DNA Assembly Cloning Kit

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1. Product Introduction

The DNA Assembly Cloning Kit is a simple, fast, and efficient seamless cloning kit for DNA that can direct the insertion fragment to any site on any vector. Linearize the vector in any manner, and introduce the end sequence of the linearized vector into the 5' ends of the insertion fragment's forward/reverse PCR primers, so that the 5' and 3' ends of the PCR product carry sequences consistent with both ends of the linearized vector (25-40 bp).This type of PCR product, which has a carrier end sequence at both ends, can be mixed with linearized vectors in a certain ratio. Under the catalysis of recombinase, transformation can be carried out by reacting at 50°C for 15 minutes to complete directional cloning.

1.1 product components:

Components	50 rxn
2 x enzymes mixture	500 μL
Positive control(linearized vector and 2 DNA fragments)	20 μL

1.2 Storage conditions:

-20°C can be stored for a long time without repeated freezing and thawing.

1.3 Scope of application:

Quick cloning
High-throughput cloning
Seamless cloning
fixed-point mutation

1.4 Self-provided materials:

DNA fragment;
linearization vector;
Chemically competent cells: Chemically competent cells prepared from cloned strains;
DH5α Competent E.coli Strain for routine cloning, suitable for plasmids <15 kb;
Large fragment cloning of XL10 competent E. coli strain, suitable for plasmids >10 kb;
Other materials: ddH_2O, PCR tubes, PCR instrument, etc.

2. Use Instructions

2.1 Configure the following reaction system on ice

	Cloning reaction	Positive control
Each DNA fragment	X μL	10 μL
linearization vector	Y μL	
2 × enzymes mixture	10 μL	10 μL
Deionized water	To 20 μL	

Note:The positive control contains the vector and two DNA fragments.

In each cloning reaction system, the recommended usage amounts of linearized vector and single DNA fragment are 0.1 pmol each.

If you use unpurified PCR products for recombinant reactions, the volume of unpurified DNA fragments should not exceed 10% of the total reaction volume.

2.2 Gently mix the reactants with a pipette.

2.3 Place the reaction tube in a PCR instrument and incubate at 50°C for 15 minutes (>6 fragment splicing, with the time extended to 30 minutes).

2.4 Absorb 2 μL of the reaction solution to transform Escherichia coli competent cells. It is recommended to use highly efficient competent cells, meaning that at least >2×10^8 transformants can be obtained after transforming each 1 μg of pUC19 vector.

2.5 If electroporation is required, dilute the reaction solution by 5 times and then pipette 1 μL into it to transform competent cells.

2.6 Prepare a 1/10 volume of transformed cells for spreading. If you cloned more than 3 target fragments, it is recommended that you centrifuge the transformed competent cells before spreading them. For positive control reactions, prepare a 1/10 volume of transformed cells and spread them on an ampicillin-containing plate with 0.1 mM IPTG.

2.7 Overnight culture at 37°C.

3. Calculation of the amount of DNA fragment added

3.1 The recommended usage amount of a single DNA fragment in each reaction is 0.1 pmol.

3.2 It is recommended that the molar ratio of DNA fragments to vectors be 1:1.

3.3 When the cloned fragment is less than 200 bp, it is recommended that the molar ratio of DNA fragment to vector be 5:1.

3.4 To determine the concentration of DNA fragments for UV or fluorescence detection, use the following formula to calculate the amount of each DNA fragment used in the reaction:

$$ul of DNA fragment = \frac{0.65 \times pmol \times bp}{ng/ul}$$

Alternatively, you can refer to the table below and roughly estimate the amount of DNA fragments used in each reaction based on the length of the fragment (for example: add 0.1 pmol of DNA fragments to each reaction):

DNA fragment size	Amount of each reaction DNA added
0.5 kb	33 ng
1 kb	67 ng
1.5 kb	100 ng
2 kb	133 ng
3 kb	200 ng
5 kb	330 ng

4. Related Products

Product	Cat.No.	Size
DNA Assembly Cloning Kit	BK0024-01	50 rxn