CYCV09-CHANGYU One Tube Fast Mutagenesis Kit

Contents	10 reactions (CYCV0901)	20 reactions (CYCV0902)
SuperPfu DNA polymerase	5 μl	10 μl
10× SuperPfu Buffer	100 μl	100 μl
Dpn I	5 μl	10 μl
10 mM dNTP Mixture	25 μl	50 μl

Note: Store at -20 °C; valid for 12 months. The reaction buffer, dNTPs and other components in this kit are specifically optimized for the reaction; do not replace them with other reaction buffers or dNTPs.

Description

This site-directed mutagenesis kit uses PCR to introduce point mutations, mutations of multiple adjacent codons, or deletions or insertions of one or more adjacent codons into a target DNA fragment, generally a plasmid. Plasmid DNA amplified in common host bacteria is methylated, whereas DNA newly synthesized by PCR is unmethylated. The methylated plasmid to be mutated serves as template, and the fast, ultra-high-fidelity SuperPfu polymerase synthesizes the mutated plasmid (unmethylated, containing the mutation and two nicks). Dpn I then selectively degrades the methylated template plasmid. After the remaining newly synthesized unmethylated mutant plasmid is transformed into *E. coli*, the two nicks can be repaired by the bacteria, giving clones containing the intended mutant plasmid. Mutation efficiency is generally greater than 90%.

Scope of application: mutation of nucleotides in methylated plasmids smaller than 12 kb.

Workflow

Step 1. PCR synthesis of the mutant strand (PCR cycling)

a. Denature the template DNA.
b. Anneal the template and mutagenic primers.
c. Extend the primers with SuperPfu.

Step 2. Dpn I digestion of the template

Dpn I digests the single-stranded and double-stranded methylated plasmid DNA template.

Step 3. Transformation

Transform the nicked, mutated double-stranded DNA into high-efficiency competent cells for screening.

Features

1. Simple: all operations are completed in one PCR tube, for the fastest workflow.

2. Partially overlapping primer design gives exponential PCR amplification; the amplified product is visible by gel electrophoresis, increasing the success rate.

3. High efficiency: Dpn I removes non-mutated template, with mutation efficiency up to 90%; no special competent cells are needed to degrade non-mutated plasmid.

4. SuperPfu, engineered by molecular evolution, provides a 4- to 6-fold higher amplification rate than Pfu and 3-fold higher fidelity than Pfu.

Primer Design

1. Each primer contains a 5' overlap region and a 3' extension region. The 5' overlap region is about 18-20 bp and the 3' extension region is about 10-12 bp.

2. Place the mutation site in the middle of the 5' overlap region, with about 7-9 bp on its 5' side and 20-22 bp on its 3' side.

3. Use PAGE or higher-grade primer purification. The 3' end should finish with one or more G/C bases.

4. Example: amino acid V (GTA), three consecutive bases mutated to R (CGT).

Sequence before mutation (original codon in bold):

GTATCTGGAGCACCCACAGGAT**GTA**CCGACAATTCGTGAAAAAGTG

CATAGACCTCGTGGGTGTCCTA**CAT**GGCTGTTAAGCACTTTTTCAC

Designed mutagenic primers, aligned to the sequence above (mutated bases in bold):

ACAGGAT**CGT**CCGACAATTCGTGAAAAAG

GACCTCGTGGGTGTCCTA**GCA**GGCTGTTA

Primer as shown above	5' overlap region	3' extension region
Upper primer	ACAGGAT**CGT**CCGACAAT	TCGTGAAAAAG
Lower primer	TGTCCTA**GCA**GGCTGTTA	GACCTCGTGGG

Recommended PCR Conditions (25 µl reaction)

Template	2-10 ng
Forward Primer (10 µM)	1 µl
Reverse Primer (10 µM)	1 µl
10× SuperPfu Buffer	2.5 µl
10 mM dNTPs	1 µl
SuperPfu DNA Polymerase	0.5 µl
ddH_2O to final volume	25 µl

PCR Cycling

94 °C	3-5 min
94 °C	20 sec
55 °C	20 sec (25-30 cycles of the three steps 94 °C / 55 °C / 72 °C)
72 °C	2 kb/min
72 °C	10 min

Note: 2 kb/min is generally chosen, but the amplification time can usually be adjusted within 1 kb per 20-40 seconds.

Electrophoresis

Analyze 5 µl of PCR product on a 1% agarose gel. If the target band is visible (or the target band is visible with other bands), success can be provisionally expected. Even if no band is visible, the following steps can still be performed, but the chance of success is greatly reduced.

Digestion of PCR Product

Add 0.5 µl of Dpn I directly to the PCR product (about 20 µl), mix thoroughly, and incubate on the PCR instrument at 37 °C for 1 hour (without heated lid).

Note: Dpn I contains glycerol and sinks easily; be sure to pipette to mix. If there is a large amount of non-mutated plasmid, digestion can be extended to 3-5 hours.

Transformation

Add 5-10 µl of Dpn I-digested product to 50-100 µl of competent cells (efficiency at least 10^8), flick gently to mix, and place on ice for 30 minutes. Transform by the standard procedure, then plate the culture and incubate overnight. To obtain more colonies, centrifuge at 4000 rpm for 1 min, discard part of the supernatant, keep 100-150 µl, flick gently to resuspend the cells, plate all of the culture, and incubate overnight.

Note: Choose competent cells of high efficiency. If no colonies or few colonies grow, transform all 20 µl of digested product into 200 µl of competent cells, or concentrate the Dpn I-digested product by conventional ethanol precipitation so that all of the product can be used for transformation.

This product is furnished for LABORATORY RESEARCH USE ONLY. Not for diagnostic or therapeutic use.