



A Mu-Based Transposition System that Is At Least 50-Fold More Efficient than the Competition

The first *in vitro* transposition system was developed by Dr. Kiyoshi Mizuuchi using the well characterized temperate bacteriophage Mu.^{1,2} However, until now, commercially-available Mu-based systems have used a MuA transposase that has a transposition efficiency 50-100 times lower than EPICENTRE's Tn5-based EZ::TN™ Transposon Tools. High transposition efficiencies are critical for obtaining a sufficient number of transposon insertions to completely sequence a clone, especially those with large inserts, as well as other applications.

Now, EPICENTRE is pleased to introduce HyperMu™ Transposon Tools that use HyperMu™ Transposase, a hyperactive enzyme that retains the highly random insertion characteristics of MuA transposase³ but is at least 50-times more active *in vitro* than the enzyme available from other suppliers. Thus, the high quality and superior performance of EZ::TN Transposon Tools are now available in these new Mu-based Transposomics™ products.

HyperMu Transposon Tools are now available for strategies that simplify and speed up DNA sequencing and analysis of gene function. HyperMu Transposase, which recognizes the same R1 and R2 end sequences as MuA Transposase, is also sold separately so that these strategies can be used with EPICENTRE's HyperMu Transposons as well as other artificial Mu Transposons.

The *In Vitro* Insertion Strategy for Sequencing Cloned DNA

A primary application of the *In Vitro* Insertion Strategy and the HyperMu™ <KAN-1> Insertion Kit is for sequencing any clone that is too large to sequence with a single set of sequencing reactions. A simple, one-step reaction catalyzed by HyperMu Transposase randomly inserts the HyperMu <KAN-1> Transposon containing sequencing primer binding sites and a kanamycin-selectable marker into any DNA molecule *in vitro*. Then, transform *E. coli* and select on kanamycin plates (Figure 1). Up to millions of independent insertion clones are obtained, each of which can be sequenced bidirectionally using only the sequencing primers provided in the kit that anneal to each end of the HyperMu Transposon.

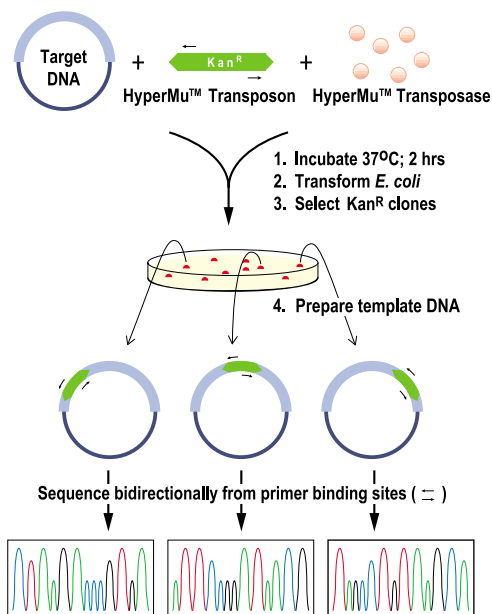


Figure 1. The process for complete sequencing of a target DNA using the HyperMu™ <KAN-1> Insertion Kit. Sequence even the largest BAC clone without the time and expense of subcloning or primer walking.

Consistently high transposition efficiencies are required for complete, overlapping sequencing of target DNA. A 150-kb BAC, for example, would require at least 700 insertion clones to approximate 100% coverage (assuming 1 kb of DNA sequenced per transposon; 500 bases in each direction). As shown in Table 1, only EPICENTRE's HyperMu <KAN-1> Insertion Kit produces enough templates to reliably sequence a large BAC clone. Similar efficiencies for both the 6-kb and 150-kb target DNAs were also obtained using Competitor A's artificial Mu transposon and EPICENTRE's HyperMu Transposase (data not shown).

Table 1. The HyperMu™ <KAN-1> Insertion Kit generates more transposon insertion clones per µg of target DNA than a leading competitor's kit.

Target DNA	EPICENTRE* (cfu/µg)	Competitor A* (cfu/µg)
6-kb plasmid (300 ng)	> 5X10 ⁶	< 10 ⁵
150-kb BAC (1µg)	> 10 ⁵	< 10 ²

*Transposition reactions (20µl) were performed using the manufacturer's protocol and electroporated into TransforMax™ EC100™ Electrocompetent *E. coli*.

The Transposome™ Strategy for Making Random Insertions into Living Cells

HyperMu Transposase and an artificial Mu transposon can also be used to make a HyperMu™ Transposome™ complex that can be electroporated into living cells to generate random transposon insertion clones *in vivo* (Figure 2).^{*} There is no need for cell conjugation, suicide vectors, or specific host factors, thus HyperMu Transposomes can be used to create insertion mutants (e.g., "gene knockouts") in species that have poorly described genetic systems or lack adequate molecular tools. Gene knockouts created with the ready-to-use HyperMu <R6K_γori/KAN>Tnp Transposome can be sequenced directly or "rescued" as a plasmid propagated from the R6K_γ origin of replication (see the center insert).

Although the increased efficiency of the HyperMu Transposase relative to MuA transposase enables practical use of HyperMu Transposome systems, researchers should consider using an EZ::TN™ Transposome™ system in lieu of or in addition to a HyperMu Transposome system. EZ::TN Transposomes typically generate 10-100 times more *in vivo* insertions using *E. coli* than a HyperMu Transposome.

