GenRec Assembly Master Mix Kit

Product Specificition

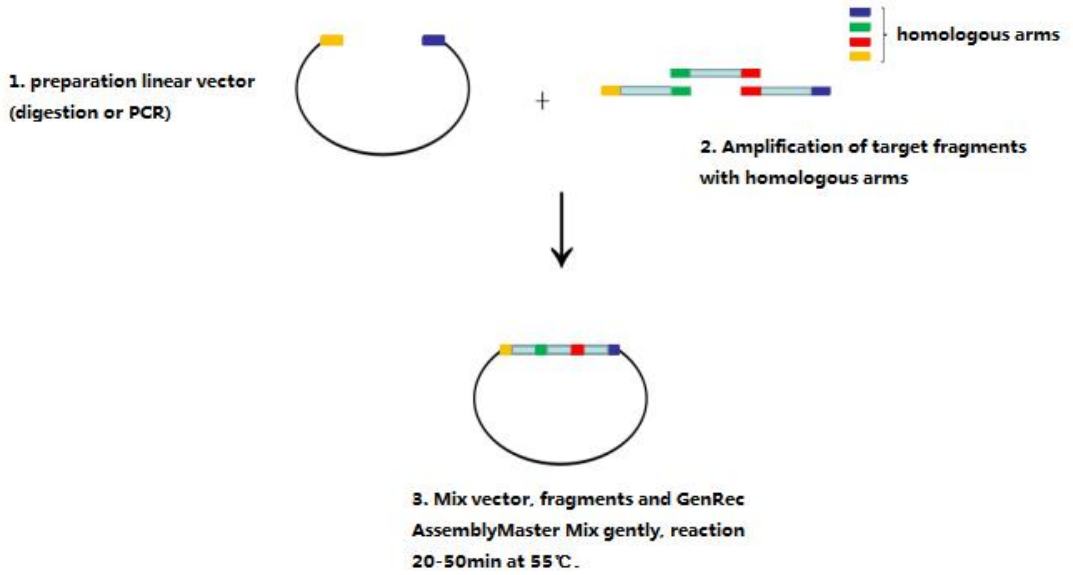
Product name	Catalog Number	Specification
2×GenRec Recombinant kit	CL08010	10 T
2×GenRec Recombinant kit	CL08020	20 T
2×GenRec Recombinant kit	CL08050	50 T

Product introduction

The GenRec Assembly Master Mix Kit is a simple, fast and efficient multifragment DNA determination cloning products, this kit can be used to clone the PCR product directly to any site of any vector. Linearizing the vector at the cloning site and to introduce linear cloning vector terminal sequence to the blocks PCR products 5' and 3 'terminal.So that the terminal of the PCR products 5' and 3 'of the insert were respectively aligned with the both ends of the linearized cloning vector (20 bp to 40 bp).The PCR products with the vector terminal sequence at both ends and the linearized cloning vector were mixed in a certain proportion. Under the catalysis of the recombinase, the reaction was carried out for 20-50 min to complete the directional cloning. Cloning positive rate can reach 90% or more.Kit of 2×GenRec Assembly Master Mix mixes the recombinase and the buffer required for the recombinant reaction and adds a special ingredient , can significantly improve the efficiency of recombinant cloning and can achieve 3-5 fragments sequential splicing at a time.

The optimum reaction temperature of the enzyme is 55 ℃. transportsed by the ice bag , product must stored at -20 ℃

Principle of cloning



References

1. Reaction system

Configuration reaction system on the ice into the bottom of the tube

2×GenRec Assembly Master Mix	10μL
Linear cloning vector	A ng
insert fragment amplified product	B ng
ddH_2O	Up to 20μL

ATTENTION： If you accidentally stick the liquid to the tube wall, you can sink it to the bottom of the tube by briefly centrifuging.

The optimum amount of DNA used in the recombinant reaction system is 0.03 pmol for each linearized fragment. The mass of 0.03 pmol is calculated by the following formula:

0.03 pmol fragment (ng) = 0.02 × number of base pairs (BP)

2. Recombination reaction

(1) Thaw 2×GenRec Assembly Master Mix on ice.

(2) Add 10 μL of 2×GenRec Assembly Master Mix to the sterile tube, add the carrier and the fragment (according to the above requirements), make up to 20 μL with sterile water, and gently suck the mixture with the pipette to avoid the air bubble .

(3) 55 ℃ constant temperature reaction 25-50min, placed on the ice about 3-5min.

3. Conversion

(1)Thaw transformed competent cells on the ice.

(2)Add 10 μL of the above reaction solution to the competent cells and mix the solution thoroughly. Place on the ice for 30min.

(3)Place centrifuge tube in 42 ℃ water bath and heat shock 90s, and then put it back to the ice immediately, cooling the cells about 2～3 min .

(4)Add preheated sterile LB culture medium 600μL to the centrifuge tube, 37 ℃ constant temperature shaking culture 45 ～60min.

(5) Remove the supernatant after brief centrifugation, absorb 100 μL of fresh LB medium and make it resuspended,s pread the bacteria evenly on the plate which containing the appropriate antibiotic.Upside down the tablet , cell culture overnight at 37℃.

4. Cloning identification

Colony PCR. Use a sterile tip or toothpick to pick up a single colony to 20 to 50 μL of LB medium and mix the solution thoroughly ,pick1μL directly as a PCR template.

Notes

1. To achieve a better PCR amplification effect, please ensure the DNA content of the template.

2. EDTA and other metal ion chelators have inhibitory effect on the amplification reaction of the enzyme, and it is necessary to ensure that no such chelating agent is included in the reaction system.

3. This product is limited to the scientific research of professional personnel and shall not be used for clinical diagnosis or treatment, and shall not be used for food or medicine.For your safety and health, please wear lab clothes and wear disposable gloves.