Product Information

Name: Minerva Super Fusion Cloning Kit

Catalog Number: M2026

Packaging Size: 20T、50T

Product content

Components	20T	50T
Super Fusion Cloning Mix (2×)	100 μL	250 μL
Control Plasmid, linearized (50 ng/μL)	5 μL	5 μL
1000 bp Control Fragment (50 ng/μL)	5 μL	5 μL

Note: For micro-volume reagents, please perform instantaneous centrifugation before the start of the formal experiment.

Storage and Handling

Store at -20 °C, The Minerva Super Fusion Cloning Kit is stable for 2 years from the date of shipping when stored and handled properly.

Product Description

Fusion cloning is a simple, speedy and efficient DNA directed cloning technology, which can clone the inserted fragment to any position in any vector. Minerva Super Fusion Cloning Mix can efficiently and accurately fuse the DNA fragments and the linearized vectors by identifying 15-25bp homlogous sequences at the terminal of linearized vectors, and reacting them at 50℃ for 15-60 min to complete directional cloning, and the positive rate of cloning can reached more than 95%.

Product features

1. Simple, speedy and efficient, which can clone the inserted fragment to any position in any linear vectors.

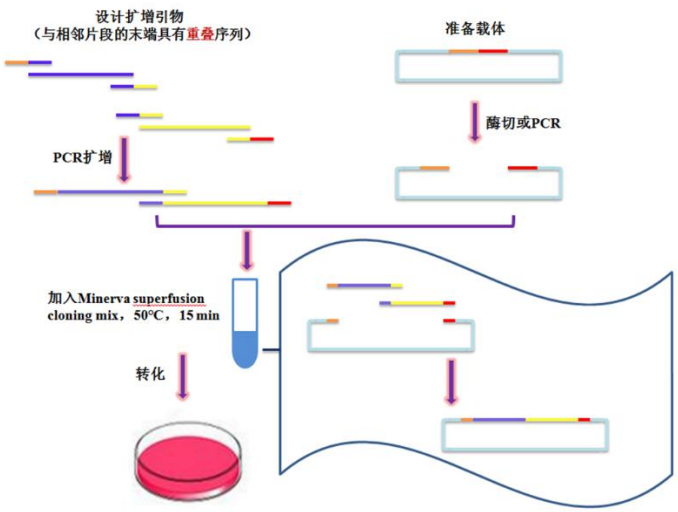
2. Independent on ligase, without carrier self-ligation, and the positive rate of cloning can reached more than 95%.

3. There is no need to consider the enzyme cleavage site carried by the insert fragment itself;

4. One-to-many fragment recombination can be done in one reaction.

Application Protocols

The process of outline diagram



Linearized cloning vector construction

The vector is linearized by selecting a suitable cloning site, and the linearized vector can be completed by restriction enzyme digestion or reverse PCR amplification.

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（1）Enzymatic digestion preparation

Double digestion: linearization is complete, and the transformation background (false positive clone) is low;

Single digestion: The degree of linearization is poor, and the residue of circular plasmid can be reduced by extending the digestion time appropriately.

Notes:

(1) Double digestion does not require dephosphorylation, while single digestion requires dephosphorylation;

(2) After the enzyme digestion is completed, the rapid endonuclease should be inactivated or purified for the desired product and then used for the recombination reaction.

（2）Construction of reverse PCR amplification

Designing a pair of reverse primers, using vector plasmid DNA as a template and cloning site was the demarcation point. It is recommended to use a high-fidelity PCR Mix for amplification.

Design insertion of PCR primer fragments

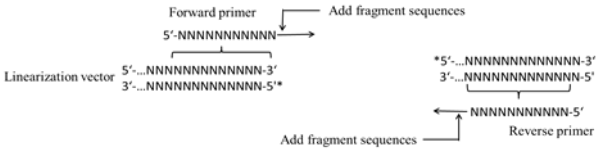
The 5' end of the PCR primer must contain a 15 to 25 nt (recommended 18 nt) sequence homologous to the end of its adjacent fragment (insert fragment or vector). If the carrier is a sticky end and the 3' end is highlighted, the primer design must include a protruding portion; if the 5' end is highlighted, the primer design may or may not include a protruding portion.

Insert forward primer:

5'- upstream vector terminal homologous sequence + restriction site (optional) + gene-specific forward amplification sequence - 3'

Insert reverse primer:

3'- gene-specific inverted amplification sequence + restriction site (optional) + downstream vector terminal homologous sequence - 5'



Note： Try to select a region without repeat sequence and uniform GC content. When the GC content is 40~60% in the 25 nt region upstream and downstream of the vector cloning site, the recombination efficiency is the highest.

PCR amplification of the insert

The insert can be amplified with any PCR enzyme (Taq enzyme or high-fidelity enzyme) without considering the presence or absence of the A-tail at the end of the product (which will be removed during recombination and will not show in the final plasmid). It is suggested that using high-fidelity polymerase for amplification to reduce the occurrence of amplification mutation. It is recommended to use the purified PCR product for seamless cloning reaction. If the PCR product is identified as a specific amplification product by agarose gel electrophoresis, it can be directly used for seamless cloning reaction, but the volume of the sample should not exceed 20% percent of the total volume of the reaction.

