



**Thermo Scientific  
Mutation Generation System Kit**

**Technical Manual**

F-701      10 reactions

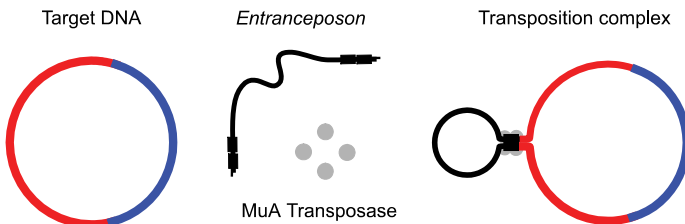


## Table of contents

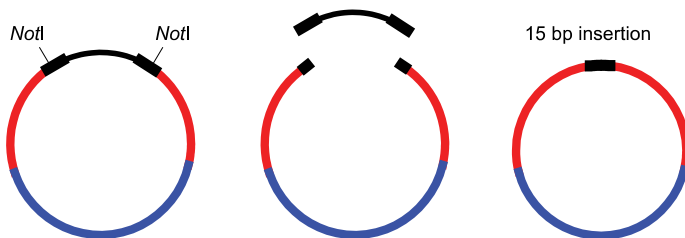
1. Description.....	4
2. Applications.....	5
3. Kit components.....	6
4. User-supplied materials .....	6
5. Reaction protocol.....	8
6. 15 bp Insertion mapping by colony PCR .....	13
6.1 Reaction conditions.....	13
6.2 Thermal cycling protocol for 15 bp insertion mapping .....	14
6.3 Agarose gel electrophoresis .....	14
Appendix I: Descriptions of kit components.....	15
Appendix II: Maps of Entranceposons .....	16
Appendix III: Translation of the 15 bp insertions .....	19
Appendix IV: DNA techniques.....	20
Appendix V: Recipes .....	21
Appendix VI: Related products.....	22
Appendix VII: References .....	23
Product use limitation.....	23

# 1. Description

The Thermo Scientific Mutation Generation System (MGS) Kit has been designed for rapid and efficient construction of linker scanning libraries for functional analyses of proteins and regulatory DNA regions. The system uses the highly efficient transposition machinery of the bacteriophage Mu to insert an artificial transposon, designated as Entranceposon, virtually into any target plasmid in random sites. The simple *in vitro* transposition reaction is catalyzed by a single purified enzyme, MuA Transposase. The insertion clones generated in the transposition reaction are digested with the rare-cutting restriction enzyme *NotI* to remove the body of the Entranceposon. Closure of the *NotI* digested clones by self-ligation results in a 15 bp insertion in the target DNA. In case the Entranceposon insertion occurs in the coding region of the target gene, the 15 bp insertion is translated into five extra amino acids.



*In vitro* transposition reaction and transformation



Plasmid preparation, *NotI* digestion, ligation and transformation

Figure 1. Flowchart of the MGS protocol.

## 2. Applications

The MGS™ kit has been designed for inserting fifteen basepairs randomly into target DNA for:

- Fast generation of in-frame five amino acid insertion libraries of any protein for functional analyses
- Rapid and random mutagenesis of cloned promoters and other regulatory DNA regions
- Insertion of a *NotI* restriction enzyme recognition site into target DNA clone

### Target DNA



### Target DNA with the 15 bp insertion

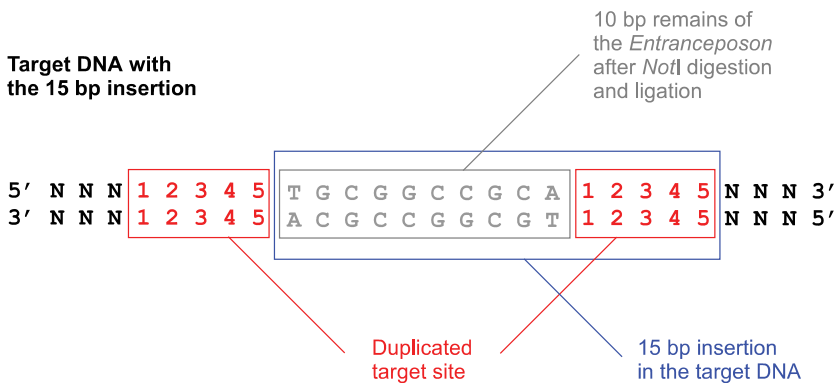


Figure 2. Structure of the 15 bp insertion generated by the MGS.

### 3. Kit components

The MGS kit contains sufficient materials for 10 reactions. See Appendix I for detailed descriptions of the kit components.

Product	Size
Entranceposon *(M1-Cam <sup>R</sup> )	10 µl
Entranceposon *(M1-Kan <sup>R</sup> )	10 µl
MuA Transposase	10 µl
5X Reaction Buffer for MuA Transposase	100 µl
Control Target DNA	10 µl
<i>NotI</i> Miniprimer	50 µl

\* See Appendix II for the complete sequences of the Entranceposons.

Store the components at -20°C.

Material safety data sheet (MSDS) is available at [www.thermoscientific.com/fzmsds](http://www.thermoscientific.com/fzmsds).

### 4. User-supplied materials

#### Target DNA

The *in vitro* transposition reaction of the MGS kit is not affected by minor impurities present in the target DNA preparation. The target DNA should be purified using standard methods, such as alkaline lysis method, or commercial plasmid DNA purification kits. Resuspend the target DNA in low-salt buffer such as TE, pH 8.0 or deionized water. The optimal amount of the target DNA per reaction depends on the size of the plasmid and can be calculated as follows:

$$\text{Amount of the target DNA (ng) per reaction} = \text{Size of the target plasmid (kb)} \times 40 \text{ ng}$$

Example: Your target DNA plasmid consists of a 1.3 kb insert cloned into a 2.8 kb vector. The size of the target plasmid is 1.3 kb + 2.8 kb = 4.1 kb, and the optimal amount per reaction is: 4.1 × 40 ng = 164 ng

Use minimum of 150 ng of the target DNA per transposition reaction.

