Table of Contents

Strategy Outline

1. Purify your target DNA *i.e.* the plasmid, cosmid, BAC or PAC clone that you wish to sequence.

   Note: The TGS 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.finnzymes.fi.

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 prior to 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.

TGS II Kit Components

The kit contains sufficient materials for 20 reactions.
See Appendix I (page 11) for more detailed information.

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	

See Appendix II (page 13) or www.finnzymes.fi for detailed maps and sequences.

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	

Store the components at -20°C.

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 or heat blocks, 30°C and 75°C

Reagents and equipment for PCR mapping

SOC medium and LB agar plates with antibiotics
See Appendix III (page 16) for the media recipes

Transposition Reaction

1. Set up the following reaction:

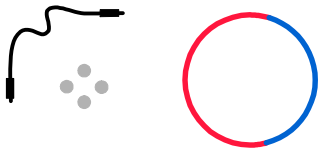
Important: MuA Transposase should be added last.

Reagent	Volume (µl)
Target DNA (see “User Supplied Materials”)	1-14
Deionized water	*ad* 14
5X Reaction Buffer for MuA Transposase	4
Entranceposon (Cam^R-3) OR (Kan^R-3) OR (Tet^R-3)	1
MuA Transposase	1
	20

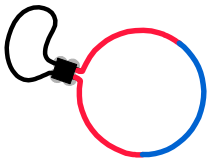
For the control reaction, use 1 µl (370 ng) of Control Target DNA supplied with the kit.

2. Mix the reagents.

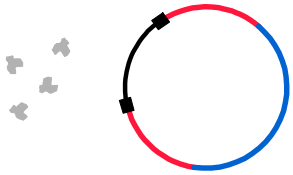
   Do not vortex.



3. Incubate 1 hour at 30°C.



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



5. Transform.

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 additionally with the antibiotic that selects your target plasmid.

Incubate the plates overnight at 37°C.

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.

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 (µl)**
Deionized water		14.8
10X Optimized DyNAzyme™ EXT buffer	1X	2
dNTPs (10 mM each)	200 µM each	0.4
pUC Fwd OR pUC Rev Primer (25 µM)	0.5 µM	0.4
Mu End Primer (25 µM)	0.5 µM	0.4
DyNAzyme™ EXT DNA Polymerase (1U/µl)	0.05 U/µl	1
		19

Note: The reaction conditions above have been optimized for the DyNAzyme™ EXT 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. For templates longer than 10 kb, modify the reaction conditions according to the DyNAzyme™ EXT Guidelines.

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 1	95°C	1 min 30 s
Step 2	95°C	30 s
Step 3	68°C	15 s
Step 4	72°C	1 min/1.3 kb of insert DNA
Repeat the steps 2-4 30 times		

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, page 13).

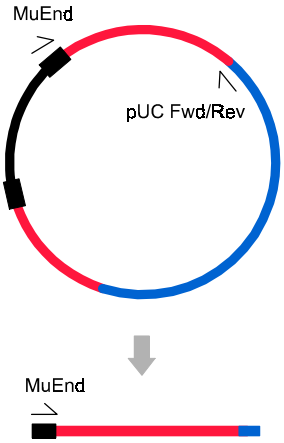
DNA Sequencing

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.

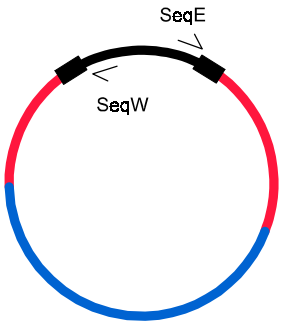


Sequencing from plasmid DNA:

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

HEPES, pH 7.6	25 mM
EDTA	0.1 mM
DTT	2 mM
KCl	300 mM
Glycerol	50% (v/v)

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.
Tris-HCl, pH 8.0	125 mM
$MgCl_2$	50 mM
NaCl	550 mM
Triton X-100	0.25%
Glycerol	50% (v/v)

