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One Step Seamless Cloning Mix

Product Number: CLN34

Shipping and Storage

-20°C

Description

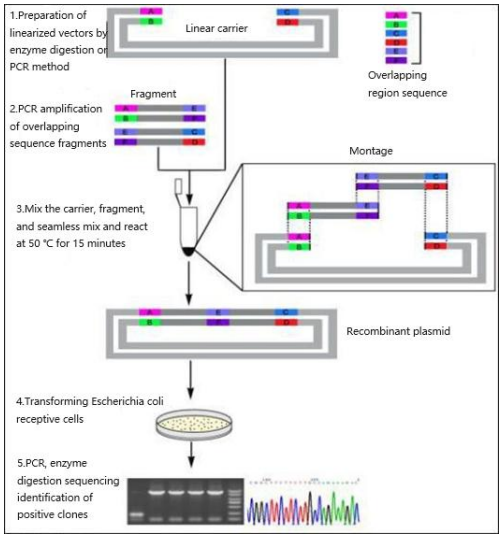
Unlike conventional cloning methods such as topological cloning, TA vector cloning, and enzyme digestion cloning, seamless cloning technology can clone fragments to any position in any vector with just one step of reaction under the action of recombinant enzymes to obtain recombinant plasmids.Seamless cloning technology, as a very powerful cloning technology, has the characteristics of fast, simple, efficient, multi segment assembly, and targeted cloning. It is used for experimental purposes such as cloning a single DNA fragment, assembling and cloning multiple DNA fragments, and constructing multiple site mutations.

Features

1. Simple, fast, precise, and targeted cloning, with a connection process that only takes 15 minutes.
2. Fragment targets can be prepared with simple PCR amplification, without the need for endonucleases, ligases, and phosphorylases.
3. The preparation of linearized vectors can be achieved through PCR amplification or selection of appropriate endonucleases.
4. Long fragments of DNA can be cloned.

Experimental Principles

(The following figure shows the assembly and cloning process of two fragments)



Protocol

1. Preparation of linearized carriers

1.1. Preparation of linearized carriers by enzymatic digestion

Linearization of clone vectors using single or double enzyme digestion is generally more effective than single enzyme tangency.The complete linearization of the carrier is the key to successful seamless connection.The false positive clones that appear after the transformation of the recombinant products of incomplete enzyme cleaved vectors are generally formed by the transformation of non linearized circular vectors. When the false positive clones are high, it is necessary to re cleave the vector.When using electrophoresis method to determine whether the carrier linearization is complete, it is necessary to use an uncut plasmid as a control for electrophoresis together.

The recombination region selected on the plasmid vector should be a region without repetitive sequences and with uniform

base distribution.The highest recombination efficiency will be achieved when the G+C% content in the recombination area ranges from 40% to 60%.Linear carriers prepared by single or double enzyme digestion do not require terminal dephosphorylation treatment.

1.2. Preparation of Linearized Clone Vector by Reverse PCR Amplification

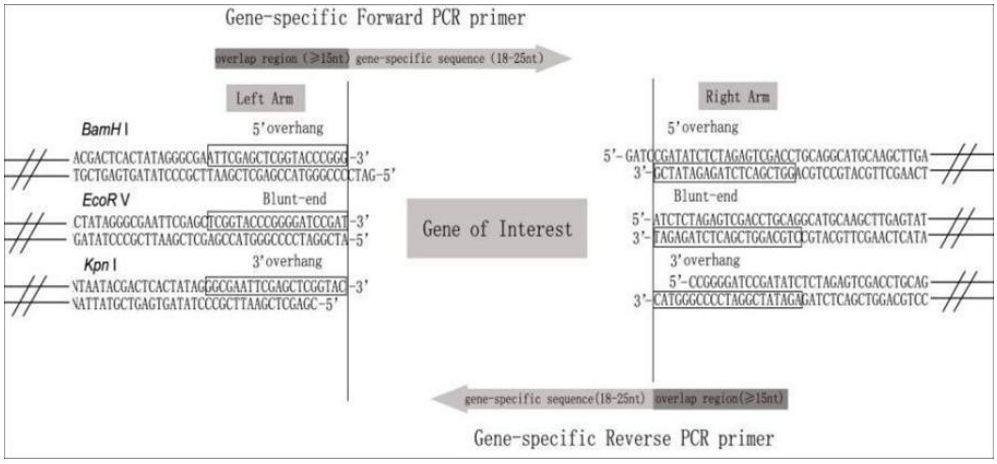
Using vector plasmid DNA as a template and clone sites as boundary points, a pair of reverse primers were designed and amplified with high fidelity DNA polymerase to prepare a linearized vector for recombination.To prevent the impact of residual circular vector plasmid templates on the cloning positive rate. The linearization carrier needs to be purified using gel recovery method or Dpn I digestion combined with gel recovery method.The high fidelity PCR amplification product is a flat terminal, and the functional group at the end of DNA is hydroxyl, without phosphate groups (except for phosphorylated primers).