**Important:** Make sure that your target DNA clone does not contain the same selectable marker gene that is present in the Entranceposon that you are using (either Cam<sup>R</sup> or Kan<sup>R</sup>).

**Important:** The target DNA clone should not contain recognition sites for the restriction enzyme *NotI* (5'-GCGGCCGC-3'). Note: Sometimes it is possible to destroy an extra *NotI* site by a fill-in reaction with a suitable DNA polymerase, such as the Klenow fragment of the *E. coli* DNA polymerase I.

The transposition reaction of the MGS has no target sequence preference. Therefore, the Entranceposon insertions occur at random locations in the target DNA clone. However, in some applications it is preferred that the Entranceposon insertions occur in the region of the DNA of interest ("insert") rather than in the vector backbone of the target clone. That can be achieved by transferring the DNA of interest with an Entranceposon insertion into a fresh cloning vector using standard DNA cloning techniques. In order to perform this, one should release the DNA of interest from the vector with specific restriction enzymes that do not cut inside the Entranceposon sequence. A selection of the non-cutting restriction enzymes for each Entranceposon is listed in Appendix II. Additionally, there should be sufficient difference in the sizes of the cloning vector and the insert ("DNA of interest") to allow separation of the restriction fragments by agarose gel electrophoresis.

### Competent *E. coli* cells

Any standard chloramphenicol- or kanamycin-sensitive laboratory strain of *E. coli* that is suitable for high-efficiency DNA cloning can be used as a transformation host. It is important that the efficiency of the transformation is high (>10<sup>8</sup> cfu/μg pUC19 for electrocompetent cells) in order to maximize the number of independent clones in the insertion scanning libraries. Therefore, electroporation is the recommended transformation method. Note: Entranceposons contain 50 bp inverted terminal repeats. To avoid potential homologous recombination between the repeats, the use of a *recA*<sup>-</sup> *E. coli* strain is recommended.

**Thermal cycler:** For transposition reaction (alternatively: heat blocks, 30°C and 75°C) and colony-PCR reactions

**Enzymes:** *NotI*, to cut out body of the Entranceposon  
Optional: restriction enzymes that cleave the DNA of interest out of the vector backbone  
DNA ligase

**Media:** LB or SOC medium  
LB agar with antibiotics

**Other reagents:** Reagents for plasmid DNA isolation  
Optional: reagents for extraction of DNA from agarose gel

## 5. Reaction protocol

Perform the *in vitro* transposition reaction:

1. Set up the following reaction in a microcentrifuge tube.  
Important: The MuA Transposase should be added last.  
See the flowchart of the *in vitro* transposition reaction in figure 1.

Reagent	Volume
H <sub>2</sub> O	add to 20 $\mu$ l
Target DNA (see "User Supplied Materials")	1 to 14 $\mu$ l
5X Reaction Buffer for MuA Transposase	4 $\mu$ l
Entranceposon (M1-Cam <sup>R</sup> ) OR (M1-Kan <sup>R</sup> ), 100 ng	1 $\mu$ l
MuA Transposase	1 $\mu$ l
	<b>20 <math>\mu</math>l</b>

For the control reaction, use 370 ng (1  $\mu$ l) of the Control Target DNA supplied with the kit.

2. Mix the components gently
3. Allow the transposition reaction to proceed for one hour at 30°C
4. Inactivate the MuA Transposase by incubating at 75°C for 10 minutes

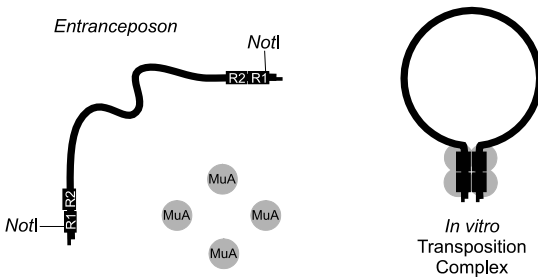


Figure 3 a. The *in vitro* Mu transposition complex is assembled as four monomers of the MuA Transposase protein are bound to the R1 and R2 sites in the ends of the Entranceposon.



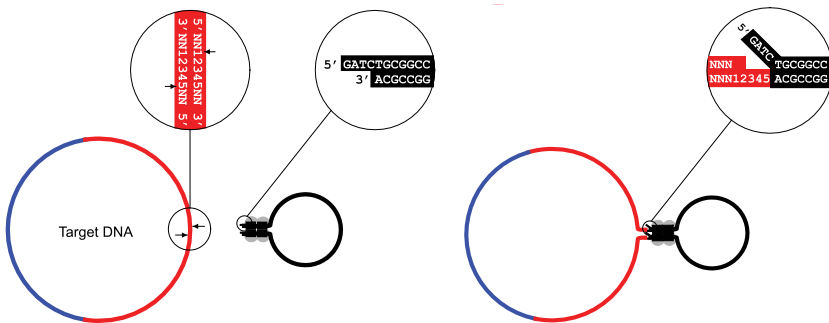


Figure 3 b. The MuA Transposase makes 5 bp staggered cuts in the target DNA and, at the same time, the 3' ends of the Entranceposon are covalently joined to the 5' phosphates in the target site.

