Premium™ Master Assembly Mix

User's Instruction

Description

Premium™ Master Assembly Mix is a ready-to-use reaction mix for seamless cloning reaction. It can simultaneously insert one or more PCR fragments composed of any sequence into any linearized vector in approximately 15 min with high efficiency and is not limited by the restriction sites of either the vector or the target fragment. Premium™ Master Assembly Mix is ideal for the construction of long DNA fragments and can be used in point mutation, construction of a mutation library, and other molecular cloning experiments.

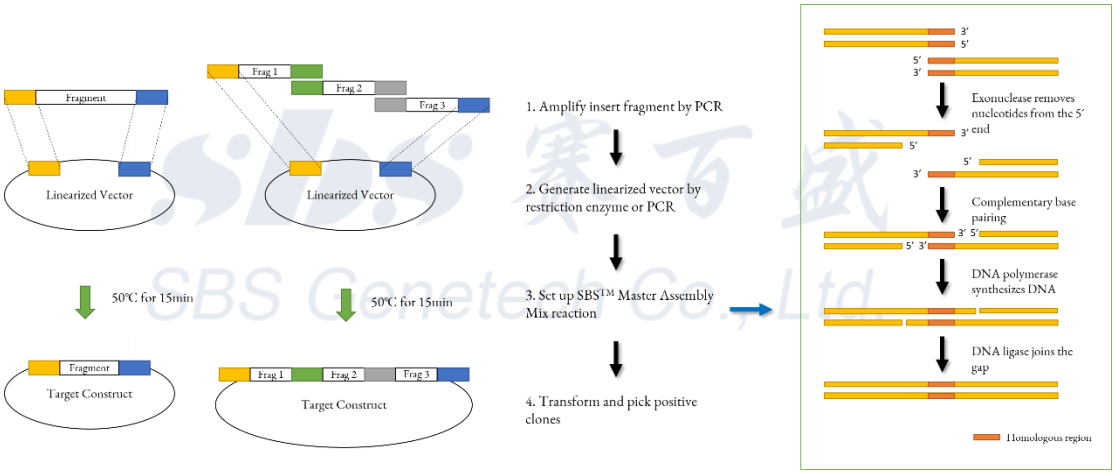
By using Premium™ Master Assembly Mix, you can insert multiple DNA fragments into a single vector in a single procedure, completely free from cumbersome and complex experiments. We hope that this can give you more time to think about interesting applications of this technology, such as constructing fusion protein, deleting or replacing DNA fragments, adding UTR to cDNA, inserting restriction sites, and so on. Enjoy the fun of scientific research from now on!

Kit Contents

	20 preps
1. 2 × Master Assembly Mix	100 μl
2. pGEM3ZEVL (control vector, 50 ng/μl)	10 μl
3. Kan Insert (control insert, 50ng/μl)	5 μl

	60 preps
1. 2 × Master Assembly Mix	100 μl × 3
2. pGEM3ZEVL (control vector, 50 ng/μl)	10 μl × 3
3. Kan Insert (control insert, 50ng/μl)	5 μl × 3

Detection Principle



Features

- **Seamless:** No extra base sequence would be introduced in your final product.
- **High Efficiency:** Only a single reaction is needed to insert multiple fragments into any designated location of your vector, which offers substantial time savings for these types of projects.
- **Extensive:** DNA fragments as short as 20bp or as long as 10kb can be cloned with high efficiency.
- **Fast:** Based on the high efficiency of enzymatic reactions, a single fragment can be cloned in 15 min.
- **High Success Rate:** The average positive rate for single fragment cloning is nearly 100% and the average positive rate for four fragment cloning is about 55%-65%.

