Thermo Scientific
Template Generation System II Kit

Technical Manual

F-702 20 reactions

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1. Strategy outline

1. Purify your target DNA, i.e., the plasmid, cosmid, BAC or PAC clone that you wish to sequence. **Note:** The Thermo Scientific Template Generation System II Kit can also be used to insert Entranceposons into linear target DNA (e.g., a restriction fragment or a PCR product). A slightly modified reaction protocol is available at www.thermoscientific.com/tgs.
2. Choose one of the three Entranceposons included in the kit.
3. Perform the transposition reaction.
4. Transform competent *E. coli* cells with the transposition reaction mixture using either a chemical transformation method or electroporation.
5. Grow transformants on selection plates.
6. Pick clones for DNA sequencing. Choose either a "shotgun" approach, i.e., the clones are not analyzed before sequencing reactions or a "directed" approach, i.e., Entranceposon insertion sites in the clones are mapped by performing colony-PCR reactions. **Note:** The PCR products from the mapping reactions can also be used directly as templates for DNA sequencing reactions.
7. Prepare plasmid DNA from the clones that you plan to sequence.
8. Use primers SeqE and SeqW for bidirectional DNA sequencing in a single reaction or in two separate reactions, depending on the DNA sequencing chemistry at hand.

2. Kit components

The kit contains sufficient materials for 20 reactions. See Appendix I for more detailed information.

Product #	Product	Size
Artificial transposons:		
F-778	Entranceposon* (Cam^R-3)	20 µl
F-779	Entranceposon* (Kan^R-3)	20 µl
F-784	Entranceposon* (Tet^R-3)	20 µl
F-750	MuA Transposase	20 µl
F-752	5X Reaction Buffer for MuA Transposase	100 µl
F-753	Control Target DNA	10 µl
Primers for insertion mapping:		
F-754	pUC Fwd Primer	400 µl
F-755	pUC Rev Primer	400 µl
F-756	MuEnd Primer	800 µl
Primers for DNA sequencing:		
F-780	SeqE Primer	250 µl
F-781	SeqW Primer	250 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.

3. User-supplied materials

Target DNA

Use 60 fmoles of target DNA per a 20 µl reaction. The amount equals approximately 40 ng DNA per kb of target.

Example: Your target DNA plasmid consists of a 6 kb insert cloned into a 2.8 kb vector. The size of the target plasmid is 6 kb + 2.8 kb = 8.8 kb. The optimal amount of target DNA per reaction is: 8.8 kb × 40 ng/kb = 352 ng. Therefore, you should use 300-400 ng of the target plasmid per reaction.

For large DNA constructs (cosmids, BACs or PACs) use maximum of 2 µg target DNA per reaction.

Important: Make sure that your target DNA replicon does not contain the same selection marker as the Entranceposon that you plan to use (Cam^R,Kan^R or Tet^R).

Purify target DNA using standard methods such as alkaline lysis or commercial DNA purification kits. Target DNA should be in a low-salt buffer such as 1X TE buffer or in deionized water.

Competent cells

Any standard *E. coli* strain that is suitable for DNA cloning can be used as a transformation host.

Important: Make sure that the *E. coli* strain is not resistant to the antibiotic that you plan to use for selecting the insertion clones after transformation (chloramphenicol, kanamycin or tetracycline).

Both electrocompetent and chemically competent *E. coli* cells may be used. Electroporation is the recommended transformation method for large target DNA molecules (>20 kb).

Thermal Cycler:	Thermal cycler or heat blocks, 30°C and 75°C
Reagents:	Reagents and equipment for PCR mapping
Media:	SOC medium and LB agar plates with antibiotics

See Appendix III for the media recipes.

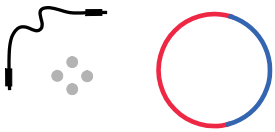
4. Transposition reaction protocol

1. Set up the following reaction:
   Important: MuA Transposase should be added last.

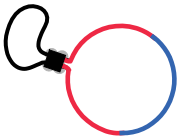
Reagent	Volume
Target DNA (see "User-Supplied Materials")	1 to 14 µl
H_2O	add to 20 µl
5X Reaction Buffer for MuA Transposase	4 µl
Entranceposon (Cam^R-3) OR (Kan^R-3) OR (Tet^R-3)	1 µl
MuA Transposase	1 µl
	20 µl

For the control reaction, use 1 µl (370 ng) of Control Target DNA supplied with the kit.

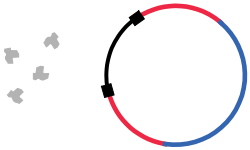
2. Mix the reagents. Do not vortex.



