

Transposon Tools

For sequencing and
mutagenesis



More hits
Less effort

Transposon Tools

Accelerating DNA sequencing and protein analysis

Introduction

The *in vitro* transposition technology enables the creation of full-coverage insertion libraries for various applications, offering greater flexibility for DNA and protein analysis. Finnzymes' **Template Generation System™ II (TGS II kit)** allows you to generate DNA sequencing templates faster and more efficiently than conventional methods. **The Stop Generation System™ (STOP)** and **Mutation Generation System™ (MGS)** kits allow you to generate large libraries of mutated proteins in a single reaction with less hands-on time than any other method.

Finnzymes' transposon tools are based on the transposition machinery of the bacteriophage Mu. During the lytic phase of the phage's life cycle the machinery replicates its genome by transposing repeatedly inside the host genome. The Mu transposition reaction has been modified into an *in vitro* reaction catalyzed by a single enzyme - **MuA Transposase**. In this system, one *in vitro* reaction is capable of generating more than a million transposon insertion clones.

The other essential component of Finnzymes' transposon tools is an artificial DNA transposon called an **Entranceposon™**. The Entranceposons are available as ready-to-use linear transposons or replicable plasmids. The plasmid Entranceposons allow the user to create transposons containing additional sequences of interest, e.g. marker genes. These custom transposons can then easily be inserted into the target DNA by taking advantage of the efficient Mu transposition reaction.

ADVANTAGES

- **Efficient** - Create saturated insertion libraries for sequencing and protein analysis in a single reaction.
- **Fast** - Decrease hands-on time compared to conventional methods.
- **Random** - Eliminate target site preference or insertion hot-spots.
- **Versatile** - Choose from a wide range of transposons for various applications.

In vitro transposition reaction

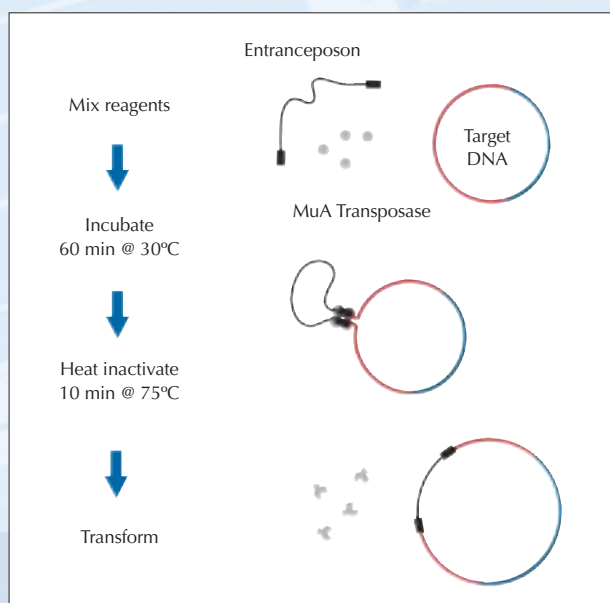


Figure 1. During a short 60 minute incubation, MuA Transposase binds to the Entranceposon and rapidly assembles into an active transposition complex. The complex attacks circular, as well as linear, target DNA and covalently joins the Entranceposon into the target DNA at random locations. The location of the transposon insertion in each individual clone can be mapped either by PCR* or by sequencing.

*The PCR process is covered by patents owned by Hoffmann-La Roche.

Template Generation System™ II (TGS™ II kit)

The fastest method available for preparing templates for DNA sequencing - Get the complete sequence in just three days!*

Has your DNA of interest ever been out of reach of your sequencing primers? Have you struggled with methods like the nested deletion, primer walking or piece-by-piece subcloning?

The Template Generation System™ II enables the creation of sequencing templates faster than any other method. The complete sequence of any DNA clone can be obtained in three days regardless of the template length. With the transposon based method there is no need to deal with primer walking nor order any template specific primers. You can sequence the entire DNA template using the universal sequencing primers included in the kit thus reducing the cost and time required for your sequencing project. Since the TGS II system is random, transposons also insert well into GC-rich regions of the target DNA. This facilitates and accelerates the sequencing of problematic DNA structures often experienced in sequence finishing. Large BAC clones can also be used as targets for the TGS II system thus avoiding the need for lengthy subcloning.

