Fast MultiSite Mutagenesis System

Cat. No. FM201

Storage: DMT Chemically Competent Cell at -70°C for six months, others at -20°C for one year

Description

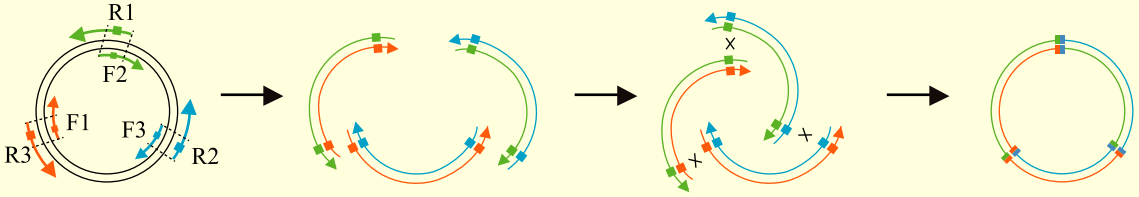
Fast MultiSite Mutagenesis System is used for generating mutated PCR fragments by introducing mutation sites on overlapping regions. High fidelity *TransStart*® *FastPfu* Fly PCR SuperMix is included for amplification. This kit uses proprietary assembly mix and homologous recombination to seamlessly assemble up to six mutagenesis fragments.

- Fast: Amplified with fast & high-fidelity 2×*TransStart*® *FastPfu* Fly PCR SuperMix; only 15 minutes for recombination.
- Flexible: Able to be cloned into any site to realize single-site/ multi-site, continuous/non-continuous mutagenesis.
- Efficient: >90% mutagenesis efficiency.

Kit Contents

Component	FM201-01 (10 rxns)
2×*TransStart*® *FastPfu* Fly PCR SuperMix	1 ml
DMT Enzyme (10 units/μl)	30 μl
2×Assembly Mix	50 μl
DMT Chemically Competent Cell	10×50 μl
Nuclease-free Water	1 ml

Design Principle



Amplify mutated fragments with F1/R1, F2/R2,….Fn/Rn primer sets

Recombination of mutagenic fragments

Transformation of the mutated plasmids

Preparation of multisite mutagenic fragment

(1) Primer Design

- Both primers contain overlapping region at the 5' ends and extension region at the 3' ends, with mutation site on overlapping region, as shown in figure 1.
- Primer length: Both primers (forward and reverse) should be approximately at 25-40 nucleotides in length, excluding the mutation site. Primers should have an overlapping region of 15-25 nucleotides and have an extension region of at least 10 nucleotides.

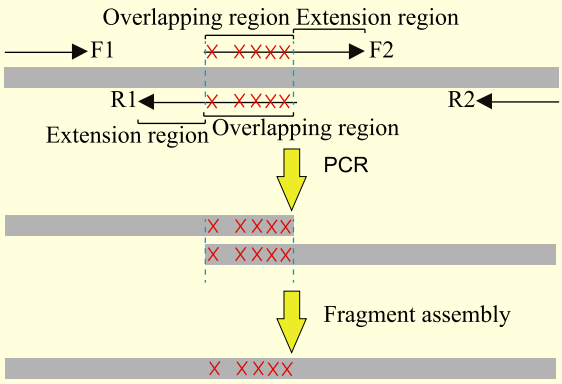


Figure 1

(2) Preparation of mutated fragment

A: The mutation sites are located on one pair of primers, as shown in figure 2.

B: The mutation sites are located on multiple pairs of primers, with F1/R1, F2/R2,….Fn/Rn for amplification, as shown in figure 3.

