DATENBLATT

Site-Directed Mutagenesis Kit

Catalog #: SDM-15

Description

Muta-direct™ Site-Directed Mutagenesis Kit can induce mutagenesis at the specific point of sequence that cloned on plasmid DNA. It guarantees 100% of efficiency in theory. Also it is very convenient and simple because it takes just two steps for all experimental procedures. Muta-direct™ Site-Directed Mutagenesis Kit does not necessary using M13 vector and methylation step.Indeed, Muta-direct™ Kit can induce mutation of nucleotide, re-mutation to wild type, mutation of codon and insertion even deletion. As Muta-direct™ Kit has these characteristics, it is applicable to analysis for genomic/proteomic function. Also as inducing mutagenesis of specific gene, it can be used for protein engineering like protein development or improving productivity.

When you use this Muta-direct™ Kit, you can have mutated clone as doing simple steps. (Design primer with own protocol, use Muta-direct™ Enzyme for 15~18 cycles of PCR. Proceed transformation step after Muta-direct™ Mutazyme treatment for mutated clone selection) In this theory, clones on LB agar plate are mutated around 100% and after sequencing, you can proceed to the next step.

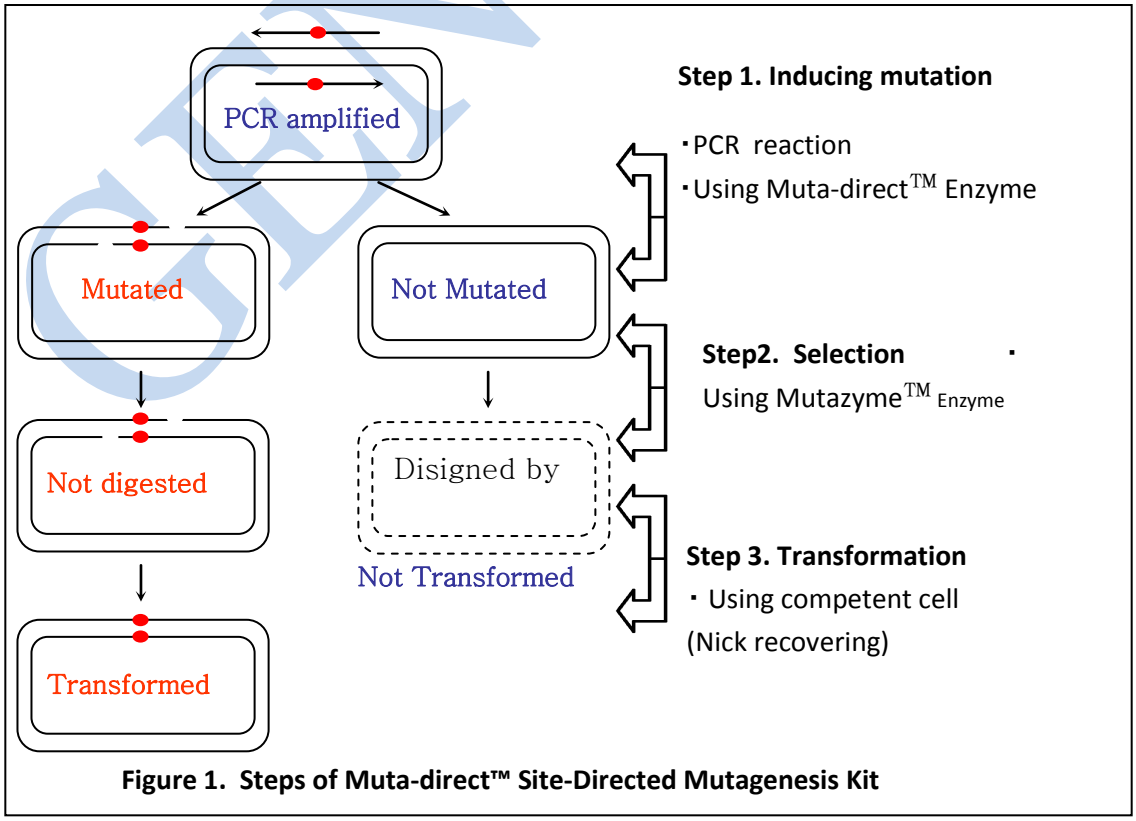

Figure 1. Steps of Muta-direct™ Site-Directed Mutagenesis Kit

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Characteristics▪

▪ Without special skill, easy to use.
▪ Can induce mutagenesis within 2~3 days.
▪ Use only two enzymes: Muta-direct™ Enzyme and Mutazyme™ Enzyme.
▪ Can use for various experiment: Point mutation, Deletion, Insertion and etc.
▪ 100% of mutation efficiency.
▪ Reasonable price.
▪ Technical assist by research agents.

Storage and Stability

All component should be stored at -20℃. The reaction buffer and dNTP mixture have been optimized for the Muta-direct™ protocols.

Kit Contents

For Research use. The Muta-direct™ kit contains sufficient reagents to perform approximately 15×50$\mu\ell$ mutagenesis reactions. The kits contain enough control template and primer mix for 5 control reactions, and enough reagents for 15 reactions total (control and experimental reactions combined).