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 dd water
5'-AGCTGGCGAAAGGGGGATGTG-3'
T_m 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 dd water
5'-TTATGCTTCCGGCTCGTATGTTGTGT-3'
T_m 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 dd water
5'-GTTTTTCGTGCGCCGCTTCA-3'
T_m 72.5°C (0.5 µM)

Important: The binding site of the MuEnd primer is present at each end of Entranceposon. Therefore MuEnd can not 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 dd water
5'-CGACACACTCCAATCTTTCC-3'
T_m 59.1°C (0.1 µM)

F-781 SeqW Primer

250 µl 10 µM in dd water
5'-GGTGGCTGGAGTTAGACATC-3'
T_m 58.1°C (0.1 µM)

* T_m calculations were done essentially as described by Breslauer *et al*. (*PNAS* 83, 3746-50) using the Oligonucleotide T_m determination program at the Virtual Genome Center (http://alces.med.umn.edu/rawtm.html).

Appendix II: Maps and Sequences of Entranceposons

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

*Pme*I *Bam*HI

1 TGAAGCGGCGCACGAAAAACGCGAAAGCGTTTCACGATAAATGCGAAAACGATGTCTAACTCCAGCCACCGTTTAAACGGATCCTTTTCGACCGAATAAA

MuEnd SeqW

101 TACCTGTGACGGAAGATCACTTCGCAGAATAAATAAATCCTGGTGTCCCTGTTGATACCGGGAAGCCCTGGGCCAACTTTTGGCGAAAATGAGACGTTGA

201 TCGGCACGTAAGAGGTTCCAACTTTCACCATAATGAAATAAGATCACTACCGGGCGTATTTTTTGAGTTGTCGAGATTTTCAGGAGCTAAGGAAGCTAAA

cat (Cam^R)