Protocol

Preparation of linearized vector

1. Enzyme digestion: Prepare the linearized E. coli cloning vector using restriction enzymes (single or two restriction enzyme digestion). In general, we recommend that you digest the vector with two restriction enzymes rather than a single enzyme to reduce the amount of background. The complete linearization of the vector is the key to the success of seamless cloning reaction. The false-positive clones of the recombinant products of incomplete enzyme cleavage vectors are generally formed by the transformation of non-linearized vectors. When the proportion of false positive clone is high, it is necessary to digest the vector again. Therefore, we recommend

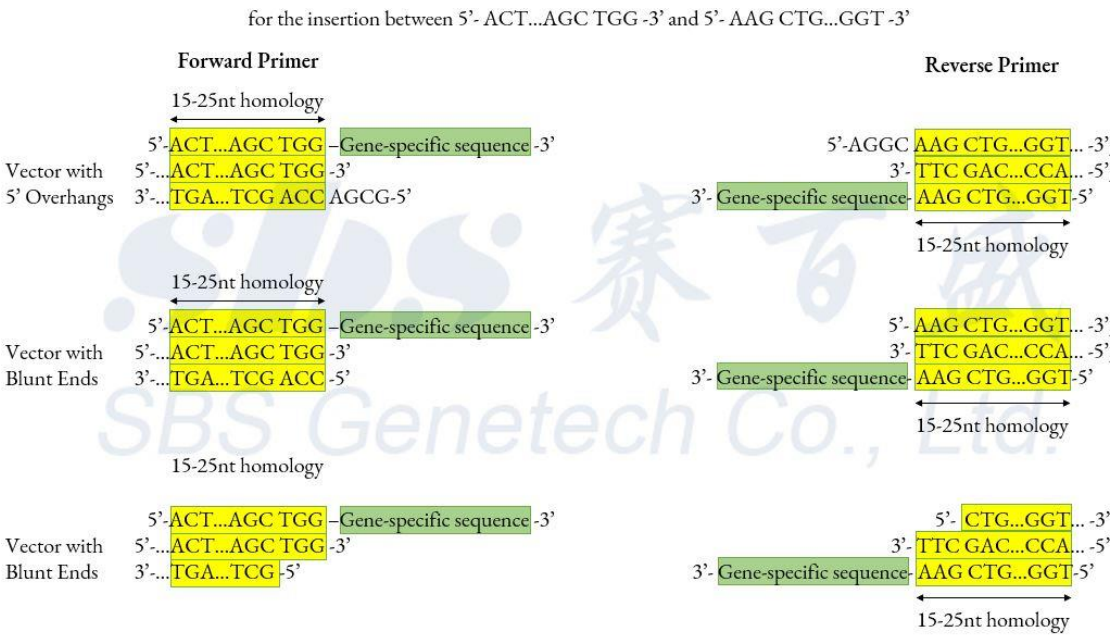
increasing the enzyme digestion time and the reaction volume. When determining whether the vector linearization is complete by electrophoresis method, it is necessary to use the plasmid without enzyme digestion as the control for electrophoresis. Purify the digested vector using SiMax™ Agarose Gel Purification kit. The recombinant region selected on the vector should have uniform base distribution and avoid repeat sequence. The recombination efficiency will be the highest when the content of G + C% is in the range of 40% - 60%. There is no need to dephosphorylate the ends of the linearized carrier.

2. PCR amplification: Prepare the linearized vector by SuperGold™ High Fidelity PCR EasyMasterMix with high fidelity (69 times as high as Taq DNA polymerase). The amplification product of SuperGold™ High Fidelity PCR EasyMasterMix should have blunt ends. The functional group of DNA terminal is hydroxyl group, and there is no phosphate group, except for the primer modified by phosphorylation. If a single expected band is generated, SiMax™ PCR Products Purification kit is recommended for purification. If multiple bands are generated, SiMax™ Agarose Gel Purification kit is recommended to recover the product.

