Fast Mutagenesis System

Please read the manual carefully before use.

Cat. No. FM111

Version No. Version 2.1

Storage: DMT Chemically Competent Cell at -70°C or below for one year, others at -18°C or below for two years

Description

This product uses methylated plasmid as the template with partially overlapping primers (both containing the mutation) and 2× *TransStart® FastPfu* Fly PCR SuperMix for amplification. The PCR products are subjected to *in vitro* digestion with DMT enzyme and *in vitro* degradation by DMT Chemically Competent Cells to remove the non-mutated parental plasmid. The system features high mutation efficiency and a simple workflow.

Features

- Partially overlapping primers enable exponential DNA amplification, yielding circular PCR products that are visible on agarose gels and readily transformed.
- The 2× *TransStart® FastPfu* Fly PCR SuperMix enables rapid amplification and exhibits high fidelity.
- Double digestion (*in vitro* and *in vitro*) of the parental plasmid enhances mutation efficiency, with a control mutation efficiency of up to 90%.

Kit Contents

Component	FM111-01 (10 rxns)	FM111-02 (20 rxns)
2×*TransStart® FastPfu* Fly PCR SuperMix	250 µl	500 µl
DMT Enzyme (10 units/µl)	10 µl	20 µl
DMT Chemically Competent Cell	10×50 µl	20×50 µl
Nuclease-free Water	1 ml	1 ml
SControl Plasmid (5 ng/µl)	10 µl	20 µl
SControl Primers (10 µM)	10 µl	20 µl

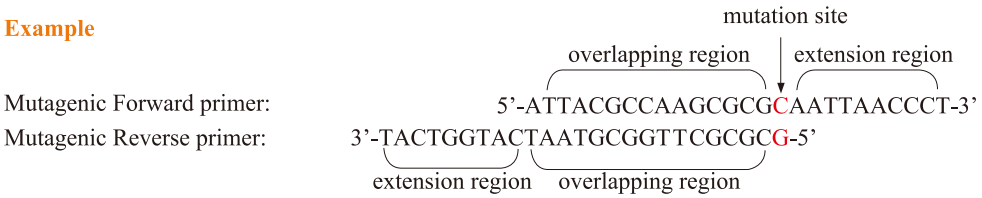
Design Principle

- Primers anneal to the DNA template, mutant strands are synthesized with 2×*TransStart® FastPfu* Fly PCR SuperMix.
- *In vitro* digestion of non-mutated parental plasmid (methylated plasmid) with DMT enzyme and *in vivo* degradation of non-mutated parental plasmid (methylated plasmid) with DMT Chemically Competent Cell, so as to efficiently select mutant clones.

Primer Design

- The primers contain a 5′ overlapping region and a 3′ extension region.
- Primer length: excluding the mutation site, both primers (forward and reverse) should be approximately 25-30 nucleotides in length. The 5′ overlapping region should contain 15-20 nucleotides, and the 3′ extension region should contain at least 10 nucleotides.
- Mutation primers: the mutation site should be located on both primers. For the forward mutagenic primer, the mutation site should be positioned downstream of and immediately adjacent to the overlapping region; for the reverse mutagenic primer, it should be located at the 5′ end.
- The mutated bases should be excluded from the annealing temperature calculation.

Example



Reaction Components

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

Thermal Cycling Conditions

94°C	2-5 min	
94°C	20 sec	20-25 cycles
55°C	20 sec	
72°C	6 kb/min	
72°C	10 min	

Electrophoresis Analysis

Amplification may be checked by electrophoresis with 10 μl of the product on a 1% agarose gel.

Note: Proceed with DMT enzyme digestion and transformation if the expected size product can be visualized on the gel.

Digestion of PCR Product

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

Transformation

a. Add 2-5 μl of DMT enzyme-treated PCR product into 50 μl of DMT Chemically Competent Cell (PCR product should be added immediately after thawing the cells on ice) and mix by tapping gently. Incubate on ice for 20-30 minutes.

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

c. Add 250 μl of SOC/LB medium (equilibrated to room temperature), and shake at 200 rpm at 37°C for 1 hour.

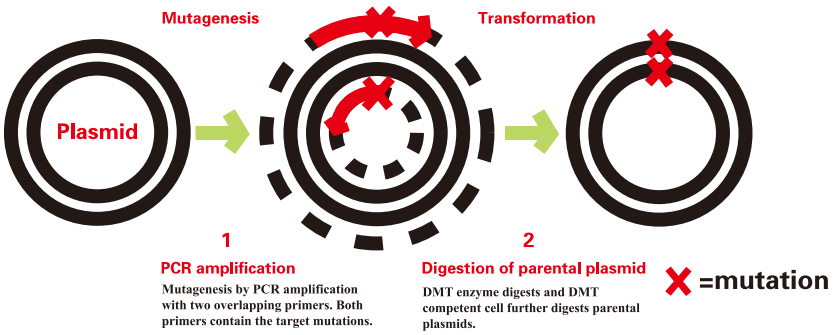
d. Pre-warm the appropriate antibiotic-containing plates in a 37°C incubator.

e. Spread 100-200 μl of the bacterial culture evenly onto the plate, and incubate overnight at 37°C.

Notes

- If no colony or low numbers of colonies are observed, it is suggested to purify the DMT enzyme-treated DNA with PCR purification kit, then perform transformation with 2-5 μl of the purified product.
- If using the control plasmid (4.5 kb) to test the mutation efficiency, spread cells on agar plates containing ampicillin, 8 μl of 500 mM IPTG and 40 μl of 40 mg/ml X-gal, successful transformation is indicated by the observation of blue colonies.

Principle of Site-Directed Mutagenesis



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