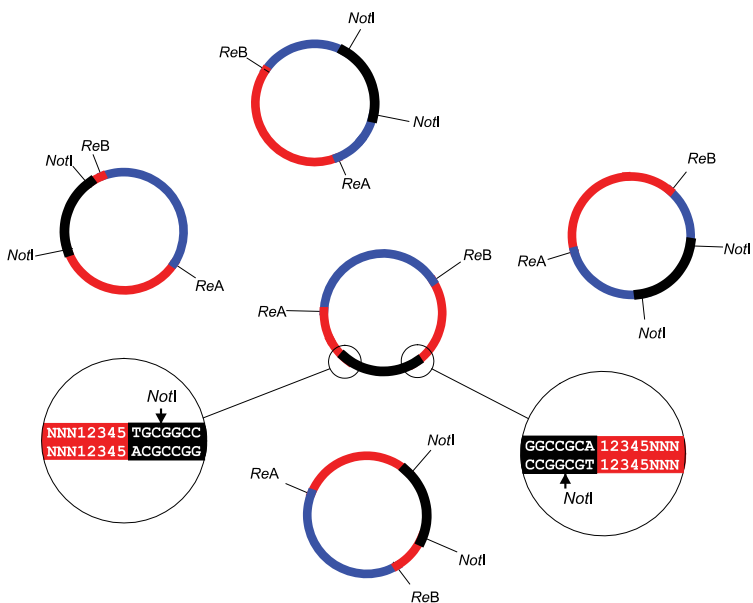


Figure 3 c. The single-stranded gaps are repaired in *E. coli*, resulting in the duplication of the 5 bp target site.

5. Proceed to the transformation step (see Appendix IV for details).  
Dilute the reaction mixture ten-fold in deionized water and use maximum of 10  $\mu$ l per single electroporation. Alternatively, precipitate the DNA in the reaction mixture, resuspend in deionized water and use aliquots for electroporation. For chemical transformation use 5-10  $\mu$ l of reaction per transformation.
6. Plate out on several LB agar plates supplemented with 10  $\mu$ g/ml chloramphenicol OR 10  $\mu$ g/ml kanamycin and additionally with the antibiotic that selects for the target DNA clone (Appendix V).

**Important:** The Entranceposon insertions occur in the vector backbone of your target DNA plasmid as well as in the insert (“DNA of interest”). For some applications it is necessary that there are no Entranceposon insertions in the vector. To transfer the DNA of interest with an Entranceposon insertion into a “fresh” cloning vector, continue through the steps 7-11. If the cloning step is not necessary for your application, proceed directly into the step 12.

7. Scrape the transformants from the plates, pool bacteria and prepare plasmid DNA using the standard alkaline lysis method or any commercial DNA preparation kit (Appendix IV).
8. Digest the plasmid preparation with the restriction enzymes that release the DNA of interest from the cloning vector. A selection of the non-cutting restriction enzymes for each Entranceposon is listed in Appendix II. **Note:** In case the cloning vector and the DNA of interest are of the same size, it may be necessary to digest the plasmid additionally with a restriction enzyme that cuts the vector backbone in smaller pieces to be able to separate the fragments by agarose gel electrophoresis.

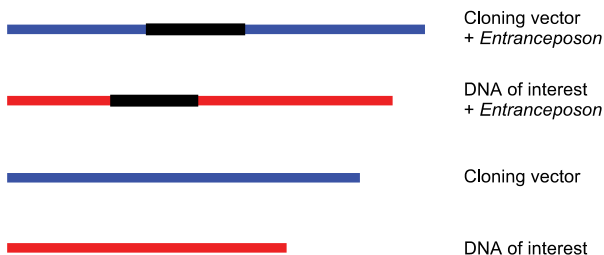


Figure 3 d. The fragments resulting from the digestion of the plasmid DNA pool with the restriction enzymes that cut out the DNA of interest from the vector.

9. Separate the resulting fragments by standard agarose gel electrophoresis. Extract the DNA of interest with an Entranceposon insertion (Appendix IV).
10. Clone the extracted DNA fragment into "fresh" cloning vector.
11. Transform and plate out on several LB agar plates supplemented with either 10 µg/ml chloramphenicol OR 10 µg/ml kanamycin and additionally with the antibiotic that selects for the cloning vector.
12. Scrape the transformants from the plates, pool the bacteria and prepare plasmid DNA using the standard alkaline lysis method or a commercial plasmid DNA isolation kit.

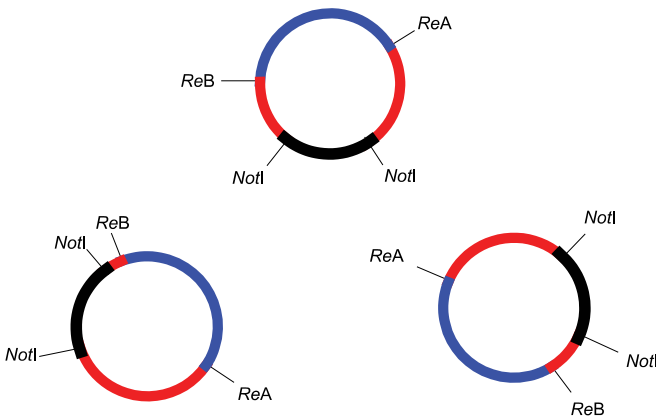


Figure 3 e. Pool of target DNA clones carrying an Entranceposon insertion at random location.

13. Digest the plasmid preparation with *NotI* to cut out body of the Entranceposon. Optional: Separate the resulting fragments by standard agarose electrophoresis. Extract the fragment with the size of your cloning vector and the DNA of interest.

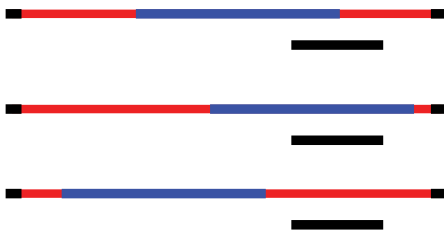


Figure 3 f. *NotI* digestion cuts out body of the Entranceposon.