3. Incubate 1 hour at 30°C.



4. Heat inactivate at 75°C for 10 minutes.



5. Transform.

5. Transformation

Chemical transformation

Transform 1-10 µl of the reaction mixture per 50-100 µl competent *E. coli* cells. Follow standard transformation protocols or proceed as instructed by the manufacturer of your competent cells.

Electroporation

Dilute the reaction mixture 10-fold in deionized water. Use 1-10 µl of the dilution per one electroporation shot (typically 40-50 µl of electrocompetent *E. coli* cells).

Before plating on selective plates, it is necessary to grow the cells in 1 ml SOC medium for one hour at 37°C to ensure marker gene expression from the inserted Entranceposon.

Plate 5, 50 and 500 µl of the transformation mixture (1 ml) on selection plates:

LB+ chloramphenicol (20 µg/ml)
OR
LB+ kanamycin (20 µg/ml)
OR
LB+ tetracycline (10 µg/ml)

Note: To reduce the number of insertions in the vector backbone, supplement the selection plates with the antibiotic that selects your target plasmid.

Incubate the plates overnight at 37°C.

6. Insertion mapping by colony-PCR

When the target plasmid is relatively small (<10 kb) it is feasible to apply the "directed" approach, i.e., to map the Entranceposon insertion sites first by colony-PCR and then, based on the mapping data, to choose a minimal set of overlapping templates for DNA sequencing.

6.1 DNA amplification

Perform two separate 20-µl PCR reactions per an insertion clone to ensure reliable mapping of Entranceposon insertion sites.

1. Prepare two PCR reaction master mixes, one with the primers MuEnd and pUC Fwd and the other with MuEnd and pUC Rev.

Reagent	Final conc.	Volume
H_2O		14.4 µl
5X Phire Reaction Buffer	1 ×	4 µl
dNTPs (10 mM each)	200 µM each	0.4 µl
pUC Fwd OR pUC Rev Primer (25 µM)	0.5 µM	0.4 µl
Mu End Primer (25 µM)	0.5 µM	0.4 µl
Phire Hot Start II DNA Polymerase		0.4 µl
		20 µl

Note: The reaction conditions above have been optimized for Thermo Scientific Phire Hot Start II DNA Polymerase. Efficient amplification of most GC-rich sequences can be achieved by supplementing the reaction mixture with 5% DMSO and by decreasing the annealing temperature 2-3°C.

2. Aliquot 19 µl of the PCR reaction master mixes into reaction tubes on ice.
3. Touch a colony on a selection plate with a pipet tip. Dip the pipet tip into 50 µl deionized water to suspend the cells.
4. Pipet 1 µl of the cell suspension into two separate reaction tubes. Also transfer a small amount of the dilution on an appropriate selection plate to "replica-plate" the colonies picked for the PCR mapping reactions.

5. Use the following thermal cycling protocol for DNA amplification:

Step	Temperature	Time
Step 1	98°C	30 s
Step 2	98°C	5 s
Step 3	72°C	10-15 s/kb
Repeat the steps 2-3 30 times		

Note: If using primers not provided in the kit, please refer to Phire Hot Start II DNA Polymerase instruction manual for information on annealing determination and Tm calculation for primers.

6.2 Agarose gel electrophoresis

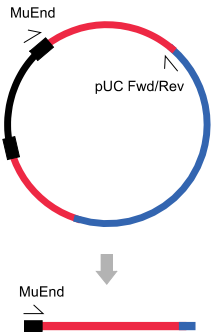
Analyze the PCR products by standard agarose gel electrophoresis. The lengths of the PCR products obtained from a given clone with the primer pairs MuEnd+pUC Fwd and MuEnd+pUC Rev equal to the distance between the Entranceposon insertion site and the pUC Fwd and pUC Rev primer, respectively. If the Entranceposon insertion in a given clone lies in the insert DNA (in the sequence between the primers pUC Fwd and pUC Rev) the sizes of the two PCR products from that clone should add up to the size of the insert DNA fragment. If the additive size of the two PCR products for a clone is bigger than the size of the insert DNA fragment, the Entranceposon insertion has most likely occurred in the vector backbone of the target plasmid.

Note: The Entranceposon insertion sites in target DNA can also be mapped using restriction enzymes. There are several unique restriction enzyme sites for that purpose in each Entranceposon (see Appendix II).

7. DNA sequencing

7.1 Sequencing from PCR products

Select clones for DNA sequencing using the information obtained from the colony PCR mapping reactions. Purify PCR-amplified DNA using standard techniques such as gel filtration if that is required.

