Product Specification

Product Name	Seamless Cloning Kit
Catalog Number	CSB-DKT036
Formation	Liquid
Storage Conditions	Store at -20℃, avoid repeated freezing and thawing
Transportation	≤0℃
Contents	DNA exonuclease, DNA polymerase and DNA ligase
Quality Control	The components are tested to be free of RNase residues
Period of validity	12 months

Product Description

The Seamless Cloning Kit is a gene cloning kit based on homologous recombination of the ends of inserts and linearized vectors. The vector is linearized, and the end sequence of the linearized vector is introduced into the 5' end of the forward/reverse PCR primer of the insert, so that the 5' and 3' ends of the PCR product respectively have sequences consistent with the two ends of the linearized vector (15 to 20 bp), the PCR product with the vector end sequences at both ends and the linearized vector are mixed in a certain proportion, and under the catalysis of a specific enzyme mixture, the transformation can be carried out at 50 °C for 15~60 minutes, and the directional cloning is completed.

Contents

Label	*Component*	**Specifications**(20/50/100 rxn)
1	2×Seamless Cloning Mix	100μL/250μL/500μL

Instructions

1. Preparation of linearized vectors

It is recommended to use single/double digestion or inverse PCR amplification to linearize the plasmid, and then recover and purify the linearized vector by the method of gel recovery. If PCR amplification is used, it is recommended to use a high-fidelity polymerase to minimize the introduction of mutations. When the linearized vector is obtained by PCR, it is

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recommended to use DpnI enzyme for digestion after PCR to eliminate the interference of the template plasmid for the subsequent acquisition of the recombinant plasmid.

2. Preparation of inserts

(1) Principles of Primer Design

Introduce a sequence homologous to the end of the linearized vector (15 - 25 bp, excluding the restriction site) at the 5' end of the forward/reverse primer of the insert, so that the end of the amplified insert has and the linearized vector End-to-end homologous sequences.

Primer design method:

Forward primer: 5'—Overlapping region with the upstream end of the vector + Restriction site (optional) + Gene insert forward primer sequence—3'

Reverse primer: 5'—overlapping region with the downstream end of the vector + Restriction site (optional) + Gene insert reverse primer sequence—3'

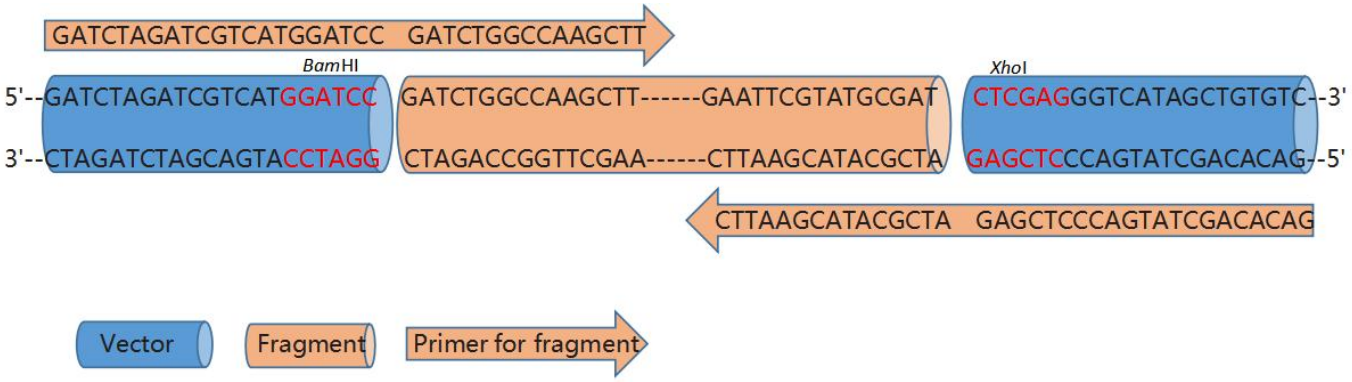
Notice:

a. If the carrier has sticky ends and the 3' end overhangs, the primer design must include the overhang; if the 5' end overhangs, the primer design may or may not include the overhang;

b. Try to select a region with no repetitive sequences and uniform GC content for cloning. It is better if the GC content in the 25bp upstream and downstream regions of the vector cloning site is 40%~60%.;

c. When calculating the annealing temperature of the PCR amplification primers of the target fragment, the homologous sequence does not participate in the calculation of the Tm value.

(2) Primer design for a single insert (Bam HI/Xho I double-digested linearized vector)



Design insert primers:

