# Muta-Direct™ Site-Directed Mutagenesis Kit

**Cat. No. 15071 15 Rxn**

## DESCRIPTION

*In vitro* site-directed mutagenesis is an invaluable molecular biology technique. Muta-Direct™ Site-Directed Mutagenesis Kit can be used for creating a mutation at a defined site in a plasmid. Muta-Direct™ Site-Directed Mutagenesis Kit has convenient and simple three steps for all experimental procedures.

Muta-Direct™ Site-Directed Mutagenesis Kit can be used to make a single-point mutation, switch amino acid sequence and delete or insert nucleotide sequence(s). Muta-Direct™ Site-Directed Mutagenesis Kit 's characteristics enable the kit to be applicable to protein engineering including the improvement of protein function or protein productivity as well as analysis of gene function.

The creation of a mutation is possibly to complete through just simple three-steps including performance of PCR using the prepared mutagenic primers and use of Muta-direct™ Enzyme which has a very low error rate; digestion of non-mutated parental DNA template by treatment with Muta-direct™ Mutazyme; and transformation of the mutated plasmid.

Fig 1. It can be checked whether mutagenesis is completed or not by sequencing of mutated plasmid if necessary.

Muta-Direct™ Site-Directed Mutagenesis Kit provides a simplified user-friendly protocol for convenient use of those who are unfamiliar with the Muta-Direct™ Site-Directed Mutagenesis Kit or new to site-directed mutagenesis.

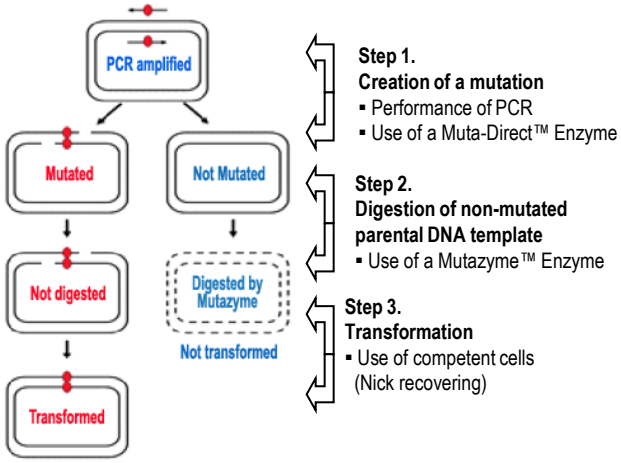



Fig 1. Overview of the Muta-Direct™ Site-Directed Mutagenesis kit

## CHARACTERISTICS

- Easy to use - No special skills required
- Simple – Three steps in two days
- High mutation efficiency

## APPLICATION

- Codon switch
- Creation of a mutation at a defined site in the sequence of protein expression regulatory elements
- Re-mutation to wild type of plasmid
- Functional analysis of a gene or protein
- Protein engineering

## ADDITIONAL REQUIRED EQUIPMENT

- PCR machine
- Standard tabletop micro-centrifuge
- Pipette set
- Incubator

## KIT CONTENTS AND STORAGE

| Material[1,2] | Quantity |
|---|---|
| Muta-Direct™ Enzyme (2.5 U/μl) | 15 μl |
| Muta-Direct™ Reaction Buffer (10×) | 100 μl |
| dNTP Mixture | 30 μl |
| Mutazyme™ Enzyme (10 U/μl) | 15 μl |
| pUC18 Control Plasmid (10 ng/μl)[3] | 10 μl |
| Control Primer Mix (20 pmole/μl)[3] | 10 μl |

[1] All components should be stored at -20°C.

[2] Muta-Direct™ Site-Directed Mutagenesis Kit contains sufficient to perform approximately 15 × 50 μl mutagenesis reactions.

[3] pUC18 Control Plasmid and Control Primer Mix contained in this kit are sufficient to perform 5 control reactions.

## PROTOCOL

### A. Muta-Direct™ Control Reaction

The pUC18 Control Plasmid and Control Primer Mix contained in the **Muta-Direct™ Site-Directed Mutagenesis Kit** are provided to check whether the mutagenesis experiment is performed completely. The pUC18 Control Plasmid and Control Primer Mix should be used in the Muta-Direct™ Control Reaction. Through the Muta-Direct™ Control Reaction, a translational termination codon is introduced into the *lac*Z gene contained in pUC18 Control Plasmid. The change from serine (TCG) to translational termination codon (TAA) can block the expression of protein product of the *lac*Z gene. Only white colonies are formed after transformation when the experiment completes properly.

### B. Primer Design

At first, design of the mutagenic primers is required for the use of the Muta-Direct™ Site-Directed Mutagenesis Kit.

It is generally accepted that the length of mutagenic primers is 25~45 bp. We recommend the use of the mutagenic primer which is 30-35 bp in length. It is important that the nucleotide desired to be mutated should be settled in the middle of the mutagenic primers.

