Universal One-Step Cloning kit

（M003）

Product Description

This product is a DNA seamless cloning kit developed based on the principle of homologous recombination. Featuring a unique ligase-independent system, the kit enables directional cloning of exogenous DNA fragments into any site of any vector. The enhanced recombinase and optimized reaction buffer significantly boost the recombination efficiency of cloning and its tolerance to impurities, thus achieving simple, rapid and highly efficient recombinant cloning. Driven by recombinase, the insert fragment and linearized vector can complete recombination with only a 5–30 min incubation at 50℃. The recombinant product is ligase-independent and can be directly transformed into competent cells, yielding a positive clone rate of over 95%. **Applications**: gene cloning, site-directed mutagenesis, DNA splicing, etc.

Product Components

Cat. No.	Product Name	Volumes（50 rxns） M003
SC001-A	2 × Universal One-Step Cloning kit	500 μL
SC001-B	Control(1:1):4.6 kb vector + 1 kb insert(50 ng/μL)	20 μL

Note: The Control contains a 4.6 kb linearized vector and a 1 kb insert fragment with Ampicillin (Amp) resistance. For use, directly add 10 μL of the Control to 10 μL of 2× One-Step Cloning Kit to prepare a 20 μL recombination reaction system.

Storage Condition

Store at -30 ~ -15℃ and transport at ≤0℃.

Experimental Principle and Procedure

1. Vector linearization: Prepare the linearized vector by restriction enzyme digestion or inverse PCR.
2. Preparation of insert fragment: Prepare by PCR. The amplification primers used should be designed with homologous sequences added to their 5’ ends (marked in red and blue in the figure), such that a homologous sequence is present between the amplification products, as well as between the amplification products and the linearized vector.
3. Recombination reaction: Mix the linearized vector and insert fragment in proportion, and the recombination can be completed by incubating at 50℃ for 15 min under the catalysis of recombinase.
4. Transformation: Transform the recombinant product into competent cells, and hundreds of single colonies will form on the plate for subsequent positive screening.