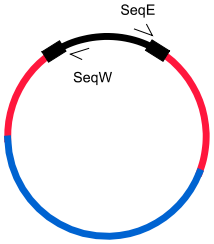


Important: Use the primer MuEnd for sequencing directly from PCR-amplified DNA. A linear PCR product amplified with MuEnd and a vector primer does not contain binding sites for the DNA sequencing primers SeqE and SeqW.

The recommended annealing temperature in a cycle sequencing reaction for the primer MuEnd is 60°C.

7.2 Sequencing from plasmid DNA

Prepare plasmid DNA from insertion clones using standard techniques.



Important: Use the primers SeqW or SeqE for sequencing from plasmid DNA. The primer MuEnd anneals on the terminal repeat sequences present at each end of the Entranceposon.

The recommended annealing temperature in a cycle sequencing reaction for the primers SeqW and SeqE is 50°C.

Important: The transposition reaction generates a 5 bp sequence duplication at the Entranceposon insertion site.

Appendix I: Descriptions of kit components

F-778 Entranceposon (Cam^R-3)
20 µl 20 ng/µl in TE, pH 8.0

F-779 Entranceposon (Kan^R-3)
20 µl 20 ng/µl in TE, pH 8.0

F-784 Entranceposon (Tet^R-3)
20 µl 25 ng/µl in TE, pH 8.0

Entranceposons are composed of inverted repeats of the bacteriophage Mu right end sequences flanking a selectable marker gene. The marker genes *cat, npt* and *tet* confer resistance to chloramphenicol, kanamycin and tetracycline, respectively.

F-750 MuA Transposase
20 µl 0.22 µg/µl in MuA Storage Buffer

A single purified polypeptide that catalyzes the chemical reactions of transposition. Isolated from an *E. coli* strain carrying the MuA gene.

F-752 5X Reaction Buffer for MuA Transposase
100 µl 5X conc.

F-753 Control Target DNA
10 µl 370 ng/µl in TE, pH 8.0

A 6.6 kb *Hin*dIII fragment of bacteriophage lambda DNA cloned into the *Hin*dIII site of pUC19.

Primers for insertion mapping

F-754 pUC Fwd Primer

400 µl 25 µM in H_2O
5′-AGCTGGCGAAAGGGGGATGTG-3′
Tm 73.5°C (0.5 µM)*

Binding site in pUC19 between the basepairs 307 and 327.

F-755 pUC Rev Primer

400 µl 25 µM in H_2O
5′-TTATGCTTCCGGCTCGTATGTTGTGT-3′
Tm 71.6°C (0.5 µM)

Binding site in pUC19 between the basepairs 535 and 510.

Note: The majority of commonly used cloning vectors are pUC-derivatives and most of them have preserved the binding sites for pUC Fwd and pUC Rev.

F-756 MuEnd Primer

800 µl 25 µM in H_2O
5′-GTTTTTCGTGCGCCGCTTCA-3′
Tm 72.5°C (0.5 µM)

Important: The binding site of the MuEnd primer is present at each end of Entranceposon. Therefore, MuEnd cannot be used for sequencing from a plasmid clone that contains an intact copy of Entranceposon.

Primers for DNA sequencing

F-780 SeqE Primer

250 µl 10 µM in H_2O
5′-CGACACACTCCAATCTTTCC-3′
Tm 59.1°C (0.1 µM)

F-781 SeqW Primer

250 µl 10 µM in H_2O
5′-GGTGGCTGGAGTTAGACATC-3′
Tm 58.1°C (0.1 µM)

* Tm calculations were done with Thermo Scientific Tm calculator available in www.thermoscientific.com/pcrwebtools).

Appendix II: Maps and sequences of entranceposons

Entranceposon (Cam^R-3), 1302 bp:

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                                                                                 PmeI     BamHI
1     TGAAGCGGCGCACGAAAAACGCGAAAGCGTTTCACGATAAATGCGAAAACGATGTCTAACTCCAGCCACCGTTTAAACGGATCCTTTTCGACCGAATAAA
              MuEnd                                             SeqW
101   TACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGA

201   TCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTGTCGAGATTTTCAGGAGCTAAGGAAGCTAAA
                        cat (CamR)
301   ATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATA
                 PvuI
401   ACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGAT

501   GAATGCTCATCCGGAATTACGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAA

601   ACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATT

701   TCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTT
                     NcoI     SspI
801   CGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCAT
                              ScaI
901   GTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAATTTTTTTAAGGCAGTTATTGGTGCCCTTAAACGCCTGGTT