14. Use an aliquot of the *NotI* digestion mixture for re-ligation reaction. To favor self-ligation of the *NotI* digestion fragments, increase the volume of the ligation reaction to adjust the DNA concentration of the mixture to 1-5 ng/μl. This is especially important if the digestion fragments are not separated by agarose gel electrophoresis.
15. Transform into competent *E. coli* cells.
16. Plate on LB agar plates supplemented only with the antibiotic that selects for the cloning vector of the plasmid. **Important!** Do not select with chloramphenicol or kanamycin after the Entranceposon has been cut out with *NotI* digestion.
17. Scrape the transformants from the plates, pool the bacteria and prepare plasmid DNA. The insertion library is now ready for your specific application. Alternatively, map the 15 bp insertions in the individual clones by performing a colony-PCR analysis on the transformants. Prepare plasmid DNA only from the clones that carry the 15 bp insertion in the desired region and proceed according to your application.

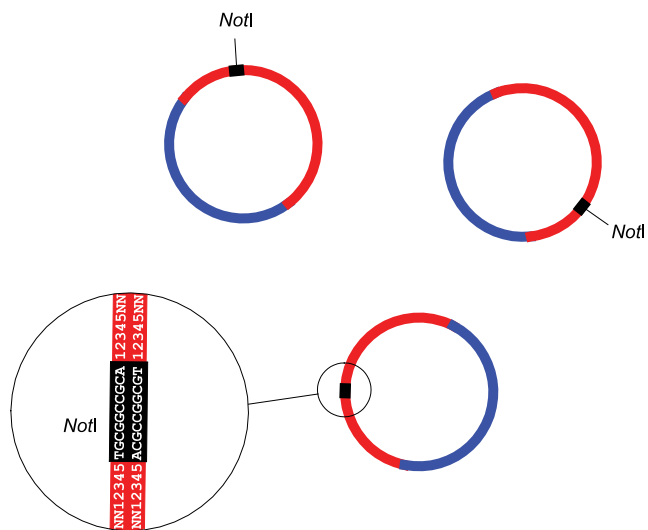


Figure 3 g. Fifteen basepair insertion in the target DNA.

## 6. 15 bp Insertion mapping by colony PCR

### 6.1 Reaction conditions

For reliable mapping of the 15 bp insertion in the target DNA, perform two separate 20  $\mu$ l PCR reactions per insertion clone. In addition to the *NotI* Miniprimer included in the kit, use primers that anneal to the sequence flanking the DNA of interest at opposite sides (see figure 4).

1. Prepare two PCR reaction mixtures, one with the *NotI* miniprimer and vector-binding forward primer (Fwd mix) and the other with *NotI* miniprimer and vector-binding reverse primer (Rev mix). **Note:** If other DNA polymerase than Thermo Scientific Phire Hot Start II DNA Polymerase is used, modify the reaction conditions according to the manufacturer's instructions.

Reagent	Final conc.	Volume
H <sub>2</sub> O		14.4
5X Phire Reaction Buffer	1 $\times$	4
dNTPs (10 mM each)	200 $\mu$ M each	0.4
Vector forward OR reverse primer	0.5 $\mu$ M	0.4
<i>NotI</i> miniprimer	0.5 $\mu$ M	0.4
Phire Hot Start II DNA Polymerase		0.4 $\mu$ l
		<b>20 <math>\mu</math>l</b>

**Note:** If using Phire<sup>®</sup> Hot Start II DNA Polymerase for insertion mapping, difficult PCR templates, such as a GC-rich target DNA, can be managed simply by supplementing the reaction mixture with 5% DMSO and by decreasing the annealing temperature 2-3°C.

2. Place the required number of reaction tubes on ice.
3. Using a toothpick or a pipet tip, transfer bacteria from a single chloramphenicol- or kanamycin-resistant colony into 50  $\mu$ l of sterile water and vortex the solution. Transfer 1  $\mu$ l of the cell dilution into two separate reaction tubes, one labeled for the Fwd mix and the other for the Rev mix. Also, at this point be sure to make a new replicate plate on each colony picked.
4. Add 19  $\mu$ l of the mixtures into the PCR tubes on ice.
5. Amplify according to the following thermal cycling protocol.

## 6.2 Thermal cycling protocol for 15 bp insertion mapping

Step	Temperature	Time
Step 1	98°C	30 s
Step 2	98°C	5 s
Step 3	X°C*	5 s
Step 4	72°C	10-15 s/kb
Step 5	Go to the step 2 for 29 times	

\* As a basic rule, for primers >20 nt, anneal for 5 seconds at a  $T_m + 3^\circ\text{C}$  of the lower  $T_m$  primer. For primers  $\leq 20$  nt, use an annealing temperature equal to the  $T_m$  of the lower  $T_m$  primer. If necessary, use a temperature gradient to find the optimal annealing temperature for each template-primer pair combination. Use the  $T_m$  calculator and instructions on website [www.thermoscientific.com/pcrwebtools](http://www.thermoscientific.com/pcrwebtools) to determine  $T_m$  for your vector-binding primer.  $T_m$  for the *NotI* miniprimer 57.5°C

## 6.3 Agarose gel electrophoresis

Analyze the PCR products by standard agarose gel electrophoresis. The length of a PCR product equals to the distance between the 15 bp insertion site and the vector-binding primer in a given clone.

Note: Since the palindromic *NotI* miniprimer anneals to each strand of the template DNA, a DNA fragment with the length of the whole target plasmid may also be produced in the PCR reaction.

