

LightNing® DNA Assembly Mix Plus

REF: EG21202S

Storage Condition

-20°C

Components

Component	Amount
LightNing® DNA Assembly Mix Plus	250 µl
pUC19 Control Plasmid, Linearized (Amp ^r , 40 ng/µl)	5 µl
500 bp Control Fragment (20 ng/µl)	5 µl

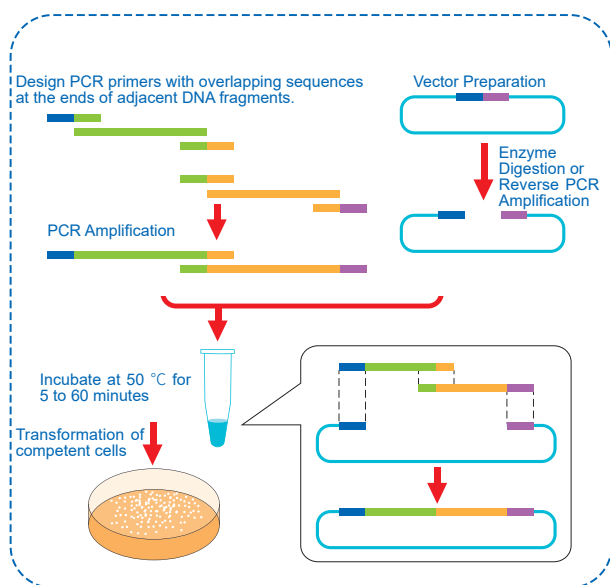
Description

LightNing® DNA Assembly Mix Plus is a kind of seamless assembly method, digest, ligate or end repair are not required. This method has been used to assemble different sizes of DNA fragments with varied overlaps (15~30 bp). And it offers a easy, fast and efficient DNA cloning technique.

This method allows for seamless assembly of multiple DNA fragments, regardless of fragment length or end compatibility. It can accomplish single fragment assembly in as little as 5 minutes with a high positive rate exceeding 95%. The mix contains enhancers that improve the cloning efficiency, while the reaction system was optimized, so no PCR clean-up step is required.

Protocol

1. Summary of Experimental Workflow



2. Prepare Linearized Vector

Select suitable cloning sites and linearize the vector. The vector can be linearized through enzyme digestion or reverse PCR amplification.

① Linearized vector Acquire

Some restriction endonucleases may not digest supercoiled DNA completely, which result in part of vector DNA undigested, leading to decreased cloning efficiency. We recommend LightNing® Restriction Enzymes for digestion (single or double digestion) to ensure complete linearization of the vector and reduce false positive colonies.

Note 1: Linearized vectors obtained through enzyme digestion do not require dephosphorylation, and double digestion is recommended.

Note 2: After digestion, it is advisable to inactivate the restriction enzymes or purify the product before the recombination reaction.

② PCR-Generated Vector Acquire

Recommend high-fidelity PCR Mix for amplification to reduce mutation. The template DNA should be linearized to reduce false colonies.

Note 1: If PCR products show non-specific bands, purify the PCR products before use.

Note 2: For multi-fragment cloning, it is advisable to purify the PCR products before use.

3. PCR Primer Design for Insert Fragment

The 5' end of PCR primers must contain a 15~25 nt (recommended 18 nt) sequence that is homologous to the adjacent fragment (insert fragment or vector) end. If the vector is digested by restriction enzymes and has a sticky end, the primer design must include the the 3' end of the overhang, the primer design may or may not include the 5' end of the overhang.

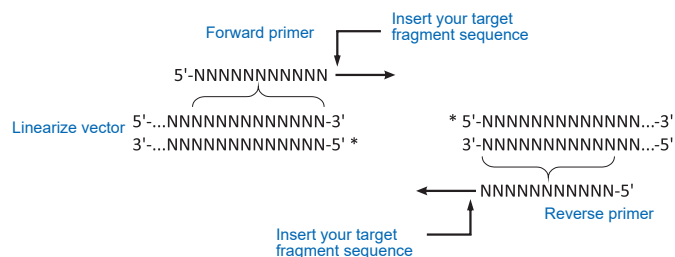
Forward Amplification Primer for Insert Fragment:

5'—Upstream vector homologous sequence + Restriction enzyme site (optional) + Gene-specific forward amplification sequence— 3'

Reverse Amplification Primer for Insert Fragment:

3'—Gene-specific reverse amplification sequence + Restriction enzyme site (optional) + Downstream vector homologous sequence— 5'

Note 1: Choose regions for cloning with minimal repetitive sequences and even GC content. The highest recombination efficiency is achieved when the GC content within the 25 nt region upstream and downstream of the vector cloning site is between 40% and 60%.



Note 2: The end sequence for the provided pUC19 vector (Amp^r) in this kit is as follows:

```

EcoRI
5' -ATGACCATGATTACGCCA-3'   5' -AATTCAGTGGCCGTCGTTTTAC-3'
3' -TACTGGTACTAATGCGGTTCCA-5' 3' -GTGACCGGCAGCAAAATG-5'
HindIII
    
```

4. PCR Amplification of Insert Fragment

Recommend high-fidelity PCR Mix for amplification to reduce mutation. Purified PCR products are advised for seamless cloning reactions. If the PCR products are identified as specific amplification products through agarose gel electrophoresis, they can be used directly. However, the volume of the sample added should not exceed 20% of the total reaction volume.