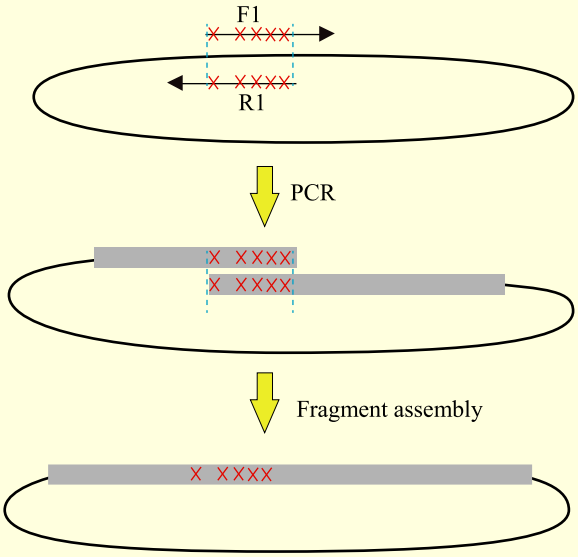


Figure 2

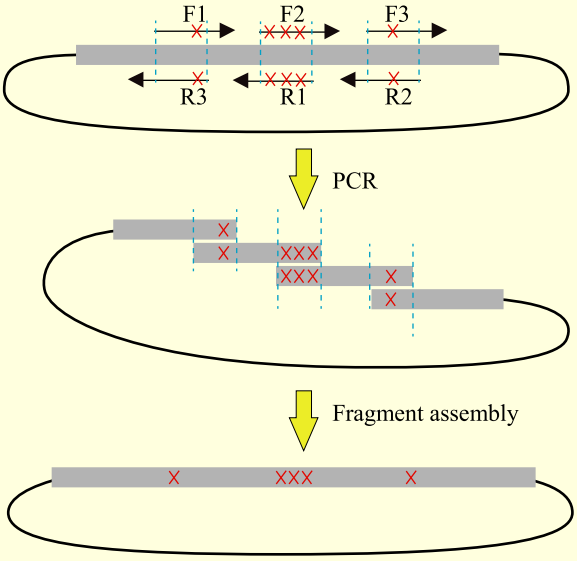


Figure 3

PCR System

Component	Volume	Final Concentration
Plasmid	1-10 ng	as required
Forward Primer (10 μM)	1 μl	0.2 μM
Reverse Primer (10 μM)	1 μl	0.2 μM
2×*TransStart® FastPfu* Fly PCR SuperMix	25 μl	1×
Nuclease-free Water	to 50 μl	Not applicable

PCR

95℃	3 min	
95℃	20 sec	25 cycles*2
55℃-65℃*1	20 sec	
72℃	2-4 kb/min	
72℃	5-10 min	

Notes

*1. Annealing temperature depends on primers.

*2. We suggest performing 25 cycles for PCR. For low yield PCR products, we suggest using up to 30 cycles.

Electrophoresis Analysis

Amplified PCR products can be checked by electrophoresis with 10 µl of PCR product on a 1% agarose gel.

(3) Digestion of PCR Product with DMT

Add 1 µl of DMT enzyme into PCR product, mix thoroughly and incubate at 37°C for 1 hour.

(4) Purification of PCR products

For PCR product with a single expected band, it is suggested to use PCR Purification Kit to purify PCR products; for PCR product with multibands, we suggest using Quick Gel Extraction Kit to purify PCR products.

Assembly of Mutated Fragments

Component	Volume
2×Assembly Mix	5 µl
Amplified fragment A	x µl*
Amplified fragment B	y µl*
……	……
Amplified fragment N	z µl*
Nuclease-free Water	to 10 µl

*Suggested amount is 20-150 ng

Gently mix and perform reaction at 50°C for 15 minutes. After reaction, transfer the reaction tube on ice for a few seconds.

Transformation

(1) Add 2 µl of assembly products into 50 µl of DMT Chemically Competent Cell (DNA should be added immediately after thawing the cells on ice) and mix by tapping gently. Incubate on ice for 20-30 minutes.

(2) Heat-shock at 42°C for exactly 45 seconds, quickly remove from 42°C water bath and place on ice for 2 minutes.

(3) Add 250 µl of SOC or LB medium (pre-warm to room temperature), and incubate at 37°C for 1 hour with shaking at 200 rpm.

(4) Pre-warm a selective plate at 37°C for 30 minutes.

(5) Spread 100-200 µl of transformants on the plate and incubate at 37°C overnight.

Positive Clone Analysis

Analyze the clones by sequencing.

Note

We suggest performing 25 cycles for PCR. For low yield PCR products, we suggest using up to 30 cycles.

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