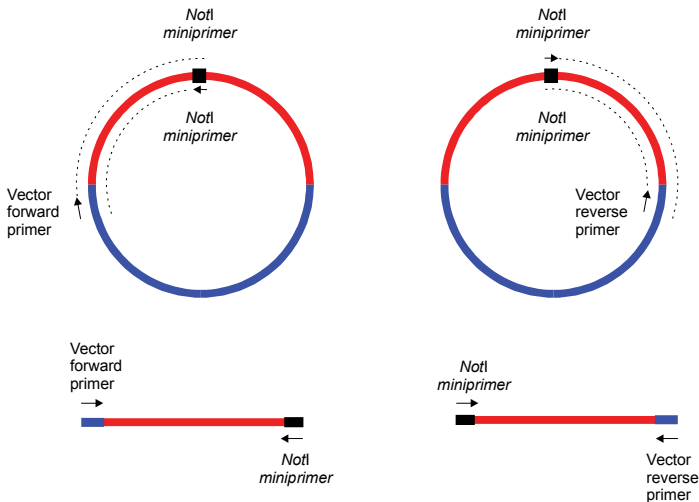


Figure 4. Colony-PCR mapping of the 15 bp insertion in the target DNA clone using the *NotI* miniprimer pairwise with primers that anneal to the vector sequence.

## Appendix I: Descriptions of kit components

### Entranceposon (M1-Cam<sup>R</sup>)

10 µl     100 ng/µl in TE, pH 8.0

### Entranceposon (M1-Kan<sup>R</sup>)

10 µl     100 ng/µl in TE, pH 8.0

Entranceposon (M1-Cam<sup>R</sup>) and (M1-Kan<sup>R</sup>) are composed of inverted terminal repeats of modified bacteriophage Mu right end sequence with a *NotI* site, flanking the selectable marker gene, either *cat* (Cam<sup>R</sup>) or *npt* (Kan<sup>R</sup>). The marker genes code for resistance to the antibiotics chloramphenicol and kanamycin, respectively (see Appendix II for complete sequences).

### MuA Transposase

10 µl     0.22 µg/µl in MuA Storage Buffer

A single purified polypeptide that catalyzes the in vitro transposition reaction. Isolated from an *E. coli* strain carrying the cloned bacteriophage MuA gene.

### 5X Reaction Buffer for MuA Transposase

100 µl     Component     5X conc.

### Control Target DNA

10 µl     370 ng/µl in TE, pH 8.0

A 6.6 kb *HindIII* fragment of bacteriophage lambda DNA cloned into the *HindIII* site of the vector pUC19.

### *NotI* miniprimer

50 µl     25 µM in H<sub>2</sub>O

5'-TGCGGCCGCA-3'     T<sub>m</sub> 57.5°C

The miniprimer anneals to each DNA strand at the 15 bp insertion site generated by the MGS. Due to the small size of the primer some unspecific amplification products may appear in the colony-PCR.

## Appendix II: Maps of Entranceposons

### a. Sequence of Entranceposon (M1-Cam<sup>R</sup>), 1 254 bp

*NotI* *Clal*  
 1 GATCT**CGGGCCGC**GCACGAAAAACGCGAAAGCGTTTTACAGATAAATGCGAAAAACGGATCGATCCTATCGT  
 71 CAATTATTACCTCCACGGGGAGAGCCTGAGCAAACGGCCTCAGGCATTTGAGAAGCACACGGTCACACT  
*AgeI*  
 141 GCTCCGGTAGTCAATAAACCGGTAAACCAGCAATAGACATAAGCGGCTATTTAACGACCCCTGCCTGAA  
 211 CCGACGACCGGGTCAAAATTTGCTTTTGAATTTCTGCCATTCATCCGCTTATTATCACTTATTCAGGCGTA  
*Scal*  
 281 GCAACCAGGCGTTTAAAGGCACCAATAACTGCCTTAAAAAAAT**TACGCCCCGCCCTGCCACTCATCGCAG**  
 351 **TACTGTTGTAATTCATTAAGCATTCTGCCGACATGGAAGCCATCACAAACGGCATGATGAACCTGAATCG**  
*SspI* *NcoI*  
 421 **CCAGCGGCATCAGCACCTTGTGCGCTTGCCTATAATATTTGCCCATGGTAAAACGGGGCGAAGAAGTT**  
 491 **GTCCATATGGCCACGTTTTAAATCAAACCTGGTGAACCTACCCAGGGATGGCTGAGACGAAAAACATA**  
 561 **TTCTCAATAAACCCTTTAGGGAAATAGGCCAGGTTTTACCGTAACACGCCACATCTTGCGAATATATGT**  
 631 **GTAGAAACTGCCGAAATCGTCGTGGTATTCCTCCAGAGCGATGAAAACGTTTCAGTTTGTCATGGAA**  
 701 **AACGGTGTAAACAAGGGTGAACACTATCCCATATCACCAGGTCACCGTCTTTCATTGCCATACGTAATCC**  
 771 **GGATGAGCATTCATCAGGCGGCAAGAATGTGAATAAAGGCCGATAAAACTTGTGCTTATTTTTCTTTA**  
*PvuII*  
 841 **CGGTCTTTAAAAGGCCGTAATATCCAGTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAA**  
← *cat* (Cam<sup>R</sup>)  
 911 **TGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTCTCC**  
 981 **ATTTTAGTTCCTTAGCTCCTGAAAATCTCGACAACCTCAAAAAATACGCCCGGTAGTGATCTTATTTTCAT**  
 1051 **TATGGTGAAGTTGGAACCTCTTACGTGCCGATCAACGTCTCATTTTCGCCAAAAGTTGGCCAGGGCTT**  
 1121 **CCCGGTATCAACAGGGACACCAGGATTTATTTATTCGCGAAGTGATCTTCCGTCACAGGTTATTTATTCG**  
*Clal* *NotI*  
 1191 **GTCGAAAAGGATCGATCCGTTTTTCGCAATTTATCGTGAAACGCTTTCGCGTTTTTCGTCG**CGGGCCGC**AGA**  
 1261 TC