5. Recombination Reaction

① Prepare the following reaction mixture on ice:

Component	Reaction System	Negative Control	Positive Control (if necessary)
LightNing [®] DNA Assembly Mix Plus	5 μ l	5 μ l	5 μ l
Linearized Vector ^a	50~200 ng	50~100 ng	pUC 19 Control Plasmid, Linearized, 1 μ l
Insert Fragment ^b	10~200 ng	-	500 bp Control, Fragment, 1 μ l
ddH ₂ O	To 10 μ l		

a. Optimal amount of vector (ng) = 0.02 × number of vector base pairs.

b. Optimal amount of insert fragment (ng) for a single fragment = 0.04 × number of fragment base pairs; for multiple fragments, optimal amount per fragment (ng) = 0.02 × number of fragment base pairs.

Note 1: If the length of the insert fragment is greater than that of the vector, exchange the amounts of vector and insert fragment.

Note 2: If the length of the insert fragment is less than 200 bp, use 5 times the amount of vector.

Note 3: If the calculated amounts exceed the minimum/maximum values, it is recommended to directly use the minimum/maximum amounts.

Note 4: Cloning efficiency may decrease with excessively long vector or insert fragments or a high number of fragments.

After preparing the recombination reaction system, gently pipette to mix the components, avoiding bubble formation.

② Incubate the reaction system at 50°C for 5~60 minutes.

Note 1: It is recommended to use a PCR machine or other accurate temperature-controlled instrument for the reaction. Insufficient or excessive reaction time may reduce cloning efficiency.

Note 2: For inserting 1~2 fragments, a reaction time of 5~15 minutes is recommended; for 3~5 fragments, a reaction time of 15~30 minutes is suggested.

Note 3: If the vector backbone is above 10 kb or the insert fragment is above 4 kb, extend the reaction time to 30~60 minutes.

③ Place the reaction tube on ice for cooling, and then proceed with transformation or store at -20°C.

Note 1: Recombinant products stored at -20°C are recommended to be used within 1 week.

6. Transformation of Recombinant Products

Take 5~10 μ l of the reaction liquid and add it to 100 μ l of competent cells. Mix gently and slowly, place on ice for 30 minutes. Heat shock at 42°C for 45~60 seconds, then place on ice for 5 minutes. Add 500 μ l of SOC or LB medium, shake at 37°C for 40~60 minutes (200 rpm). Spread the bacterial solution evenly on a plate containing the corresponding antibiotic and incubate it upside down at 37°C overnight.

Note 1: Different competent cells may yield different cloning efficiencies. It is recommended to use competent cells with transformation efficiency > 10⁸ CFU/ μ g.

Note 2: The number of colonies depends on the quantity and purity of PCR products and linearized vector.

Note 3: Positive control plates usually show abundant white colonies, while negative control plates show very few colonies.

7. Positive Clone Assay

Pick a single colony and mix it in 10 μ l of ddH₂O. After a 95°C incubation for 10 minutes, take 1 μ l of the lysate as a template for colony PCR identification. Alternatively, inoculate a single colony in antibiotic medium and incubate it overnight, then extract the plasmid for enzyme digestion identification.

For positive clone detection of the positive control, use primers M13F and M13R for colony PCR, and HindIII, EcoRI for enzyme digestion.

Note 1: If necessary, further sequence verification can be performed.

Note 2: M13F: TGTAACGACGGCCAGT
M13R: CAGGAAACAGCTATGAC

F&Q

Descriptipon	Reason	Solution
Low transformation efficiency	Low efficiency of competent cells	Use newly prepared or properly stored competent cells.
	Unfavorable ratio of DNA fragments	Prepare the reaction system according to the recommended optimal amounts and ratios in the instructions. Determine the concentrations of the vector and insert fragments as follows: If the linearized vector and insert fragment have been purified and show a single band or no remaining smear on agarose gel electrophoresis, their concentrations can be measured using instruments based on spectrophotometric methods. However, the concentration value is reliable only when the A260/A280 ratio is between 1.8 and 2.0. If the linearized vector and insert fragment have not been purified, sample concentrations can be measured using agarose gel electrophoresis.
	Insufficient purity of DNA fragments	Perform gel extraction purification for both the vector and insert fragments. As chelating agents such as EDTA can inhibit seamless cloning reactions, the purified products should be dissolved in ddH ₂ O. Do not use Tris-EDTA or similar buffer solutions.
	Excessive reaction products	In the transformation system, the volume of seamless cloning reaction products should not exceed 10% of the volume of competent cells.
Large numbers of clones lack the insert fragment	Incomplete linearization of the vector	When preparing linearized vectors using restriction enzymes, increase the amount of the fast restriction enzyme used, extend the reaction time, and perform gel extraction purification of the digestion product.
	Contamination with identical resistant plasmids	When performing PCR amplification of the insert fragment using plasmids as templates, use pre-linearized plasmids as amplification templates, and treat the amplification products with methylation-sensitive restriction enzymes such as DpnI, or perform gel extraction purification of the products.
	Insufficient antibiotic resistance on plates	Ensure the correct use of antibiotics and use freshly prepared antibiotic plates.
Large numbers of clones contain incorrect insert fragments.	Non-specific PCR amplification products	Optimize the PCR system to improve amplification specificity, or perform gel extraction purification of PCR products containing overlapping sequences of primers.