Servicebio® 2×In-Fusion Cloning Mix Plus

Cat. #：G3351-20T

Product Information

Product Name	Cat. No.	Spec.
2×In-Fusion Cloning Mix Plus	G3351-20T	20 T
	G3351-100T	100 T

Product Description/Introduction

The 2×In-Fusion Cloning Mix Plus offer increased cloning efficiency over previous generations of In-Fusion kits (G3350,2×In-Fusion Cloning Mix), especially for multiple fragments. It is designed for fast, directional cloning of one or 2~5 multiple fragments of DNA into any vector. It fuses DNA fragments (e.g., PCR-generated inserts and linearized vectors) efficiently and precisely by recognizing 15-bp overlaps at their ends. These 15-bp overlaps can be engineered by designing primers for amplification of the desired sequences.The simultaneous insertion of multiple fragments greatly simplifies the experimental steps, improves cloning efficiency and saves your time.

Storage and Shipping Conditions

Ship with wet ice; stored at -20°C, valid for 12 months.

Product Contents

Component	G3351-20T	G3351-100T
2×In-Fusion Cloning Mix Plus	100 μL	5 x 100 μL
pUC19 (Linearized, Control Vector, 5 ng/μL)	10 μL	10 μL
Control Inset (10 ng/μL)	10 μL	10 μL
Manual	One copy	

Assay Protocol / Procedures

Perform ligation reaction

1. To an autoclaved, 1.5-ml microcentrifuge tube, add the following(recommend 10-uL reaction system):

Component	Volume
2×In-Fusion Cloning Mix Plus	5 μL
Vector DNA	X μL
Insert DNA	Y μL
Nuclease-Free Water	Add to 10 μL

2. Mix gently and centrifuge briefly to bring the contents to the bottom of the tube.
3. For single-fragment recombination reactions, incubate at 50°C for 15 minutes; For the multi-fragment recombination, incubate at 50°C for 30 minutes.

NOTE: For 3-5 multi-fragment recombination, increase the reaction time will yield more colonies, but should not exceed 1 h).

4. Place the tube on ice and Proceed immediately to "Perform transformation reaction".

Perform transformation reaction

5. Add appropriate ligation reaction into Chemically Competent *E. coli* (such as DH5α, Top10, etc.)and mix gently. Do not mix by pipetting up and down.
6. Incubate for 30 minutes on ice.

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7. Heat-shock the cells for 30 seconds in a 42°C water bath.
8. Immediately place the tubes on ice and incubate for 2 minutes.
9. Add 900 µL of room temperature SOC or LB medium. Cap the tube tightly and shake the tube horizontally at 225 rpm for 1 hour at 37°C.
10. Spread required volume of transformation reaction on a prewarmed LB plate containing corresponding antibiotics.
11. Incubate plates overnight at 37°C.

Analyze transformants

Pick an individual colony from the transformation plate, analyze transformants by colony PCR or restriction enzyme digestion.

Note

1. The vector DNA and insert DNA should be gel purified and analyse their quality and concentration by electrophoresis. Water can be omitted in ligation reaction if the concentration is low.
2. The Tm value between the overlapping regions of multiple fragments should be consistent and >60℃.
3. It is recommended that the molar ratio of vector and insert is 1:1~1:3; when 2-3 fragments are connected, the molar ratio between each fragment is 1:1, and the ligation reaction system can be scaled up in equal proportions.
4. If the total volume of vector and insert is more than 5 µL, you may scale the ligation system to 20 µL.
5. The volume of the ligation product should not exceed 1/10 of the volume of the competent cells, otherwise the transformation efficiency will be significantly reduced. The volume of the ligation product and the competent cells can be increased in equal proportions (for example, 20 µL of ligation system transforms 200 µL of competent cells).
6. The 2×Universal Ligation Mix shoule be kept at -20°C until within 5-10 minutes of use and returned immediately to -20°C after use. It is recommended to freeze in aliquots to reduce freeze-thaw cycles.
7. If electroporation is used for transformation, vector DNA and insert DNA should be purified by column method or ethanol precipitation method.

For Research Use Only!

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