is recommended to perform the reaction on an instrument with precise temperature control such as a PCR machine.

This product is compatible with the input amount of 0.01 - 0.25 pmol vectors and inserts, so when the total volume of vectors and inserts is greater than 5 μl, the input amount can be appropriately reduced, but the reaction time should not exceed the recommended time.

The recombination product can be stored at -20°C for one week. Thaw the product before transformation.

2. Transformation

2.1 Thaw the competent cells on ice (e.g., Fast-T1 Competent Cell).

2.2 Pipette 5 - 10 μl of the recombination products to 100 μl of competent cells, flick the tube wall to mix thoroughly (DO NOT VORTEX), and then place the tube still on ice for 30 min.

Note: The volume of recombination products should be ≤ 1/10 of the volume of competent cells.

2.3 Heat shock at 42°C water bath for 30 sec and then immediately place on ice for 2 - 3 min.

2.4 Add 900 μl of SOC or LB liquid medium (without antibiotics). Then, shake at 37°C for 1 h at 200 - 250 rpm.

2.5 Preheat the corresponding resistant LB solid medium plates in a 37°C incubator.

2.6 Centrifuge the culture at 5,000 rpm (2,500× g) for 5 min, discard 900 μl of supernatant. Then, use the remaining medium to suspend the bacteria and use a sterile spreading rod to gently spread on an agar plate which contains appropriate selection antibiotic.

2.7 Incubate at 37°C for 12 - 16 h.

3. Recombinant Product Identification

3.1 Colony PCR method: Pick several single colonies and mix them with 10 μl ddH2O as the template; use appropriate forward and reverse primers for colony PCR identification.

Note: Please use at least one primer from the vector for Colony PCR.

3.2 Enzyme digestion method: Pick several single colonies and culture them overnight in a liquid medium with appropriate resistance, then extract the plasmid for restriction endonuclease digestion identification.

3.3 Sequencing identification: Sequencing analysis with appropriate primers on the vector.

Troubleshooting

How to design primers?

Primer design: It is recommended to use online primer design software - CE Design, and select the corresponding module for design.

Three parts of primers: Homology arms (15 - 20 bp, exclude restriction sites and base residues, the content of GC is 40% - 60%) + restriction sites (optional) + specific primers (when calculating the Tm value of primers, the homology arms should be excluded).

Few clones or no clone are formed on the plate

Incorrect primer design: The primer includes 15 - 20 bp homology arms (exclude restriction sites) and the content of GC is 40% - 60%.

The amount of linearized vectors and amplified inserts are too low/high in the recombination reaction or the ratio is not appropriate. Please use the amount and ratio according to specification recommended.

Contamination in vector and insert inhibits the recombination: The total volume of unpurified DNA should be ≤2 μl (1/5 of the total volume of reaction system). It is recommended that the linearized vector and PCR products are purified by gel extraction. Then, dissolve the purified product in ddH_2O (pH 8.0).