Fast Mutagenesis System

Cat. No. FM111

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

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.

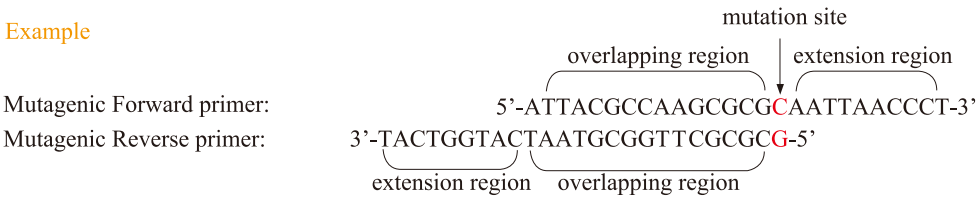
Highlights

- Mutation sites on both primers to improve mutation efficiency.
- Partially overlapping primers for exponential DNA amplification.
- Fast (4 kb/min) and high fidelity (54-fold fidelity as compared to *EasyTaq®* DNA Polymerase) 2×*TransStart® FastPfu* Fly PCR SuperMix for DNA amplification.
- Double digestions (in vitro and in vivo) of parental plasmids to enhance mutation efficiency.

Primer Design

- Both primers (forward and reverse) should be approximately at 25-30 nucleotides in length.
- Primers should have an overlapping region of 15-20 nucleotides for exponential amplification.
- Primers should have an extension region of at least 10 nucleotides.
- The mutation site should be located on both primers.

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	2-6 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 30 minutes.

b. Heat-shock at 42°C for exactly 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 225 rpm at 37°C for 1 hour.

d. Spread 200 μl of transformants on the plate and incubate overnight (to obtain more colonies, centrifuge the transformation vial at 4000 rpm for 1 minute, discard a portion of supernatant and keep 100-150 μl of it. Gently tapping to suspend the cells, plate all the cells and incubate overnight).

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 use the control plasmid (4.5 kb) to test the mutation efficiency, spread cells on agar plates containing 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.

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