Entranceposon (M1-Cam<sup>R</sup>) and (M1-Kan<sup>R</sup>) are composed of inverted terminal repeats of the modified bacteriophage Mu right end sequence with a *NotI* site, flanking the selectable marker gene, either (a.) *cat* (Cam<sup>R</sup>) or (b.) *npt* (Kan<sup>R</sup>).



b. Sequence of Entranceposon (M1-Kan<sup>R</sup>), 1 131 bp

*NotI* *Clal*

1 GATCTGCGG CCGCGCACGAAAAACGCGAAAG CGTTTCACGATAAATGCGAAAA CCGGATCGATCCTAGTAA

71 GCCACGTTGTGTCTCAAATCTCTGATGTTACATTGCACAAGATAAAAAATATATCATCATGAACAATAAA  
*npt (Kan<sup>R</sup>)* → *XhoI*

141 ACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATGAGCCATATTC AACGGGAAACGCTCTTGCTCG

211 AGGCCGCGATTAAATTC AACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGC

281 AATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAA

351 AGGTAGCGTTGCCAATGATGTTACAGATGAGATGGTCAGACTAACTGGCTGACGGAATTTATGCCTCTT

421 CCGACCATCAAGCATT TTTATCCGTACTCCTGATGATGCATGGTTACTCACCAC TGCGATCCCGGGAAAA

*SspI*

491 CAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCT

561 GCGCCGGTTGCATTTCGATTCCTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTTCGCTCTCGCTCAG

631 GCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGACGAGCGTAATGGCTGGCCTG

*HindIII*

701 TTGAACAAGTCTGGAAGAAATGCATAAGCTTTTGCCATTCTACCCGATT CAGTCGTCAC TCATGGTGA

771 TTTCTCACTTGATAACCTTATTTT GACGAGGGGAAATTAATAGTTGTATTGATGTTGGACGAGTCGGA

841 ATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACGCCTCGGTGAGTTTCTCCTT CATTACAGA

911 AACGGCTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATG CAGTTTCATTGATGCTCGA

981 TGAGTTTTTCTAA TCAGAATTGGTTAATTGGTTGTAACACTGCGAGACATTACGCTGACTTGACGGGAC

1051 GCGGCTTTGTTGAATAAATCGAACTGGATCATCCGTTTTCGCATTTATCGTGAACGCTTTCGCGTTTT

*NotI*

1121 TCGTGCGCGGCCGCAGATC

### Entranceposon (M1-Cam<sup>R</sup>), non-cutting enzymes:

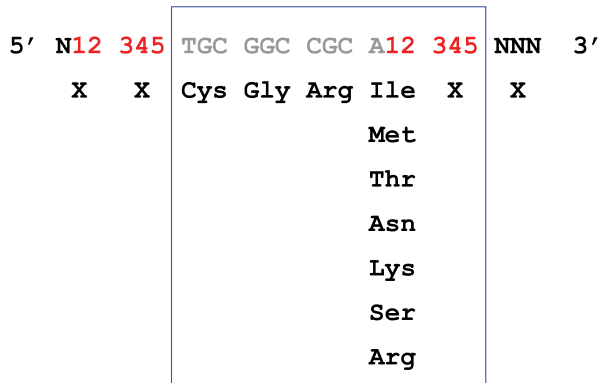
<i>AatII</i>	<i>AccI</i>	<i>Acc65I</i>	<i>AflII</i>	<i>AlwNI</i>
<i>ApaI</i>	<i>ApaLI</i>	<i>AscI</i>	<i>Asel</i>	<i>AvaI</i>
<i>Avall</i>	<i>AvrII</i>	<i>BamHI</i>	<i>BanII</i>	<i>BbsI</i>
<i>BbvI</i>	<i>BcgI</i>	<i>BclVI</i>	<i>BclI</i>	<i>BfaI</i>
<i>BglI</i>	<i>BglII</i>	<i>BlpI</i>	<i>BmrI</i>	<i>BsaI</i>
<i>BsaBI</i>	<i>BseRI</i>	<i>BsgI</i>	<i>BsiHKA1</i>	<i>BsiVI</i>
<i>BspHI</i>	<i>BspMI</i>	<i>BsrBI</i>	<i>BsrGI</i>	<i>BssHII</i>
<i>BssSI</i>	<i>BstAPI</i>	<i>BstEII</i>	<i>BstXI</i>	<i>DrallI</i>
<i>EarI</i>	<i>EcoNI</i>	<i>EcoO109I</i>	<i>EcoRI</i>	<i>EcoRV</i>
<i>FseI</i>	<i>FspI</i>	<i>HaeII</i>	<i>HgaI</i>	<i>HincII</i>
<i>HindIII</i>	<i>HpaI</i>	<i>KasI</i>	<i>KpnI</i>	<i>MfeI</i>
<i>MluI</i>	<i>NaeI</i>	<i>NarI</i>	<i>NdeI</i>	<i>NheI</i>
<i>NruI</i>	<i>NsiI</i>	<i>NspI</i>	<i>PacI</i>	<i>PleI</i>
<i>PmeI</i>	<i>PpuMI</i>	<i>PstI</i>	<i>PvuI</i>	<i>SacI</i>
<i>SacII</i>	<i>SalI</i>	<i>SapI</i>	<i>SexAI</i>	<i>SfcI</i>
<i>SfiI</i>	<i>SgrAI</i>	<i>SmaI</i>	<i>SmlI</i>	<i>SpeI</i>
<i>SphI</i>	<i>StuI</i>	<i>SwaI</i>	<i>TseI</i>	<i>XbaI</i>
<i>XcmI</i>	<i>XhoI</i>	<i>XmaI</i>	<i>XmnI</i>	

