

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× <i>TransStart</i> [®] <i>FastPfu</i> 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.

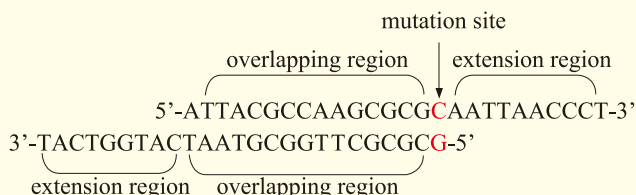
Primer Design

- 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

Mutagenic Forward primer:

Mutagenic Reverse primer:



Reaction Components

Component	Volume	Final Concentration
Plasmid	1-10 ng	as required
Forward Primer (10 μ M)	1 μ l	0.2 μ M
Reverse Primer (10 μ M)	1 μ l	0.2 μ M
2 $\times$ TransStart® FastPfu Fly PCR SuperMix	25 μ l	1 $\times$
Nuclease-free Water	to 50 μ l	Not applicable

Thermal cycling conditions

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

Electrophoresis Analysis

Amplification may be checked by electrophoresis with 10 μ l 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 μ l of DMT enzyme into PCR product, mix thoroughly and incubate at 37°C for 1 hour.

Transformation

- Add 2-5 μ l of DMT enzyme-treated PCR product into 50 μ l 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.
- Heat-shock at 42°C for exactly 45 seconds, quickly remove from 42°C water bath and place on ice for 2 minutes.
- Add 250 μ l of SOC/LB medium (equilibrated to room temperature), and shake at 225 rpm at 37°C for 1 hour.
- Spread 200 μ l 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 μ l 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 μ l of the purified product.
- If use the control plasmid (4.5 kb) to test the mutation efficiency, spread cells on agar plates containing 8 μ l of 500 mM IPTG and 40 μ l of 40 mg/ml X-gal, successful transformation is indicated by the observation of blue colonies.

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