1001  GCTACGCCTGAATAAGTGATAATAAGCGGATGAATGGCAGAAATTCGAAAGCAAATTCGACCCGGTCGTCGGTTCAGGGCAGGGTCGTTAAATAGCCGCT

1101  TATGTCTATTGCTGGTTTACCGGTTTATTGACTACCGGAAGCAGTGTGACCGTGTGCTTCTCAAATGCCTGAGGCCAGTTTGCTCAGGCTCTCCCCGTGG
                       BamHI   NotI         SeqE                                          MuEnd
1201  AGGTAATAATTGACGATAGGATCCGCGGCCGCCGACACACTCCAATCTTTCCGTTTTCGCATTTATCGTGAAACGCTTTCGCGTTTTTCGTGCGCCGCTT

1301  CA
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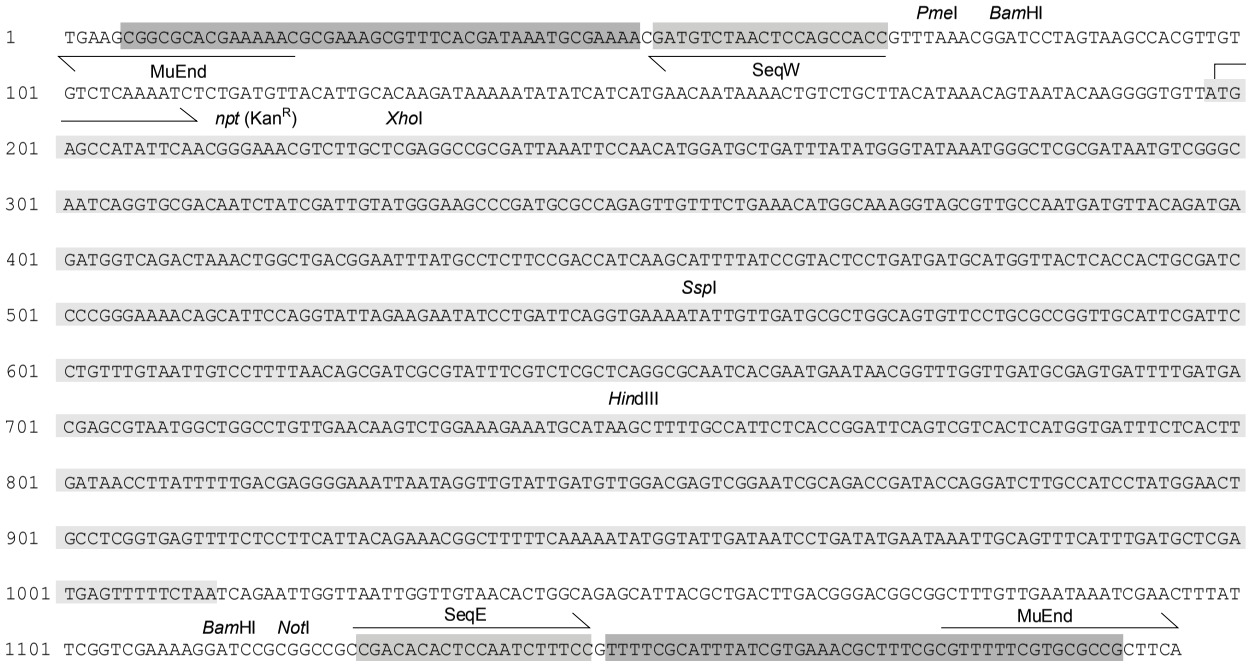
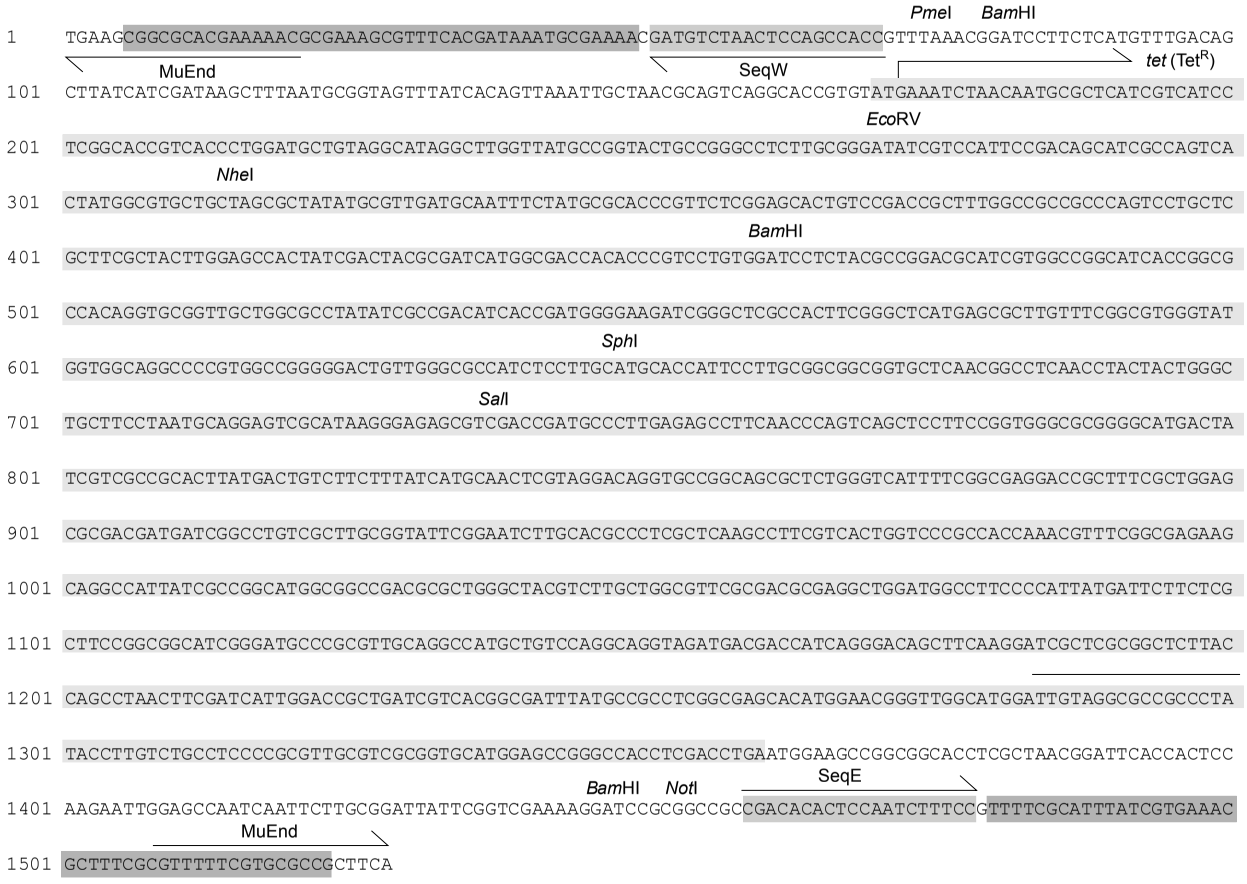