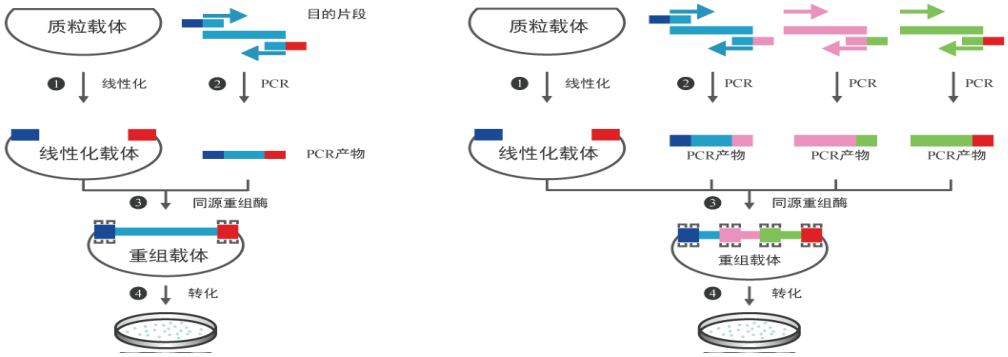


Figure. Schematic diagram of homologous recombination

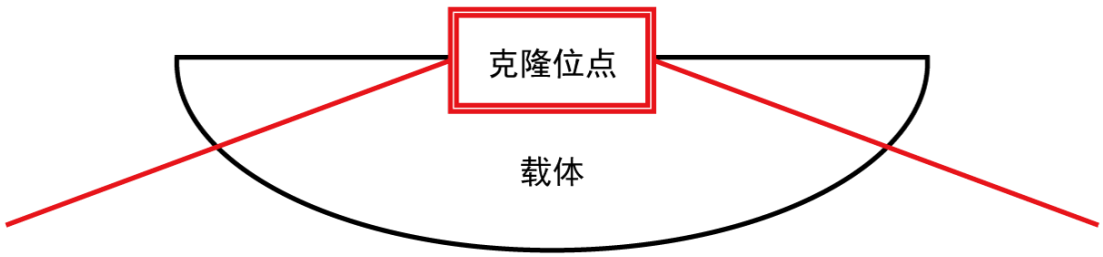
Usage Instructions

(I) Preparation of Linearized Vector

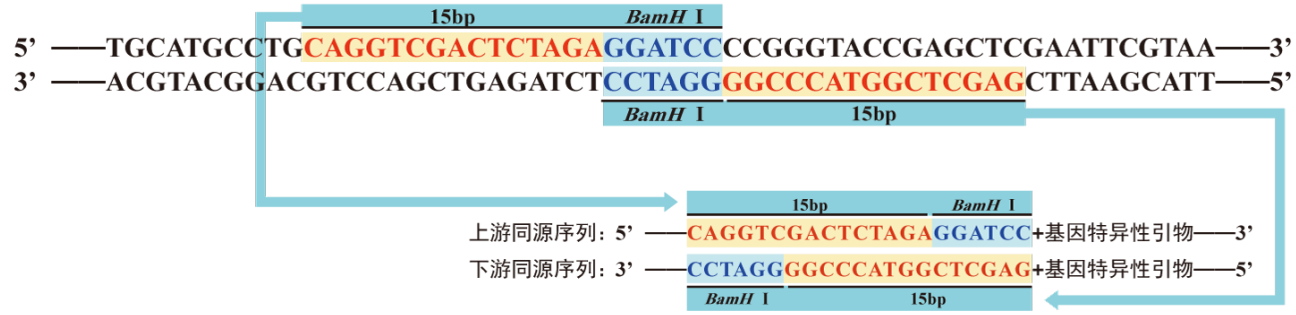
1. Select appropriate cloning sites: Choose sites without repetitive sequences as far as possible, and with a GC content of 40% ~ 60% within the 20 bp region upstream and downstream of the vector cloning site.
2. Vector linearization: Typically prepared by restriction endonuclease digestion or inverse PCR amplification.
   1） Restriction enzyme digestion：Double digestion is recommended to achieve complete vector linearization and reduce transformation background (false positive clones). If single digestion is used for linearization, appropriately extend the digestion time to minimize residual circular plasmids and lower the transformation background.
   2） Inverse PCR amplification：Pre-linearized plasmids should be used as the PCR template as much as possible to prevent residual circular plasmid templates from affecting the positive cloning rate.

(II) Preparation of Insert Fragment

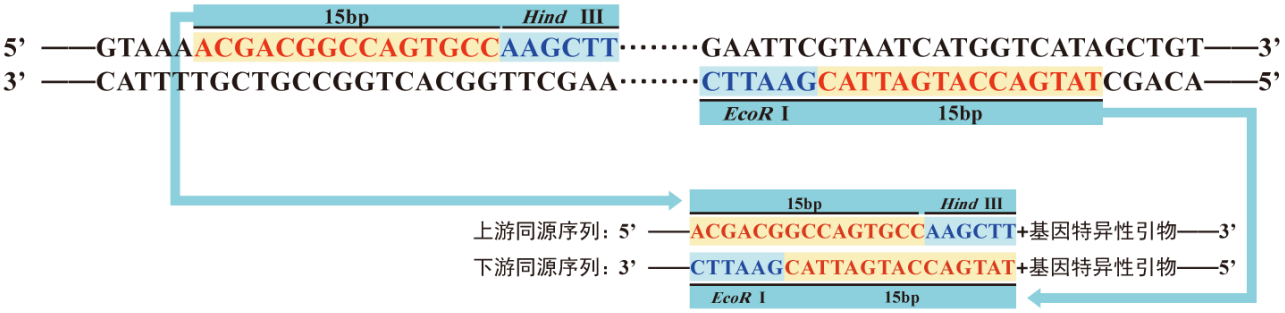
Primer design principle：Homology arm (15–20 bp, 40%–60% GC content) + restriction enzyme site (retained or removed according to experimental requirements) + gene-specific primer. Refer to the following examples:



A. Cloning vector linearized by single restriction enzyme digestion (BamH I):



B. Cloning vector linearized by double restriction enzyme digestion (Hind Ⅲ + EcoR Ⅰ):



C. Cloning vector prepared by inverse PCR amplification:

Note: If the final primer length exceeds 40 bp, **PAGE purification** is recommended for primer synthesis. When calculating the annealing temperature of amplification primers, only the Tm value of the **gene-specific amplification sequence** should be computed; the introduced homology arms and restriction enzyme sites should **not** be included in the calculation.

(III) Recombination Reaction

1. Concentration Determination

   It is recommended to quantify DNA by comparing band intensities using agarose gel electrophoresis. Prepare several

equal-volume dilution gradients of the linearized vector and the amplified insert product. Take 1 μL of both the undiluted and diluted samples for agarose gel electrophoresis, and determine their approximate concentrations by comparing band intensities with a DNA molecular weight marker (DNA Marker). If the linearized vector and amplified insert have been purified, and electrophoresis shows no obvious non-specific bands or smearing, their concentrations can be determined by absorbance using instruments such as a Nanodrop.