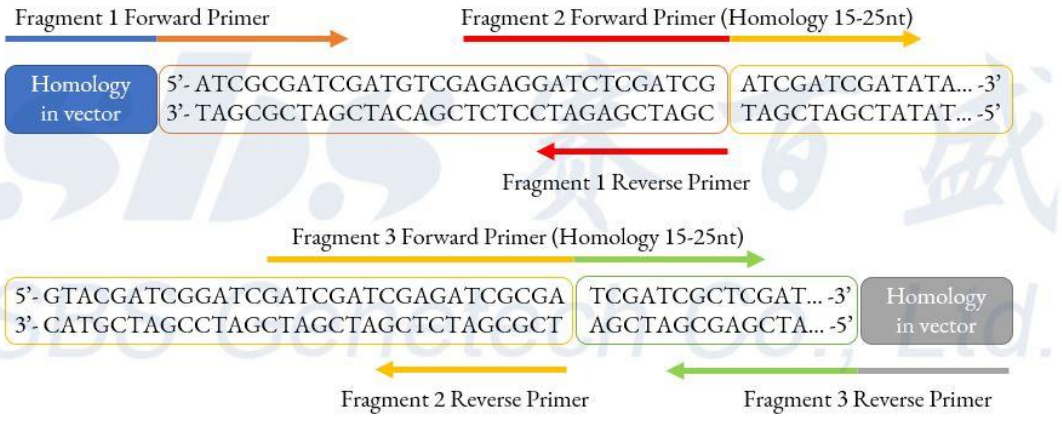
Preparation of inserts

1. The inserts can be amplified by Taq DNA polymerase if they are short enough. There is no need to worry about the 3'-dA overhangs since they will be removed in the recombination process and will not appear in the final plasmid. However, we still recommend using high fidelity polymerase (e.g. SuperGold™ High Fidelity Polymerase) to avoid unwanted mutations. After PCR amplification, we recommend you taking a small number of products for agarose electrophoresis to test the length and specificity of the amplified products. Nonspecific amplification and the presence of primer dimer will seriously affect the cloning efficiency. We recommend using SiMax™ Agarose Gel Purification kit to purify the target fragment.

2. Primers for single fragment: It is important to design primers properly to ensure the success of the seamless cloning reaction. Both forward and reverse primers are designed by 15-25nt homology of linearized vector followed with 20-25nt target specific sequence (5' – 3'). Example:



3. Primers for multiple fragments: The design method of primers for multiple fragments is the same as primers for single fragment. Example:



Setting up the reaction

Components	Volume(μl)
2 × Master Assembly Mix	5
Linearized Vector (5-100ng)	X
Inserts	Y
ddH_2O	To 10

Note:

- In a 10 μl system, we recommend using 50-100ng of vector. For optimal cloning, using 1:1 to 1:3 (vector: insert) molar ratio. When the insert is shorter than 200bp, the molar ratio can add up to 1:5 (vector: insert).
- Gently mix and incubate at 50℃ for about 15 min (no more than 60 min). Place it on ice for a few seconds. The reaction mixture can be directly used for transformation or stored at -20℃.

Transformation (using 100 μl frozen competent cells as example)

1. Thaw a vial of frozen competent cells on ice. Tap tube gently to ensure that the cells are suspended.
2. Transfer 4 μl of reaction mixture into competent cells, mix gently to ensure even distribution of the DNA solution (flicking the tube, not vortex). Leave the tube on ice for 20-30 minutes.
    a) If you use 50 μl frozen competent cells, then transfer 2 μl of reaction mixture instead of 4 μl.
3. Heat-shock at 42℃ for 90 sec, and immediately place them directly on ice for 2 min.
4. Add 500 μl of room temperature SOC/LB medium to the cells and then incubate at 37℃ for 60 min while shacking at 200 rpm.

5. Centrifuge at 4,000 rpm for 1 min. Discard the supernatant fluid. Tap tube gently to ensure that the cells are suspend with the remaining liquid (100-200 μl).

6. Spread 50 μl of cells on the selection plate and incubate overnight at 37℃.

Analysis of positive clones

1. Analyzing by PCR: Pick single colony into 10 μl of ddH_2O. Mix by vortexing or pipetting up and down. Add 1 μl of mixture into 25 μl of PCR system. Identify the positive clones by appropriate forward and reverse primer.

2. Analyzing by restriction enzyme digestion: Pick single colonies and culture them overnight in LB medium containing the appropriate selection antibiotic. Isolate plasmid DNA by SiMax™ Plasmid DNA Miniprep. Analyze the plasmids by restriction enzyme digestion.

3. Sequencing: Perform sequence analysis.