The TGS II kit provides a complete set of reagents required for creating convenient sequencing entrance points inside your template DNA for rapid and efficient sequencing. The TGS II kit includes three artificial transposons, each with a different antibiotic selectable marker containing sequencing and mapping primer binding sites. Additionally, the TGS II kit includes universal primers for bi-directional DNA sequencing and mapping of the insertion sites by PCR. Due to the high efficiency of the transposition reaction, a vast amount of insertion clones are obtained from a single reaction. The number of transposon clones to be sequenced will be proportional to the size of the target DNA. All individual clones can be sequenced at the same time using the universal primers included in the TGS II kit, allowing you to complete your sequence much faster than with conventional methods.

TGS II Entranceposon

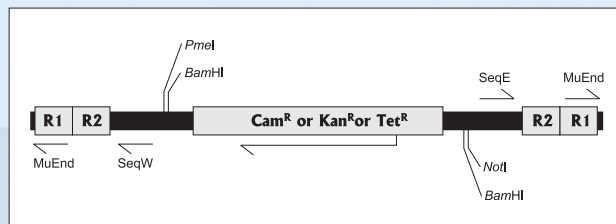


Figure 2. The linear TGS II Entranceposon has MuA transposase binding sites at either end and an antibiotic resistance marker for selection in the middle. SeqW and SeqE primer sites are included for DNA sequencing. The MuEnd sequence can be also used for PCR mapping.

*Detailed protocols available at Finnzymes web-site: www.finnzymes.fi

Sequencing from TGS II clones

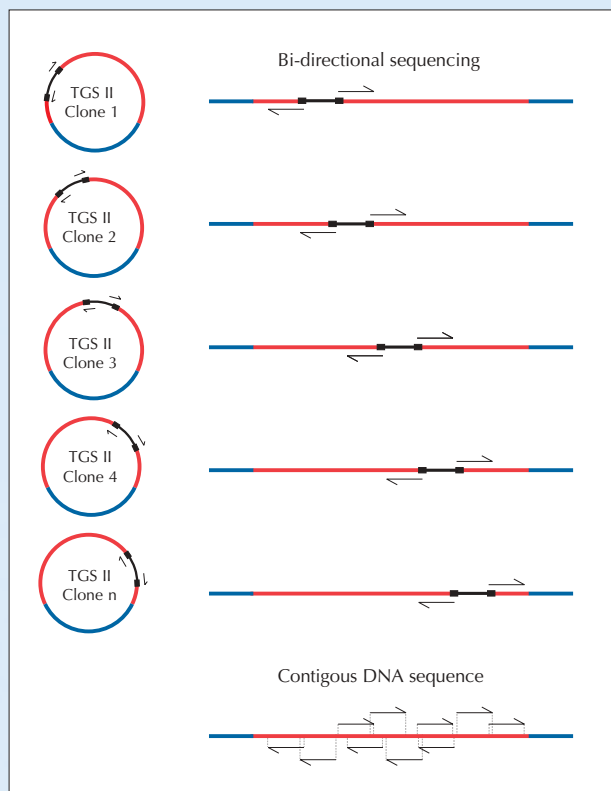


Figure 3. In the TGS II transposition reaction, Entranceposon insertions are positioned throughout the target plasmid in a random manner. Under optimized reaction conditions, MuA Transposase faithfully inserts only one Entranceposon per one target DNA clone. This efficient and random insertion reaction ensures complete sequence coverage of even a large insert.

Ordering information

F-702

Template Generation System™ II,
TGS™ II kit, 20 reactions

Tools for constructing custom-transposons for your specific applications

MuA Transposase	
F-750	MuA Transposase, 1X conc., 20 µl
F-750L	MuA Transposase, 1X conc., 100 µl
F-750C	MuA Transposase, 5X conc., 20 µl
pEntranceposons	
F-765	pEntranceposon (Cam ^R)
F-766	pEntranceposon (Kan ^R)
F-767	pEntranceposon (Tet ^R)
F-773	pEntranceposon (<i>supF</i>)

Saturated libraries for functional analysis of proteins - STOP™ and MGS™ kits

Finnzymes has developed the transposon-based STOP™ and MGS™ kits for functional analysis of proteins. These new transposon tools enable the creation of saturated libraries of mutated proteins in a single reaction with less hands-on time than any other method. The location of the transposon insertion in each mutated clone can be mapped by either PCR or sequencing. **With STOP and MGS kits, thousands of mutated clones are ready for expression studies in just three days.***

Stop Generation System™ (STOP™ kit)

Truncated proteins for functional assays

The STOP kit Entranceposons contain translational stop codons in all three reading frames within the terminal portion of the transposon sequence. The proprietary modification of the Stop Generation System makes it possible to generate a saturated C-terminal deletion library from virtually any target protein with a maximum addition of three amino acids.