Design a primer of 30 bp in length and then you have to estimate a melting temperature (Tm) with Tm formula. Tm of the mutagenic primers should be greater than or equal to 78°C (At least more than 40% of GC ratio). If the Tm is under 78°C, the change of the primer length is necessary.

Note) Check points below for the design of primer.

1) Design forward and reverse primers which are 30 bp each in length. In this step, locate the nucleotide desired to be mutated in the middle of the mutagenic primers.
2) Estimate the Tm of the mutagenic primers. If the Tm is under 78℃, adjust the length of primers for 78°C (At least more than 40% of GC ratio).
3) Avoid using of desalting-grade primers. Use of primers with FPLC- or PAGE-grade is recommended. Most of companies commonly provide FPLC-grade primer but customer are required to check this point.

The following formula is commonly used for estimating the Tm of mutagenic primers:

- **Tm formula: Tm=0.41(% GC)–675/L+81.5**
  L: Primer length in base pairs;
  % GC: GC % of primer

## C. Muta-Direct™ Protocol

**< *Inducing mutation* >**

In this step, you may create a mutation at a defined site in a plasmid performing PCR with the mutagenic primers and Muta-direct™ Enzyme.

1. Prepare plasmids for using as a PCR template.

**[Note]**

Use *dam*+ *E. coli* strains such as DH5α strain as host strain. Most of *E. coli* strains commonly used for molecular biology work are *dam*+ strains except JM110 and SCS110 strains. For *end*+ strains, low number of colonies may be obtained because of activity of endonuclease. But this may not affect the mutation efficiency. We recommend DNA-spin™ Plasmid DNA Extraction Kit or DNA-midi™ Plasmid DNA Extraction Kit when you prepare the plasmids.

2. **[Optional]**

Muta-Direct™ Control Reaction (50 µl reaction volume)

| | |
|---|---|
| Muta-Direct™ Reaction Buffer (10×) | 5 µl |
| **pUC18 Control Plasmid (10 ng/µl)** | 2 µl |
| **Control Primer Mix (20 pmole/µl)** | 2 µl |
| dNTP mixture | 2 µl |
| $dH_2O$ | 38 µl |
| Muta-Direct™ Enzyme (2.5 U/µl) | 1 µl |

3. Sample reaction (50 µl reaction volume)

| | |
|---|---|
| Muta-Direct™ Reaction Buffer (10×) | 5 µl |
| **Sample plasmid (10 ng/µl, total 20 ng)** | 2 µl |
| **Sample primer (F) (10 pmole/µl)** | 1 µl |
| **Sample primer (R) (10 pmole/µl)** | 1 µl |
| dNTP mixture | 2 µl |
| $dH_2O$ | 38 µl |
| Muta-Direct™ Enzyme (2.5 U/µl) | 1 µl |

4. PCR condition

Perform PCR according to the following PCR condition and a final extension step may be skipped.

| Segment | Cycle | Temperature | Time |
|---|---|---|---|
| 1 | 1 cycle | 95°C | 30 sec |
| 2 | 15 cycles | 95°C<br>55°C<br>72°C | 30 sec<br>1 min<br>1-2 min/ kbp of plasmid length |

**[Note]**

In the PCR condition described above, the number of PCR cycle of segment 2 can be adjusted according to the following table. In case more than 4 nucleotides are mutated, mutation efficiency may be decreased .

| Mutation | Cycles |
|---|---|
| 1~2 nucleotide | 15 cycles |
| 3 nucleotides | 18 cycles |

5. After PCR, place the PCR tube in ice for 5 minutes.

**< *Digestion of non-mutated parental DNA template* >**

In this step, you may digest the non-mutated parental DNA template (methylated plasmid) by treatment with the Mutazyme™ Enzyme after the completion of PCR.

1. Add 1 µl of Mutazyme™ Enzyme (10 U/µl) to PCR mixture.
2. After mixing shortly, incubate the mixture at 37°C for 1hour.

**[Note]**

In case of using significantly excess amount of PCR template plasmid, the treatment with the Mutazyme™ Enzyme may incomplete. This may cause difficulty in the correct selection of mutated clones. Therefore, proper amount of PCR template plasmid should be used.

**< *Transformation* >**

This step is to recover the nick on the mutated plasmid by transforming *E. coli* with the PCR mixture treated with Mutazyme™ Enzyme. Transformation can be performed according to conventional methods. Use directly 1-10 µl of Mutazyme™ Enzyme–treated PCR mixture for the transformation of aliquot 50 µl of the competent cells.

**[Note]**

To check the amount of mutated plasmid, electrophorese 10 µl of Mutazyme™ Enzyme–treated PCR mixture on a 1% agarose gel.

# EXPERIMENTAL INFORMATION

## • Primer design example

The example below is for a primer design with a mutation of **G**CG→**A**CG.