2. PCR amplification of target DNA

The inserted fragment can be amplified using any PCR enzyme (Taq enzyme or high fidelity enzyme), without considering the presence or absence of an A-tail at the end of the product (which will be removed during the recombination process and will not appear in the final plasmid). It is recommended to use high fidelity polymerase for amplification to reduce the occurrence of amplification mutations.After PCR, a small amount of the product was taken for agarose electrophoresis to test the length and specificity of the amplified product. The presence of non-specific amplification and primer dimers can seriously affect cloning efficiency. It is recommended to use gel recovery kits to purify the target fragment.

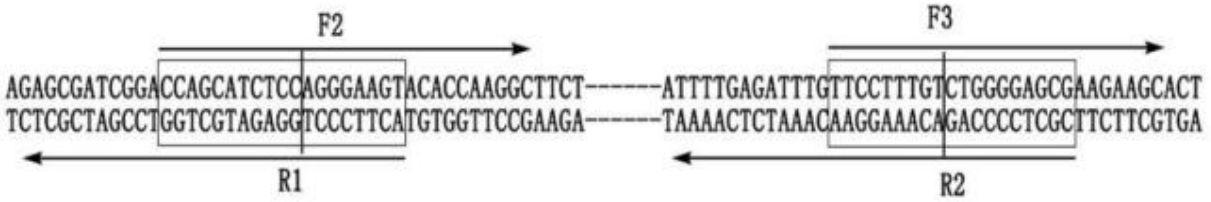
2.1. Primer design for splicing and cloning of a single fragment

This kit can clone any PCR amplification product, but the amplification primers have special design requirements.The primer sequence consists of an overlapping region of 15-25nt at the 5 'end for homologous recombination and a specific primer for the target gene at the 3' end.The following figure is a schematic diagram of primer design requirements for single fragment cloning.For example, the BamH I, EcoR V, and KpnI restriction endonucleases produce a 5 'protruding end, a flat end, and a 3' protruding end, respectively. The base length of the overlap region is designed to be between 15-25nt, with gene specific primers adjacent to the overlap region sequence, typically 18-25nt in length.According to the design method shown in the figure below, the endonuclease used in the preparation of the recombinant vector will not have the enzyme site. If the primer for amplifying the target fragment retains the restriction endonuclease site or adds additional restriction endonuclease sites, the original restriction endonuclease site or newly added restriction endonuclease site will appear in the final recombinant plasmid sequence.The following figure only shows the situation of single enzyme cleavage, and in actual operation, it can be one of three terminal structures or any combination of two.



2.2. Primer design for assembly cloning of multiple fragments

The primer design for multi fragment assembly cloning is shown in the following figure. The design method of primers at both ends of the carrier is the same as that of single fragment primers, and the design of multi fragment primers follows the principle of overlapping extension PCR primer design.



The following figure shows a partial sequence of three fragment assembly clones. Primer R1 and undisclosed primer F1 amplify the first fragment, primer F2 and primer R2 amplify the second fragment, primer F3 and undisclosed primer 3 amplify the third fragment, and the column in the direction of primer 5 '-3 in the head direction represents the overlap zone. The line in the square represents the fragment boundary

Recommend using NEB's free online design tool NEBuilder™, Experimental design that allows for multi segment assembly.

3. Recombinant connection of vector fragments

Add one by one in a 0.2ml PCR tube according to the following table

Component	Volume
PCR product (50-100ng/ul)	Xul
Linearized vector (50-100ng/ul)	Yul
2×Seamless Cloning Mix	5ul
Replenish water to total volume	10ul

Operation: Gently mix and centrifuge for a few seconds. Hold at 50℃ for 15 minutes on the PCR instrument. After the reaction is completed, the centrifuge tube is placed on ice for the conversion experiment. If the conversion experiment is not conducted temporarily, the connecting product can be frozen at -20℃.

Note:1)The carrier dosage is generally between 50-100ng, which is better. The molar ratio of carrier to fragment is 1:1 to 1:3. When the fragment size is less than 200bp, the fragment dosage can be increased to five times the amount of the carrier.

2)The reaction time at 50℃ should not exceed 60 minutes

4. Transform

4.1. Add the connecting product to the newly melted receptive cells, gently mix well, and soak in ice water for 20-30 minutes.

4.2. 42℃ water bath heat shock for 90 seconds, do not shake the water surface. Immediately after the heat shock, place it in an ice water bath for 2 minutes.

4.3. Add 500 μl of SOC or LB medium to the tube and incubate in a 37℃ shaker at around 200rpm for 60 minutes.

4.4. Centrifuge at 4000rpm for 1 minute, discard some of the supernatant, retain 100-200μl, gently blow the bacterial block with a suction head to resuspend the bacteria, take half of the bacterial solution and apply it to a LB solid culture plate containing antibiotics. After the liquid is sucked dry, invert the plate and incubate at 37℃ overnight.

Note: If the methods of using receptive cells are different, please follow the instructions for receptive cell products.

5. Positive clone identification

5.1. Colony PCR method;

5.2. Restrictive enzyme digestion analysis method;

5.3. DNA sequencing analysis methods.