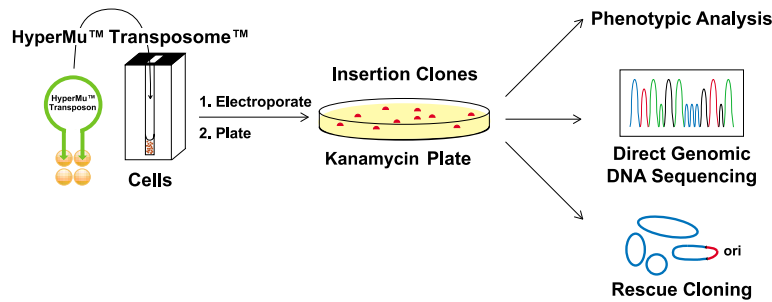


Figure 2. The HyperMu™ <R6K γ ori/KAN-1>Tnp Transposome™ can be electroporated into living cells where it randomly inserts the transposon component into the host's genomic DNA. The HyperMu Transposon insertion site can be analyzed by a variety of methods.

Strategies That Use Both EZ::TN and HyperMu Transposon Systems

Since EZ::TN and HyperMu Transposases do not recognize the same end sequences for transposition, they can also be used in strategies in which it is desirable to use more than one transposon system. For example, EPICENTRE scien-

tists have "rescued" plasmids and other episomes from heterologous bacterial systems that are not capable of replicating in *E. coli* by inserting into them an *E. coli ori*-containing EZ::TN Transposon. Then, the plasmid or episome can be completely sequenced following *in vitro* insertion of a HyperMu Transposon.

References

1. Mizuuchi, K. (1983) *Cell* **35**, 785.
2. Chaconas, G. and Harshey, R.M. (2002) In: *Mobile DNA II*, Ed. by N.L. Craig *et al.*, ASM Press, Washington, D.C., Chapter 17, pp. 385-402.
3. Butterfield, Y.S.N. *et al.* (2002) *Nucleic Acids Research* **30**, 2460.
4. Savilahti, H. *et al.* (1995) *EMBO J.* **14**, 4893.

www.epicentre.com/transposomics.asp

HyperMu™ <KAN-1> Insertion Kit

HMI032K 10 Reactions

HyperMu™ <R6K γ ori/KAN-1>Tnp Transposome™ Kit

MTS32RK 10 Reactions

HyperMu™ Transposase

THM03210 10 U

* The use of Transposome™ complexes for *in vivo* insertion of a transposon, including, but not limited to HyperMu™ and EZ::TN™ Transposome™ complexes, is covered by U.S. Patent No. 6,159,736 and related patent applications, exclusively licensed to EPICENTRE.



Phage T1-Resistant Electrocompetent *E. coli* with a Transformation Efficiency of >1 X 10¹⁰

Phage T1-Resistant TransforMax™ EC100™-T1^R Electrocompetent *E. coli*

EPICENTRE's new Phage T1-Resistant TransforMax™ EC100™-T1^R Electrocompetent *E. coli* provide complete security against loss of valuable clones and libraries by accidental phage T1 contamination of the lab. And, with a transformation efficiency of >1 X 10¹⁰ the cells are ideal for the most demanding cloning applications including:

- Genomic library construction
- cDNA library construction
- Cloning rare or limiting DNA
- Shotgun library construction

Once introduced into the lab environment, bacteriophage T1 rapidly lyses *E. coli* strains that are commonly used in cloning applications. The result can be significant lab downtime and the loss of valuable clones and entire libraries. Bacteriophage T1 is particularly difficult to eliminate from the lab and can lay dormant for many years. The *tonA* genotype protects the Phage T1-Resistant TransforMax EC100-T1^R cells, and your clones, from attack by phage T1 (and phage T5).

Phage T1-Resistant TransforMax EC100-T1^R Chemically Competent *E. coli* are also available.

Important Benefits (compare to DH10B*)

- Greater than 10¹⁰ cfu/μg DNA.
- Resistant to bacteriophages T1 and T5 (*tonA*).
- Readily accepts large DNAs for construction of large-insert genomic libraries.
- Restriction minus (*mcrA*, Δ(*mrr-hsdRMS-mcrBC*)) enables efficient cloning of methylated DNA for more complete genomic libraries.
- Endonuclease minus (*endA1*) to ensure high yields of DNA.
- Restriction minus (*recA1*) for greater stability of large cloned inserts.
- *lacZ*ΔM15 for blue/white screening of recombinants.

Genotype

F⁻ *mcrA* Δ(*mrr-hsdRMS-mcrBC*)
 φ80*dlacZ*ΔM15 Δ*lacX74 recA1 endA1*
araD139 Δ(*ara, leu*)7697 *galU galk* λ⁻
rpsL nupG tonA

www.epicentre.com/ec100t1r.asp

Phage T1-Resistant TransforMax™ EC100™-T1^R Electrocompetent *E. coli*

ECO205T1 5 X 100 μl

ECO210T1 10 X 100 μl

Transformation efficiency >1 X 10¹⁰ cfu/μg.
 Includes pUC19 control DNA.

Phage T1-Resistant TransforMax™ EC100™-T1^R Chemically Competent *E. coli*

CCT10210 10 X 50 μl

Transformation efficiency >5 X 10⁷ cfu/μg.
 Includes pUC19 control DNA.

* DH10B is a trademark of Invitrogen Corporation.