MB447_IFU_EN_V2301

NZYSupreme Mutagenesis kit

Catalogue number	Presentation
MB44701	10 mutations
MB44702	10 mutations + competent cells

Description

NZYMutagenesis kit is designed to make point mutations and delete or insert single or multiple nucleotides in a DNA sequence. The system requires the provision of two synthetic oligonucleotide primers containing the desired mutation. Incorporation of the oligonucleotide primers with NZYProof DNA polymerase generates a mutated plasmid containing staggered nicks, which resists Dpn I digestion (as the synthetic DNA is not methylated). The resulting mutated plasmid is recovered through transformation of NZYStar competent cells. For a schematic presentation of the mutagenesis protocol, see Figure 1. DNA isolated from *dam*$^-$ *Escherichia coli* strains, including JM101 and SCS110, is not a suitable template for the mutagenesis reaction.

Storage temperature

For MB44702, store competent cells at -85°C to -65°C upon arrival. Other kit components or MB44701 may be stored at -85°C to -15°C. NZYSupreme Mutagenesis kit components are stable till the expiry date when stored under the recommended conditions.

System components

MB44701, 10 mutations:

COMPONENT	CONCENTRATION	AMOUNT
Supreme NZYProof Colourless Master Mix	2x	300 µL
Dpn I	10 U/µL	500 U
Control plasmid [b]	25 ng/µL	5 µL
Mutagenesis Control primer mix	62.5 ng/µL	10 µL

MB44702, 10 mutations + competent cells:

KIT PART REFERENCE	COMPONENT	CONCENTRATION	AMOUNT
MB44701	Supreme NZYProof Colourless Master Mix	2x	300 µL
	Dpn I	10 U/µL	500 U
	Control plasmid [b]	25 ng/µL	5 µL
	Mutagenesis Control primer mix	62.5 ng/µL	10 µL
MB00503	NZYStar Competent Cells [a]	-	5 × 200 µL
	Competent Cells Control Plasmid [b]	0.1 ng/µL	10 µL

[a] ***Genotype of NZYStar competent cells***: *endA1 hsdR17(r_k-, m_k+) supE44 thi -1 recA1 gyrA96 relA1 lac[F´ proA$^+$B$^+$ lacI^{q}ZΔM15 :Tn10(Tc^R)]*

[b] ***Antibiotic resistance*** : *ampicillin*

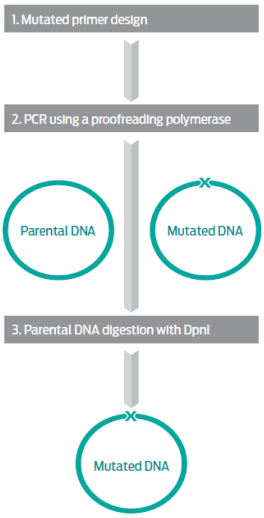

Figure 1. Overview of the NZYSupreme Mutagenesis kit protocol.

Guidelines for using NZYSupreme Mutagenesis kit

Primers specifications

Primers should be complementary at the 5'-overhangs and must contain the desired mutation(s) in both sequences. Thus, the two primers should consist of two main parts (Figure 2): (1) a 5'-complementary region of 17 nucleotides (in green), containing the desired point-mutation(s) (indicated as * in Figure 2) in the middle of the sequence; (2) 15-20 non-overlapping nucleotides (in grey) at the 3'-terminus. In case the desired mutation is a codon select the most divergent codon possible. Primers should have a melting temperature (Tm) of at least 78 °C and should terminate in one or more C or G bases. A minimum GC content of 40% is advisable. Primer purification (FPLC, PAGE or HPLC) is strongly recommended. The mutagenesis protocol uses 125 ng of each oligonucleotide primer.

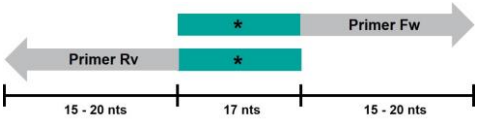

Figure 2. NZYSupreme Mutagenesis kit primer design scheme.

Plasmid specifications

The target plasmid DNA may be isolated from any source and purified using DNA purification kits (as NZYGelpure kit, MB011). No special vectors or restriction sites are required. We recommend starting with 5-25 ng of plasmid template. However, some plasmids may require higher amounts depending on the sequence and quality of the nucleic acid.

Control reaction

Control plasmid and control primer mix are included in the NZYSupreme Mutagenesis kit, allowing blue/white screening of mutagenesis reaction efficiency. The control plasmid, derived from pNZY28 vector (2.88 Kb), contains a premature stop-codon in the gene coding for *lacZα* and thereby forms white colonies on LB-ampicillin agar plates containing X-Gal and IPTG. The control primer mix is designed to revert the premature stop-codon into a functional codon, thus allowing *lacZα* expression. A positive mutagenesis control reaction forms blue colonies on LB-ampicillin agar plates containing X-Gal and IPTG.