Contents	Quantity
Muta-Direct™ Enzyme (2.5U/ul)	15ul
Muta-Direct™ Reaction Buffer (10×)	100ul
dNTP Mixture	30ul
Mutazyme™ Enzyme (10U/ul)	15ul
pUC18 Control Plasmid (10ng/ul)	10ul

Muta-direct™ Control Reaction

Control plasmid, contained in Muta-direct™, is pUC18 that informs us whether the experiment success or not. pUC18 plasmid has *lacZ* gene, so we can confirm the result as induce termination codon at *lacZ* gene by using Control Primer Mix (provided). In case of success, there must be all white colonies on LB plate. As change from serine (TCG) to stop codon (TAG) in pUC18, *lacZ* gene can be blocked. If user handles the mutation procedure for the first time, he can know about result as proceed of the control reaction step.

Primer Design

At first, it is required to design a primer. It is no matter who has experiences about designing. Just have a check the points below when you want design your primer. Normally, primer size is 25~45mer and we recommend 30~35 mer length.The important thing is that the target nucleotide on the center of primer.

Design as 30mer and next, you have to calculate the Tm value, more than 78℃ or not. (At least more than 40% of GC ratio)
If the Tm value is under 78℃, it is necessary to change the primer length.
①Design two strands, forward and reverse primers. In this step, locate the target nucleotide on the center of primer.
②Calculate the Tm value to know whether over than 78℃ or not. If the value is under 78℃, adjust the length of primer for 78℃ (Minimum GC ratio is 40%)
③Avoid desalting grade, Must use over than minimum FPLC or OPC grade. Normally, the most of companies use OPC, but it depends on the company. So customer must check this point.
Tm formula : Tm = 0.41(% of GC) – 675/L + 81.5
L : Number of oligomer in primer, % of GC : GC % of primer

Primer Design Example
Next, showing primer design. Case of **G**CG → **A**CG.
5′ CCTCCTTCAGTATGTAG**G**CGACTTACTTATTGCGG-3′
①First step, locate A (or T) to center which you want to mutate and then design 30mer for forward and reverse each.
Primer #1 : 5′-CCTTCAGTATGTAG**A**CGACTTACTTATTGC-3′
Primer #2 : 5′-GCAATAAGTAAGTCG**T**CTACATACTGAAGG-3′
②This primer contain 40% of GC and L value is 30, using these data to Tm formula, the result is 75.5℃(Tm = 0.41*40-675/30 +81.5). So we can find that the Tm value is under 78℃. This is not an appropriate primer.
③In 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′
5 mers are added to original primers (italic, under lined). In this case, the primers contain 45.7% of GC and L value is 35, using these data to Tm formula, the result is 80.952℃(Tm = 0.41*45.7-675/35 +81.5). Now you can use this primer.

Muta-direct™ PROTOCOL
[A] Induction of Mutagenesis (PCR Reaction)
In this step, you can induce mutagenesis at target nucleotide. As using synthesized primer, proceed PCR reaction with Muta-direct™ Enzyme.
1. Design each primer for Site direct mutation.
[Note] Refer to Primer design guide
2. Prepare plasmid DNA as a template.
[Note] Use *dam*+ bacteria (ex. DH5 strain) as host. (Almost *dam*+ bacteria when except JM110 and SC

S110 strain). Also, in case of *end*+ strain, sometimes it can be happen the number of colony is low. But this is not affected to mutation efficiency. We recommend to use

DNA-spin™ and DNA-midi™ Plasmid DNA extraction kit when you extract plasmid DNA

3. [Option] Control reaction (50㎕reaction volume)

10× reaction buffer 5ul

Puc18 control plasmid (10ng/, total 20ng) 2 ul㎕

Control primer mix (20pmol/ul) 2 ul㎕

dNTP mixture (each 2.5mM) 2 ul㎕

dH2O 38 ul㎕

Muta-direct™ Enzyme 1 ul

4.Sample reaction (50 reaction volume)

10× reaction buffer 5 ul㎕

Sample plasmid (10ng/ ul, total 20ng) 2 ul

Sample primer (F) (10pmol/ ul㎕) 1 ul㎕

Sample primer (R) (10pmol/ ul㎕) 1 ul㎕

dNTP mixture (each 2.5mM) 2 ul㎕

Muta-direct™ Enzyme 38 ul㎕

5. PCR condition

[Note] Follow the PCR condition described below and final extension step can be omitted.

Cycles	Temperature	Reaction Time
1 cycle	95℃	30 sec
15 cycle	95℃	30 sec
	55℃	1 min

6. After PCR, put it in the ice for 5 minutes then, store at RT.
(Avoid frequent freeze-thawing)

[Note] In the PCR condition described above, control the number of PCR cycle.
[Note] that there is very low mutagenesis efficiency in case more than 4 nucleotides are mutated or Avoid more than 4 nucleotides mutagenesis. In this case, the

efficiency can be very low.