F (5'-3') : GATCTAGATCGTCAT+**GGATCC (optional)** +GATCTGGCCAAGCTT

R (5'-3') : GACACAGCTATGACC+**CTCGAG (optional)** +ATCGCATACGAATTC

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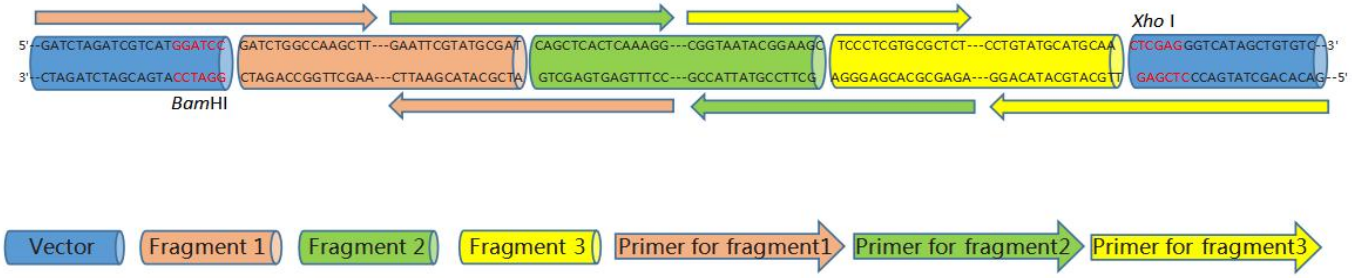
(3) Primer design for multiple inserts(Bam HI/Xho I double-digested linearized vector)



Design insert primers:

Insert 1 F (5'-3') : GATCTAGATCGTCAT**+GGATCC (optional)** +GATCTGGCCAAGCTT

Insert 1 R (5'-3') :CCTTTGAGTGAGCTG+ATCGCATACGAATTC

Insert 2 F (5'-3') : GAATTCGTATGCGAT+CAGCTCACTCAAAGG

Insert 2 R (5'-3') :AGAGCGCACGAGGGA+GCTTCCGTATTACCG

Insert 3 F (5'-3') :CGGTAATACGGAAGC+TCCCTCGTGCGCTCT

Insert 3 R (5'-3') :GACACAGCTATGACC**+CTCGAG (optional)** +TTCGATGCATACAGG

3. Perform recombination reactions

(1) Calculation of the usage of linearized vectors and inserts

In the 10 μl reaction system, for single-fragment homologous recombination reaction, the optimal amount of cloning vector used is 0.03 pmol, and the amount of insert fragment used is 0.06 pmol (the molar ratio of vector to insert fragment is 1:2 (1~10)):

The amount of plasmid vector (0.03pmoL) =[0.03×0.325×2×Number of base pairs of vector] ng

The amount of insert (0.06pmoL) = [0.09×0.325×2×Number of base pairs of inserted fragments] ng

For example, when a 1 KB target fragment is cloned into a 4 KB vector, the optimal amount of vector is:

0.03×0.325×2×4000=78 ng; The optimal usage of the target fragment is: 0.09×0.325×2×1000=58.5 ng。

Notice:

a.When the length of the insert is longer than that of the cloning vector, the calculation methods of the optimal cloning vector and the amount of the insert should be interchanged, that is, the insert is regarded as the cloning vector, and the clone vector is regarded as the insert for calculation.

b.The amount of linearized cloning vector used should be between 50-200 ng; The amount of the amplified product of the insert should be between 10-200 ng. If the length of the insert is less than 200bp, 5 times the amount of vector should be used for the insert. When the above formula is used to calculate the optimal amount of DNA out of range, the minimum/maximum is chosen directly.

c.When the linearized vector and the amplified product of the target fragment are used directly without DNA purification, the total volume added should not exceed 1/5 of the volume of the reaction system. A 10 μl system is 2 μl.

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(2) Preparation reaction system

Prepare the following reaction system in an ice-water bath:

Component	Volume（1~2 Insert fragment）	Volume（≥3 Insert fragment）
2×Seamless Cloning Mix	5μl	5μl
Insert fragment : Linearized vector	3 : 1	1 : 1
Linearized vector	X μl (50 - 200 ng)	X μl (50 - 200 ng)
Insert fragment /PCR product	Y μl (10 - 200 ng)	Y μl (10 - 200 ng)
ddH2O	Up to 10 μl	Up to 10 μl

(3) Reaction conditions

Mix gently with pipette, centrifuge briefly and collect the reaction solution to the bottom of the tube. The reaction system was incubated at **50°C for 30 min(1~2 insert fragments) or 60 min(3~5 insert fragments)**. If subsequent operations cannot be performed immediately after the completion of the reaction, the reaction sample can be stored at -20°C.

4.Transformation

(1) Thaw competent cells on ice（For example：DH5α、BL21、TOP10）.

(2) Add 10 μl of the recombinant product to 100 μl of competent cells, flick the tube wall to mix (do not shake to mix), and let stand on ice for 30 min.

(3) Heat shock in a water bath at 42°C for 90 seconds, immediately place on ice water for 3-5 minutes to cool.

(4) Add 500 μl SOC or LB medium (without antibiotics), incubate at 200 rpm for 1 hour at 37°C.

(5) Pre-warm the corresponding resistant LB solid medium plates at 37°C.

(6) Centrifuge at 5000 rpm for 3 min and discard 500 μl of supernatant. After resuspending the bacteria with the remaining medium, all of them were spread on the LB plate containing appropriate antibiotics, and the plate was placed for 5 minutes to fix the coated bacteria.

(7) Invert the LB plate in an incubator and incubate at 37°C for 12-16 hours.

5.Positive clone identification

Colony PCR: It is recommended to use at least one universal primer to avoid false positive results.

Double digestion identification: Use the restriction sites at both ends of the insert to identify the size.

Sequencing verification: sequenced with the universal primers of the vector, and performed sequence analysis.

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