2. Amount of Linearized Vector and Insert Fragment

The optimal amount of cloning vector for the recombination reaction is 0.03 pmol, and the optimal amount of insert fragment is 0.06 pmol (the optimal molar ratio of vector to foreign insert is 1:2).

The recommended rough dosage calculated by fragment length is as follows:

Optimal amount of cloning vector (ng)=[0.02×number of base pairs of cloning vector] ng

Optimal amount of insert fragment (ng)=[0.04×number of base pairs of insert fragment] ng

Note: Use a minimum of 40 ng for vectors smaller than 2 kb, and a minimum of 20 ng for foreign insert fragments smaller than 0.5 kb.

3. Recombination Reaction System (prepared on ice, recommended)

Components	Volumes
2 × Universal One-Step Cloning kit	10 μL
Linearized vector	20 ng/kb (min. 40 ng)
Foreign DNA insert	40 ng/kb (min. 20 ng per fragment)
ddH_2O	Up to 20 μL

4. Recombination Reaction Conditions

Incubate at 50 °C for 5–30 min (5–15 min for 1–2 inserts; 15–30 min for 3–5 inserts). After the reaction, place the product on ice for 5 min to terminate the reaction.

Note: It is recommended to use a PCR instrument or other equipment with precise temperature control. Excessive deviations in reaction temperature or duration will affect cloning efficiency. The reaction product can be directly used for transformation or stored at -20 °C.

(IV) Transformation

1. Thaw competent cells for cloning (e.g., DH5α competent cells) on ice.
2. Add 10 μL of the recombination reaction mixture from the previous step into 100 μL of competent cells (the

transformation volume of the recombination product should not exceed 1/10 of the competent cell volume). Mix gently and incubate on ice for 30 min.

3. After heat shock at 42 °C for 90 sec, immediately transfer to ice and incubate for 2 min.
4. Add 900 μL of antibiotic-free SOC or LB liquid medium (without antibiotics), and shake at 37℃ for 1 h (180–200 rpm).
5. Centrifuge at 5,000 rpm for 3 min, then discard 900 μL of the supernatant. Resuspend the cells with the remaining medium and spread gently on a plate containing the appropriate antibiotic using a sterile spreader.
6. Invert the plate and incubate overnight in a 37 °C incubator.

(V) Identification of Recombinant Products

1）Colony PCR identification：Pick a single colony into the PCR mixture using a sterile tip or toothpick for amplification. It is recommended to use at least one vector-specific primer for colony PCR to avoid false positive results.

2）Restriction enzyme digestion identification：Pick a single colony with a sterile tip or toothpick into LB medium containing the appropriate antibiotic and culture overnight. After plasmid extraction, perform restriction digestion with the corresponding endonucleases, and verify the fragment sizes by electrophoresis.

3） Sequencing identification：Perform sequencing analysis and identification using appropriate primers on the vector.

VI. Common Problems and Solutions

(I) No colonies or very few colonies formed on the plate

1. Incorrect primer design: The general primer design principle is: homology arm (15–20 bp, GC content 40%–60%) + restriction enzyme site (retained or deleted according to experimental requirements) + specific primer.
2. Poor ratio of linearized vector to insert fragment: Please refer to the recommended dosage and ratio in the product manual.
3. Impure linearized vector and exogenous insert fragment inhibit the reaction: The volume of unpurified DNA fragments should not exceed 1/5 of the total reaction volume. Purification of the linearized vector and exogenous insert fragment is recommended prior to use.
4. Low efficiency of competent cells: Use freshly prepared or properly stored competent cells for cloning.

(II) Most colonies do not contain the insert or contain incorrect inserts

1. Non-specific amplification: Optimize the PCR conditions and system to improve product specificity; pick more colonies for identification.
2. Incomplete linearization of cloning vector: Verify complete vector linearization using a negative control. Optimize the restriction digestion system by increasing the amount of endonuclease, prolonging digestion time, etc.
3. Reaction system contamination: The system is contaminated with circular plasmids of the same antibiotic resistance.

(III) No bands observed in colony PCR

1. Incorrect primers: It is recommended to use universal primers for the vector, or at least one universal primer for colony PCR detection.
2. Inappropriate PCR system or program: Optimize the PCR system and reaction protocol; extract plasmids and use them as templates for PCR verification; identify by restriction enzyme digestion.
3. Recombination failure: Incomplete linearization of the vector may result in empty vectors. It is recommended to optimize the restriction enzyme digestion system.