Truncated protein library

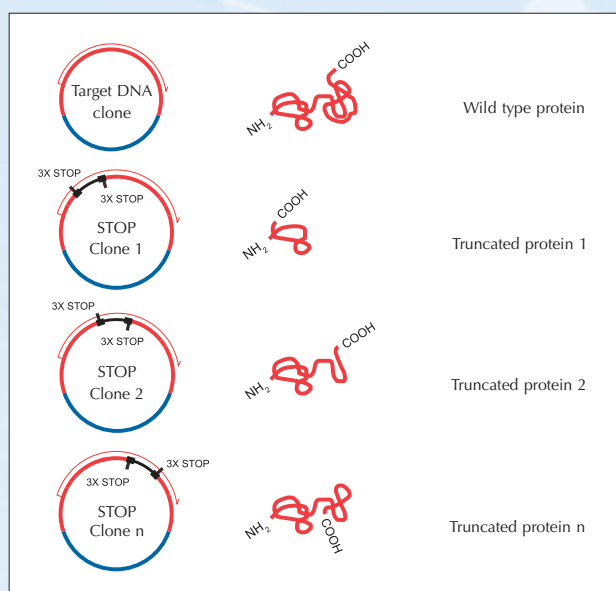


Figure 4. Protein translation from clones that contain STOP Entranceposons terminate at the site of the transposon insertion. As a result, various sizes of truncated target proteins are formed.

*Detailed protocols from Finnzymes web-site: www.finnzymes.fi

Template Generation System™, Mutation Generation System™, Stop Generation System™, TGS™, MGS™, STOP™ are trademarks of Finnzymes Oy.

Mutation Generation System™ (MGS™ kit)

Linker insertion mutants for functional assays

The MGS kit contains the complete set of reagents for transposon-based linker scanning mutagenesis of any target protein. The MGS Entranceposons are designed for making subtle changes in the structure of a target protein by inserting 15-bp in-frame linkers throughout the corresponding target gene. This in-frame insertion allows for conservation of downstream sequences.

Insertion mutation library

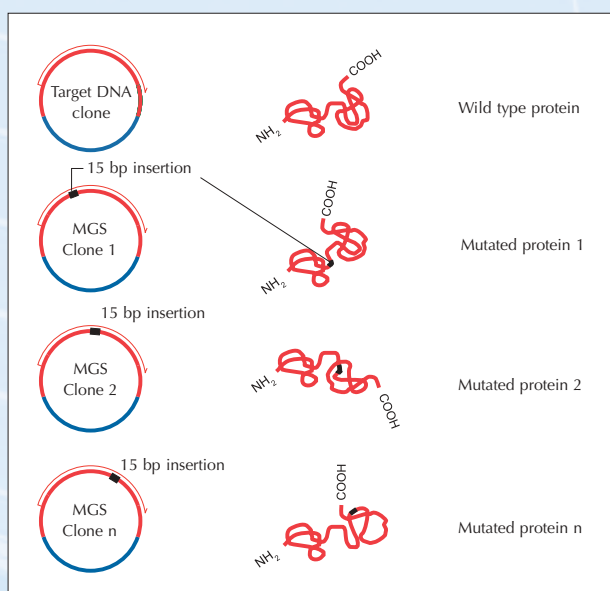


Figure 5. 15-bp linker sequence left at the site of the Entranceposon insertion site is translated into five extra amino acids altering the folding of the target protein. The subtle changes created in the protein structure with the MGS kit will help determine which regions of the polypeptide are necessary for activity and for protein interaction.

Ordering information

F-703	Stop Generation System™, STOP™ kit, 10 reactions
F-701	Mutation Generation System™, MGS™ kit, 10 reactions

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