Mutagenesis protocol

Mutagenesis reaction

1. Synthesize the two oligonucleotides containing the desired mutation(s) (see Primer specifications section).
2. On ice, in a sterile, nuclease-free microcentrifuge tube, prepare a reaction mixture, combining the following components (for a 50 µL reaction):

Supreme NZYProof 2x Colourless Master Mix	25 µL
dsDNA template (5-25 ng)*	x µL
Oligonucleotide primer #A (125 ng)*	y µL
Oligonucleotide primer #B (125 ng)*	z µL
Nuclease-free water	up to 50 µL

* ***Control reaction:*** *To test the efficiency of the system use 1 µL of the control plasmid and 2 µL of the control primer mix provided.*

3. Gently mix and centrifuge the reactions in a microcentrifuge for 5 seconds. If using a thermal cycler without a heated lid, overlay the reaction mix with 1-2 drops of mineral oil to prevent evaporation during the thermal cycling.

4. Proceed with the amplification following the cycling parameters outlined below:

CYCLE STEP	TEMP.	TIME	CYCLES
Initial denaturation	95 °C	5 min	1
Denaturation	95 °C	30 sec	18
Annealing	64 °C	30 sec	
Extension	68 °C	30 sec/kb plasmid length	
Final Extension	68 °C	10 min	1

Note: *The control plasmid is 2.88 kb in size, so a 1.5-minute extension period is recommended for the control reactions.*

5. Place reaction tubes on ice for 2 minutes. Check the efficiency of the amplification by analysing 10 µL of the reaction on a 0.7-1% agarose gel. Proceed with the Dpn I digestion even if a band is not visualized at this stage.

6. Add 5 µL of Dpn I directly into the reaction (below the mineral oil if used). Gently mix, spin down the reaction and incubate at 37 °C for 5-20 minutes to digest the non-mutated template DNA.

Transformation

1. Transfer 5-10 µL of the Dpn I treated DNA to 100 µL of the ultracompetent cells. NZYStar cells are resistant to tetracycline. If the mutagenized plasmid contains only a *tet*^{R} resistance marker, an alternative tetracycline-sensitive strain of competent cells must be used.

2. To determine the transformation efficiency, add 1 µL (10 ng) competent cells control plasmid DNA to one tube containing 100 µL competent cells. Gently tap tube to mix. Do not mix cells by pipetting.

3. Incubate transformation reaction for 30 min on ice.

4. Heat shock cells at 42 °C for exactly 40 seconds.

5. Place on ice for 2 minutes.

6. Add 900 µL of pre-warmed SOC medium (not provided).

7. Shake the tubes at 200 rpm at 37 °C for 1 hour.

8. Centrifuge at 5000 rpm for 1 min. Remove 900 µL of the supernatant.

9. Re-suspend cells by gently pipetting. Plate 100 µL of cells onto LB agar plates containing the appropriate antibiotic. For control reaction, plate 100 µL of cells onto LB agar plates containing 100 µg/mL ampicillin, 15 µg/mL tetracycline, 100 µg/mL X-gal and 0.5 mM IPTG.

10. For competent cells control plasmid transformation directly plate 100 µL without spinning, onto LB agar plates containing 100 µg/µL ampicillin.

11. Incubate inverted plates overnight at 37 °C.

12. Select 3-5 colonies and analyse by plasmid isolation, PCR, or sequencing.

Important Notes

- In case completely overlapping mutated primers are used, the following PCR program should be alternatively performed (instead of the one indicated in the Mutagenesis Protocol):

CYCLE STEP	TEMP.	TIME	CYCLES
Initial denaturation	95 °C	2 min	1
Denaturation	95 °C	1 min	18
Annealing	64 °C	1 min	
Extension	68 °C	1.5 min/kb plasmid length	
Final Extension	68 °C	15 min	1

- PCR efficacy may be improved by increasing the amount of template DNA used (to a maximum of 50 ng of plasmid DNA per reaction). In this circumstance increase the incubation time with Dpn I to 2 hours.

- The amount of reaction used for transformation may be increased to a maximum of 10 µL to 100 µL of cells.

- False priming and the formation of secondary structures may affect the mutagenesis reaction. Increasing the annealing temperature up to 68 °C may help improving the efficacy of the PCR reaction.

Quality control assays

Functional assay

All components of the NZYSupreme Mutagenesis kit are tested following the mutagenesis protocol described above. The mutagenesis system must generate mutants with an efficiency ≥ 80% of the colonies screened.

Related products

Product name	Cat. No.
NZYGelpure	MB011
SOC Broth	MB28001
LB Agar	MB11802
Ampicillin	MB021