### Entranceposon (M1-Kan<sup>R</sup>), non-cutting enzymes:

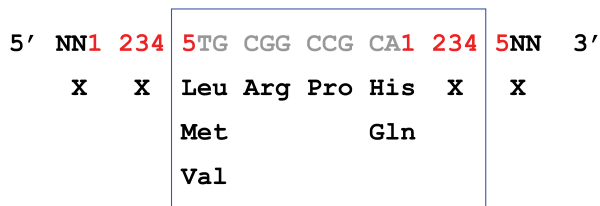
<i>AatII</i>	<i>AccI</i>	<i>AccII</i>	<i>Acc65I</i>	<i>AflII</i>
<i>AgeI</i>	<i>AlwNI</i>	<i>ApaI</i>	<i>ApaLI</i>	<i>AscI</i>
<i>Avall</i>	<i>AvrII</i>	<i>BaI</i>	<i>BamHI</i>	<i>BanI</i>
<i>BbsI</i>	<i>BbvI</i>	<i>BcgI</i>	<i>BclVI</i>	<i>BclI</i>
<i>BglI</i>	<i>BglII</i>	<i>BlpI</i>	<i>BmrI</i>	<i>BpmI</i>
<i>BsaI</i>	<i>BsaAI</i>	<i>BsaBI</i>	<i>BseRI</i>	<i>BsgI</i>
<i>BsiHKA1</i>	<i>BsNI</i>	<i>BspMI</i>	<i>BsrBI</i>	<i>BsrGI</i>
<i>BssHII</i>	<i>BssSI</i>	<i>BstAPI</i>	<i>BstEII</i>	<i>BstXI</i>
<i>Bsu36I</i>	<i>DraI</i>	<i>DrdI</i>	<i>EcoO109I</i>	<i>EcoRI</i>
<i>EcoRV</i>	<i>FauI</i>	<i>FseI</i>	<i>FspI</i>	<i>HaeII</i>
<i>HgaI</i>	<i>HincII</i>	<i>HpaI</i>	<i>KasI</i>	<i>KpnI</i>
<i>MfeI</i>	<i>MluI</i>	<i>MscI</i>	<i>MslI</i>	<i>MspA1I</i>
<i>NaeI</i>	<i>NarI</i>	<i>NcoI</i>	<i>NdeI</i>	<i>NheI</i>
<i>NlaIV</i>	<i>NspI</i>	<i>PacI</i>	<i>PmeI</i>	<i>PpuMI</i>
<i>SapI</i>	<i>PvuI</i>	<i>SacI</i>	<i>SacII</i>	<i>SalI</i>
<i>SapI</i>	<i>Sau96I</i>	<i>Scal</i>	<i>SexAI</i>	<i>SfcI</i>
<i>SfiI</i>	<i>SgrAI</i>	<i>SnaBI</i>	<i>SpeI</i>	<i>SphI</i>
<i>StuI</i>	<i>StyI</i>	<i>SwaI</i>	<i>TseI</i>	<i>Tth111I</i>
<i>XbaI</i>	<i>XcmI</i>	<i>XmnI</i>		

## Appendix III: Translation of the 15 bp insertions

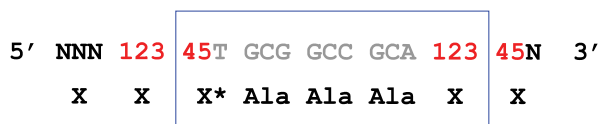
Frame 1:



Frame 2:



Frame 3:



X = Any amino acid

X\* = Any amino acid except Gln, Glu, Lys, Met, Trp

## Appendix IV: DNA techniques

### DNA precipitation

Precipitate the DNA in the reaction mixture by using the standard techniques.

1. Add 2  $\mu$ l 3 M NaAc pH 5.5 (1:10 vol) and 50  $\mu$ l 94% ethanol (2.5 vol).
2. Incubate on ice for 20 minutes and centrifuge 13000 rpm for 30 minutes at 4°C.
3. Remove the supernatant carefully and rinse the DNA pellet with 500  $\mu$ l 75% ethanol.
4. Spin briefly, remove the ethanol supernatant and air-dry the pellet.
5. Resuspend the DNA pellet in deionized water for electroporation.

### Transformation

**Electroporation:** 1X Reaction Buffer for MuA Transposase contains 125 mM NaCl. To avoid arcing that may result from excessive salt dilute the reaction mixture ten-fold in deionized water and use maximum of 10  $\mu$ l per single electroporation (typically 40-50  $\mu$ l electrocompetent cells, transformation efficiency  $>10^8$  cfu/ $\mu$ g pUC19). If it is necessary to use more than 1  $\mu$ l of the reaction mixture per single electroporation, precipitate the DNA in the reaction mixture as instructed above, resuspend in deionized water and use aliquots for transforming electrocompetent cells. Use the pulse settings instructed by the manufacturer of your electroporation system.

**Chemical transformation:** For chemical transformation use 5-10  $\mu$ l of the reaction mixture per 50-100  $\mu$ l chemically competent cells (transformation efficiency  $>10^7$  cfu/ $\mu$ g pUC19). Before plating on selective LB agar plates, it is necessary to grow the cells in 1 ml SOC medium for one hour at 37°C to ensure expression of the marker gene present in the Entranceposon.

### Preparation of plasmid DNA from transformation plates

1. Scrape small bacterial colonies from transformation plates in TE, pH 8.0. Adjust the cell density to equal an overnight liquid culture.
2. Transfer 1.5-3 ml of the suspension to a fresh tube and pellet the cells by spinning briefly in a microcentrifuge.
3. Discard the supernatant and resuspend the bacteria in 250  $\mu$ l of 15 mM Tris-HCl, pH 8.0, 10 mM EDTA, 100  $\mu$ g/ml RNase A.
4. Add 250  $\mu$ l of 0.2 M NaOH, 1% SDS to lyse the cells. Mix thoroughly but do not vortex. Incubate 5 min at RT.
5. Add 350  $\mu$ l of 3 M KAc, pH 5.5 and mix by turning the tubes vigorously immediately after KAc addition.
6. Immediately centrifuge 13000 rpm for 15 minutes at 4°C to pellet the chromosomal DNA and cell debris.
7. Transfer 400  $\mu$ l aliquot of the supernatant into fresh microcentrifuge tube.