Mutation	Cycles
1~2 Nucleotide	15 cycles
3 Nucleotides	18 cycles

[B] Selection of mutated plasmid

In this step, you can select mutated plasmid DNA by digestion of the methylated plasmid with Mutazyme™ Enzyme after PCR reaction.

1. Prepare the product from above PCR reaction
2. Incubate the sample at 37℃for 1hour with 1ul(10U/ul) of Mutazyme™ Enzyme.

[Note] In case of using much amount of plasmid DNA, sometimes Mutazyme™ Enzyme couldn't reaction with sample. So we suggest you to follow the procedure correctly for good mutation efficiency. If mutation efficiency is low, take a long time for reaction or add more amount of Mutazyme™ Enzyme.

[C] Transformation

This step recovers the nick on the plasmid DNA after reaction. When you transform into *E.coli*, use *dam+* strain competent cell like DH5a.

1. Put the 10ul sample into 50ul competent cell vial and than keep it in the ice for 30minutes
2. Follow general steps with an appropriate transformation method.

SEQUENCING ANALYSIS▪

▪ White colonies on LB plate resulted by Muta-direct™ protocol is supposed to be occurred 100% of mutation.0

▪ To confirm this result, sequencing analysis is recommended with white colonies.

MUTAGENESIS EXAMPLE

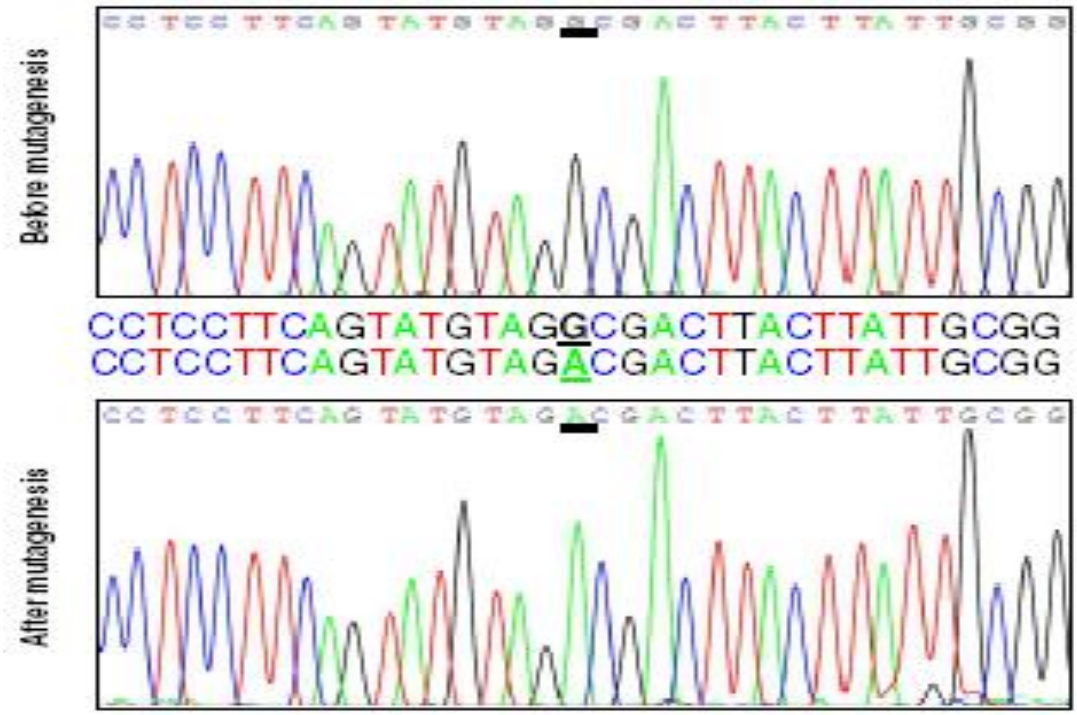
Example of mutagenesis inducing. G**G**C → G**A**C **[Reaction Mixture]**

……10× reaction buffer 5ul㎕
Sample plasmid (6.3Kb) (10ng/ul, total 20ng) 2ul
Sample primer (F) (10pmol/ ul) 1 ul
Sample primer (R) (10pmol/ ul) 1 ul
dNTP mixture (2.5mM each) 2 ul
dH2O 38 ul
Muta-direct™ Enzyme 1 ul

[PCR Condition]

Cycles	Temperature	Reaction Time
1 cycle	95℃	30 sec
15 cycles	95	30 sec
	55	1 min
	72	1 min per plasmid Kb

[Sequencing Analysis]



Trouble	Solution
No colonies	Check the PCR amplification by gel running. If the problem is PCR reaction step, adjust annealing temperature
	Check the efficiency of competent cell
Low mutation efficiency	Mutazyme™ Enzyme treatment step might be inappropriate. As this template plasmid can transform to cell, the mutation efficiency could be low. Increase the volume of Mutazyme™ Enzyme or extend reaction time.
	Check the amount of template plasmid. Excessive plasmid can affect low efficiency .
Mutant error	Check the quality of the synthesized primers

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