Seamless cloning reaction

1. Prepare the following reaction system in an ice water bath:

Components	Reaction system	Negative control	Positive control（optional）
Super Fusion Cloning Mix (2×)	5 μL	5 μL	5 μL
Linearization vector [1]	50-200 ng	50-100 ng	1μL
Insert fragment [2]	50-200 ng	-	1000 bp, 1μL
ddH_2O	To 10μL		

[1] Optimum carrier amount (ng) = 0.02 × carrier base pairs, ie 0.03 pmol.

[2] When inserting a single fragment, the optimum fragment amount (ng) = 0.04 × fragment base pair; when inserting multiple fragments, the optimum amount per fragment (ng) = 0.02 × fragment base pairs.

Notes:

(1) If the length of the inserted single fragment is longer than that of the

vectors, then the vectors and the inserted fragments dosage should be exchanged;

(2) If the length of the inserted fragment is less than 200 bp, then five times the amount of vectors should be needed;

(3) If the amount calculated according to the above formula is lower/higher than the highest value, it is recommended to use it directly according to the minimum/maximum amount;

(4) If the vector or insert is too long and the number of fragments is too large, the positive rate will be lowered.

After the system is prepared, gently pipette several times to mix the components to avoid air bubbles. Do not vortex.

2. Place the reaction system at 50℃ for 15-60 min.

Notes:

(1) It is recommended to use a more accurate temperature control instrument for reaction, such as PCR instrument, the reaction time is too short or too long will reduce the cloning efficiency;

(2) When the carrier backbone is above 10 kb or the insert is above 4 kb, it is recommended to extend the reaction time to 30-60 min;

(3) After the reaction at 50℃ is completed, it is recommended to perform instantaneous centrifugation to collect the reaction liquid to the bottom of the tube.

3. Place the reaction tube in an ice water bath and cool it for direct conversion or storage at -20℃.

Note: Recombinant products were stored at -20°C and it recommended to use within 1 week.

Cloned product transformation

Add 5-10 μL of the reaction solution to 100 μL of competent cells, gently pipette and place on ice for 30 min.

Heatshock at 42 °C for 45~60s, ice bath for 5 min. Add 500 μL of SOC or LB medium and incubate at 37 °C for 40-60 min (200 rpm). The bacterial solution was uniformly spread on a plate containing the corresponding antibiotic, and cultured overnight in a 37 ° C incubator.

Note: (1) The final clone positive rate of different competent cells will be different. It is recommended to use competent cells with transformation efficiency >10^8 cfu/μg;

(2) The number and purity of the PCR product and the linearized vector determine the number of colonies;

(3) The positive control plate usually grows a large number of white single colonies, and the negative control plate only grows few colonies.

Positive clone detection

Colony PCR identification: single colony was picked and mixed in 10 μL ddH_2O, lysed at 95 °C for 10 min, and 1 μL was used as a template for colony PCR identification. UE 2×Taq PCR Master Mix (Green) (S2045) was recommended.

Enzyme digestion identification: single colony was inoculated into resistant medium for overnight cultivation, and plasmids were extracted for enzyme digestion identification.

Note: (1) At least one universal primer is recommended for colony PCR, which can effectively avoid false positive results;

(2) The positive results can be further sequenced and identified if necessary.

Notes:

1) For Cloning reactions, the reagent used for the PCR reaction is recommended: 2×Taq PCR Master Mix（Green） (Catalog No. S2045). The above products can be combined with Minerva Super Fusion Cloning Kit to obtain highly reliable results.

2） In the final examination of electrophoresis for positive results, non-toxic, non-carcinogenic and aquatic animals friendly super gelred can be used as nucleic acid gel electrophoresis reagent.

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Questions

Problem Description	Possible reasons	Solution
Low transformation efficiency	Bacteria were not competent	You should use ⩾10^8 cfu/μg competent cells, otherwise use fresh competent cells.
	Poor DNA fragment ratio	The reaction system was prepared according to the most suitable amount and ratio recommended by the instructions. The commonly used absorbance measurement method is susceptible to factors such as DNA purity and pH value of the buffer, and the measurement deviation is large. Therefore, it is recommended to use agarose gel to purify DNA first then determine the sample concentration.
	Poor DNA fragment purity	Gel Purified your Vector and DNA fragment. Since metal ion chelators such as EDTA inhibit the cloning reaction, the purified product should be dissolved in ddH_2O, and do not use a buffer such as Tris-EDTA.
	Excessive reaction product	In the transformation system, the volume of the seamless cloning reaction product should not exceed 1/10 of the volume of the competent cells.
Large numbers of colonies obtained without insertion	Incomplete linearization of your vector	When the vector is linearized, the amount of restriction endonuclease is increased, the reaction time is prolonged, or the digestion product is purified using a gel recovery.
	Contamination of Fusion reaction by plasmid with same antibiotic resistance	When the plasmid is used as a template for PCR amplification of the insert, the pre-linearized plasmid is used as an amplification template, and the amplified product is treated with a methylation-sensitive endonuclease such as DpnI, or the product is subjected to gel recovery and purification.
	Plates too old or contained incorrect antibiotic	Be sure that your antibiotic plates are fresh. Check the antibiotic resistance of your fragment.
Large number of colonies contain incorrect insert	Your PCR product contained non-specific sequences	If your PCR product is not a single distinct band, then it may be necessary to gel purify the PCR product to ensure cloning of the correct insert.

Notes

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