8. Add 1 volume of phenol:chloroform (1:1), vortex briefly and centrifuge at full speed for five minutes to separate the aqueous and organic phases.
9. Extract the aqueous phase twice with chloroform to remove the traces of phenol.
10. Transfer the aqueous phase into a fresh tube and precipitate the DNA as instructed above.
11. Resuspend the DNA pellet in TE, pH 8.0.

## DNA extraction from agarose gel

1. Separate the fragments resulting from a restriction enzyme digestion by standard agarose gel electrophoresis.
2. Cut out the desired band from the agarose gel with a clean scalpel.
3. Melt the gel slice at 65°C for 10 min. Add TE, pH 8.0 to decrease the agarose concentration to >0.4%.
4. Add an equal volume of buffered phenol and mix vigorously for 5 min.
5. Centrifuge 13000 rpm for 10 min in a microcentrifuge.
6. Remove the aqueous phase to a fresh eppendorff tube and re-extract the phenol phase with an equal volume of TE, pH 8.0.
7. Combine the aqueous phases and extract again with an equal volume of buffered phenol.
8. Extract the aqueous phase twice with chloroform to remove the traces of phenol.
9. Transfer the aqueous phase into a fresh tube and precipitate the DNA as instructed above.
10. Resuspend the DNA pellet in deionized water for ligation reaction.

## Appendix V: Recipes

### LB agar with antibiotics, per liter

Tryptone	10 g
Yeast Extract	5 g
NaCl	10 g
Agar	15 g

Adjust pH to 7.0 with 1 M NaOH

Autoclave

Cool to 55°C and add:

Chloramphenicol (per liter) <b>OR</b>	20 mg
Kanamycin (per liter) <b>OR</b>	20 mg
Tetracycline (per liter)	10 mg

Optional: supplement the medium with the antibiotic that selects for the target DNA replicon.

## SOC medium

Tryptone	20 g
Yeast Extract	5 g
NaCl	0.5 g
KCl (final 2.5 mM)	0.186 g

Adjust pH to 7.0 with 1 M NaOH

Autoclave

Before use add sterile solutions:

1 M MgCl <sub>2</sub>	10 ml
1 M MgSO <sub>4</sub>	10 ml
1 M Glucose	20 ml

## TE

10 mM Tris-HCl, pH 8.0
1 mM EDTA

## Appendix VI: Related products

### Transposon products

- F-750 MuA Transposase, 20 µl (0.22 µg/µl)
- F-750C MuA Transposase, conc., 20 µl (1.1 µg/µl)
- F-760 Entranceposon (M1-Cam<sup>R</sup>)
- F-762 Entranceposon (M1-Kan<sup>R</sup>)
- F-702 Template Generation System II Kit
- F-703 Stop Generation System Kit
- F-764 Entranceposon (Tet<sup>R</sup>)
- F-771 Entranceposon (*supF*)
- F-774 Entranceposon (*lacZ*)
- F-765 pEntranceposon (Cam<sup>R</sup>)
- F-766 pEntranceposon (Kan<sup>R</sup>)
- F-767 pEntranceposon (Tet<sup>R</sup>)
- F-773 pEntranceposon (*supF*)

### PCR products

- F-122S/L Thermo Scientific Phire Hot Start II DNA Polymerase

## Appendix VII: References

Taira, S., Tuimala, J., Roine, E., Nurmiäho-Lassila, E.-L., Savilahti, H. and Romantschuk, M. (1999). Mutational analysis of the *Pseudomonas syringae* pv. *tomato* *hrpA* gene encoding Hrp pilus subunit. *Mol. Microbiol.* 34, 736-744.

Haapa, S., Suomalainen, S., Eerikäinen, S., Airaksinen, M., Paulin, L., and Savilahti, H. (1999). An efficient DNA sequencing strategy based on the bacteriophage Mu *in vitro* DNA transposition reaction. *Genome Res.* 9, 308-315.

Haapa, S., Taira, S., Heikkinen, E., and Savilahti, H. (1999). An efficient and accurate integration of mini-Mu transposons *in vitro*: a general methodology for functional genetic analysis and molecular biology applications. *Nucleic Acids Res.* 27, 2777-2784.

Mizuuchi, K. (1992). Transpositional recombination: mechanistic insights from studies of Mu and other elements. *Annu. Rev. Biochem.* 61, 1011-1051.

Savilahti, H., Rice, P.A., and Mizuuchi, K. (1995). The phage Mu transpososome core: DNA requirements for assembly and function. *EMBO J.* 14, 4893-4903.

### Product use limitation

This product has been developed and is sold exclusively for research purposes and *in vitro* use only. This product has not been tested for use in diagnostics or drug development, nor is it suitable for administration to humans or animals.

The quality system of Finnzymes Oy, now part of Thermo Fisher Scientific, is certified according to standard SFS-EN ISO9001:2008.

# Thermo Scientific Mutation Generation System Kit Technical Manual



© 2012 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

v2\_2012

Technical support:  
US: [techservice.genomics@thermofisher.com](mailto:techservice.genomics@thermofisher.com)

Europe, Asia, Rest of World:  
[techservice.emea.genomics@thermofisher.com](mailto:techservice.emea.genomics@thermofisher.com)

**Thermo**  
SCIENTIFIC