301 ATGGAGAAAAAAATCACTGGATATACCACCGTTGATATATCCCAATGGCATCGTAAAGAACATTTTGAGGCATTTCAGTCAGTTGCTCAATGTACCTATA

*Pvu*I

401 ACCAGACCGTTCAGCTGGATATTACGGCCTTTTTAAAGACCGTAAAGAAAAATAAGCACAAGTTTTATCCGGCCTTTATTCACATTCTTGCCCGCCTGAT

501 GAATGCTCATCCGGAATTACGTATGGCAATGAAAGACGGTGAGCTGGTGATATGGGATAGTGTTCACCCTTGTTACACCGTTTTCCATGAGCAAACTGAA

601 ACGTTTTCATCGCTCTGGAGTGAATACCACGACGATTTCCGGCAGTTTCTACACATATATTCGCAAGATGTGGCGTGTTACGGTGAAAACCTGGCCTATT

701 TCCCTAAAGGGTTTATTGAGAATATGTTTTTCGTCTCAGCCAATCCCTGGGTGAGTTTCACCAGTTTTGATTTAAACGTGGCCAATATGGACAACTTCTT

*Nco*I *Ssp*I

801 CGCCCCCGTTTTCACCATGGGCAAATATTATACGCAAGGCGACAAGGTGCTGATGCCGCTGGCGATTCAGGTTCATCATGCCGTTTGTGATGGCTTCCAT

*Sca*I

901 GTCGGCAGAATGCTTAATGAATTACAACAGTACTGCGATGAGTGGCAGGGCGGGGCGTAATTTTTTTAAGGCAGTTATTGGTGCCCTTAAACGCCTGGTT

1001 GCTACGCCTGAATAAGTGATAATAAGCGGATGAATGGCAGAAATTCGAAAGCAAATTCGACCCGGTCGTCGGTTCAGGGCAGGGTCGTTAAATAGCCGCT

1101 TATGTCTATTGCTGGTTTACCGGTTTATTGACTACCGGAAGCAGTGTGACCGTGTGCTTCTCAAATGCCTGAGGCCAGTTTGCTCAGGCTCTCCCCGTGG

*Bam*HI *Not*I SeqE MuEnd

1201 AGGTAATAATTGACGATAGGATCCGCGGCCGCCGACACACTCCAATCTTTCCGTTTTCGCATTTATCGTGAAACGCTTTCGCGTTTTTCGTGCGCCGCTT

1301 CA

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

```
                                                                                                   PmeI     BamHI
1    TGAAGCGGCGCACGAAAAACGCGAAAGCGTTTCACGATAAATGCGAAAACGATGTCTAACTCCAGCCACCGTTTAAACGGATCCTAGTAAGCCACGTTGT
            MuEnd                                                     SeqW
101  GTCTCAAAATCTCTGATGTTACATTGCACAAGATAAAAATATATCATCATGAACAATAAAACTGTCTGCTTACATAAACAGTAATACAAGGGGTGTTATG
                     npt (KanR)            XhoI
201  AGCCATATTCAACGGGAAACGTCTTGCTCGAGGCCGCGATTAAATTCCAACATGGATGCTGATTTATATGGGTATAAATGGGCTCGCGATAATGTCGGGC

301  AATCAGGTGCGACAATCTATCGATTGTATGGGAAGCCCGATGCGCCAGAGTTGTTTCTGAAACATGGCAAAGGTAGCGTTGCCAATGATGTTACAGATGA

401  GATGGTCAGACTAAACTGGCTGACGGAATTTATGCCTCTTCCGACCATCAAGCATTTTATCCGTACTCCTGATGATGCATGGTTACTCACCACTGCGATC
                                                                      SspI
501  CCCGGGAAAACAGCATTCCAGGTATTAGAAGAATATCCTGATTCAGGTGAAAATATTGTTGATGCGCTGGCAGTGTTCCTGCGCCGGTTGCATTCGATTC

601  CTGTTTGTAATTGTCCTTTTAACAGCGATCGCGTATTTCGTCTCGCTCAGGCGCAATCACGAATGAATAACGGTTTGGTTGATGCGAGTGATTTTGATGA
                                                             HindIII
701  CGAGCGTAATGGCTGGCCTGTTGAACAAGTCTGGAAAGAAATGCATAAGCTTTTGCCATTCTCACCGGATTCAGTCGTCACTCATGGTGATTTCTCACTT

801  GATAACCTTATTTTTGACGAGGGGAAATTAATAGGTTGTATTGATGTTGGACGAGTCGGAATCGCAGACCGATACCAGGATCTTGCCATCCTATGGAACT

901  GCCTCGGTGAGTTTTCTCCTTCATTACAGAAACGGCTTTTTCAAAAATATGGTATTGATAATCCTGATATGAATAAATTGCAGTTTCATTTGATGCTCGA

1001 TGAGTTTTTCTAATCAGAATTGGTTAATTGGTTGTAACACTGGCAGAGCATTACGCTGACTTGACGGGACGGCGGCTTTGTTGAATAAATCGAACTTTAT
                                     SeqE                                                   MuEnd
                 BamHI    NotI
1101 TCGGTCGAAAAGGATCCGCGGCCGCCGACACACTCCAATCTTTCCGTTTTCGCATTTATCGTGAAACGCTTTCGCGTTTTTCGTGCGCCGCTTCA
```

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 NaOH
Autoclave

Cool to 55°C and add :

Chloramphenicol (per liter)	20 mg

OR

Kanamycin (per liter)	20 mg

OR

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 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-750L	MuA Transposase, large
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

DyNAzyme™ EXT DNA Polymerase products:

F-505S	200 U
F-505L	1000 U
F-552XS	Kit for 25-50 reactions
F-552S	Kit for 100-200 reactions
F-552L	Kit for 500-1000 reactions

More information about the Finnzymes products available at:
www.finnzymes.fi.

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.

Appendix VI: Warranty

Finnzymes Oy warrants that its products will meet the specifications stated on the technical data section of the data sheets, and Finnzymes Oy agrees to replace the products free of charge if the products do not conform to the specifications. Notice for replacement must be given within 60 days of receipt. In consideration of the above commitments by Finnzymes Oy, the buyer agrees to and accepts the following conditions:

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3. That the buyer's sole remedy shall be to obtain replacement of the product free of charge from Finnzymes Oy; and
4. That this remedy is in lieu of all other remedies or claims for damages, consequential or otherwise, which the buyer may have against Finnzymes Oy.

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Prices are subject to change without notice.

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