Entranceposon (Kan^R-3), 1195 bp:

Entranceposon (Tet^R-3), 1528 bp:



Appendix III: Media 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) **OR**	20 mg
Kanamycin (per liter) **OR**	20 mg
Tetracycline (per liter)	10 mg

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

SOC medium, per liter

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 $MgCl_2$	10 ml
1 M $MgSO_4$	10 ml
1 M Glucose	20 ml

Appendix IV: Related products

Stand-alone enzymes

F-750 MuA Transposase
F-750C MuA Transposase, 5X conc.

Ready-to-use Entranceposons

F-778 Entranceposon (Cam^R-3)
F-779 Entranceposon (Kan^R-3)
F-784 Entranceposon (Tet^R-3)
F-771 Entranceposon (*supF*)
F-774 Entranceposon (*lacZ*)

Plasmid Entranceposons

F-765 pEntranceposon (Cam^R)
F-766 pEntranceposon (Kan^R)
F-767 pEntranceposon (Tet^R)
F-773 pEntranceposon (*supF*)
F-701 Mutation Generation System MGS

PCR products

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

Appendix V: Mu Transposition references

Haapa, S., Suomalainen, S., Eerikäinen, S., Airaksinen, M., Paulin, L., and Savilahti, H. (1999a). 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. (1999b). 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.

Lamberg, A., Nieminen, S., Qiao, M. and Savilahti H. (2002). Efficient insertion mutagenesis strategy for bacterial genomes involving electroporation of in vitro-assembled DNA transposition complexes of bacteriophage mu. *Appl. Environ. Microbiol.* 68, 705-12.

Vilen, H., Eerikäinen, S., Tornberg, J., Airaksinen, M.S., Savilahti, H. (2001). Construction of gene-targeting vectors: a rapid Mu in vitro DNA transposition-based strategy generating null, potentially hypomorphic, and conditional alleles. *Transgenic Res.* 10, 69-80.

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.

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Technical Manual

v2_2012

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