5' CCTCCTTCAGTATGTAG**G**CGACTTACTTATTGCGG-3'

1) First step, design the forward and reverse primers which are 30 bp each in length with locating A (or T) in the middle of the primer.

Primer #1: 5'-CCTTCAGTATGTAG**A**CGACTTACTTATTGC-3'
Primer #2: 5'-GCAATAAGTAAGTCG**T**CTACATACTGAAGG-3'

2) This primer has 40% of GC and 30 of L value. With these data, Tm is calculated to be 75.4°C (**Tm**=0.41×40-675/30+81.5). If Tm is under 78°C, that is not an appropriate primer.

3) In this case, it is necessary to adjust the length of primer.

Primer #1: 5'-*CCT*CCTTCAGTATGTAG**A**CGACTTACTTATTGC*GG*-3'
Primer #2: 5'-*CC*GCAATAAGTAAGTCG**T**CTACATACTGAAGG*AGG*-3'

The case above with adding 5 nucleotides to original primers (italic and underlined), the primers has 45.7% of GC and 35 of L value. With these data, Tm is re-calculated to be 80.952°C (**Tm**=0.41×45.7-675/35+81.5). This designed primer may be used.

## • Mutagenesis example

The example below is for mutagenesis with a mutation of **G**CG→**A**CG.

**[Reaction Mixture]**

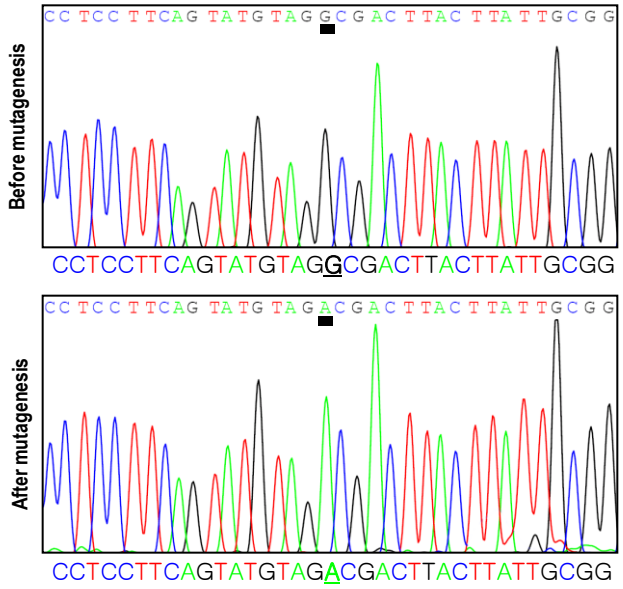
| | |
|---|---|
| Muta-Direct™ Reaction Buffer (10×) | 5 µl |
| **Sample plasmid (6.3Kbp) (10 ng/µl; total 20 ng)** | 2 µl |
| **Sample primer (F) (10 pmole/µl)** | 1 µl |
| **Sample primer (R) (10 pmole/µl)** | 1 µl |
| dNTP mixture | 2 µl |
| $dH_2O$ | 38 µl |
| Muta-Direct™ Enzyme (2.5 U/µl) | 1 µl |

**[PCR Condition]**

| Segment | Cycle | Temperature | Time |
|---|---|---|---|
| 1 | 1 cycle | 95℃ | 30 sec |
| 2 | 15 cycles | 95℃<br>55℃<br>72℃ | 30 sec<br>1 min<br>6 min 30 sec<br>(plasmid length: 6.3 kbp) |

**[Sequencing Analysis]**

Sequencing result of the mutated plasmid is as follows.



# TROUBLESHOOTING GUIDE

| Problem | Recommendation |
|---|---|
| No colony | - Check the PCR amplification by gel running. If there are problems in PCR, adjust the PCR conditions such as an annealing temperature.<br>- Check the viability of competent cells used. |
| Low mutation efficiency | - Incomplete treatment with the Mutazyme™ Enzyme may cause the decreased mutation efficiency. Increase the amount of Mutazyme™ Enzyme used in the step of digestion of the non-mutated parental DNA template or extend the treatment time.<br>- Check the amount of template plasmid used in PCR. The excessive use of template plasmid may cause low mutation efficiency. |
| Occurrence of mutation error | - Check the quality and sequence of primer used. |

# RELATED PRODUCTS

| Product Name | Cat. No. |
|---|---|
| CCC(Competent Cell for Cloning)-DH5α | 15045/15046 |
| CCC(Competent Cell for Cloning)-JM109 | 15047/15048 |
| CCC(Competent Cell for Cloning)-Top10 | 115049/15050 |
| DNA-spin™ Plasmid DNA Extraction Kit | 17096/17097/17098 |
| LINKeed® Rapid DNA Ligation Kit (Version 2.0) | 15023 |
| α-Complementation Solution | 15032 |