



Transposon-Based Method to Create and Express N-Terminal and C-Terminal Protein Deletions

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Introduction

Characterizing a protein, whether to understand the relationship between structure and function, map a domain or epitope, or ultimately create a chimeric enzyme, often requires a set of nested deletion mutants. Creating deletions using pre-existing restriction enzyme sites limits the depth of such a collection. Timed nuclease digestion methods are tedious and require careful monitoring at each reaction step.

EPICENTRE offers two new transposon-based kits, the EZ-Tn5™ Protein Truncation Kit and the Mu-End™ Protein Truncation Kit, as simple and reliable alternatives. Used consecutively, these kits can generate a library of unidirectional deletions from the N-terminal and C-terminal ends of any protein coding sequence. The transposon-derived deletions are propagated in *E. coli* as kanamycin-resistant “rescue” clones that can be expressed from a T7 promoter.

Methods and Results

Creating transposon insertions

The EZ-Tn5 Protein Truncation Kit features the EZ-Tn5™ <p15Aori / KAN-2 / T7Exp> Transposon, which contains the low-copy p15A origin of replication, a kanamycin resistance marker, and a T7-promoter region (Figure 1). First, a simple, *in vitro* reaction catalyzed by EZ-Tn5™ Transposase is used to randomly insert this EZ-Tn5 Transposon into any target DNA. In this work and in a similar study,¹ the *E. coli* DNA polymerase I gene (*polA*), which contains three well-characterized domains, was chosen as a model target. A *polA* PCR product was used in the 2-hour transposition reaction, but the target DNA can be almost any DNA containing the desired sequence (e.g., an existing plasmid, a fosmid or cosmid clone, or a restriction fragment).

PCR amplification and cloning

Next, the transposition reaction is amplified by PCR (Figure 2) using one primer to the transposon end and the other primer to a fixed point in the target sequence. In this example, the transposition reaction was amplified with the EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon primer, P15 FP-1, and the 3'-end *polA* primer, POL RP. Since the transposon is randomly inserted along

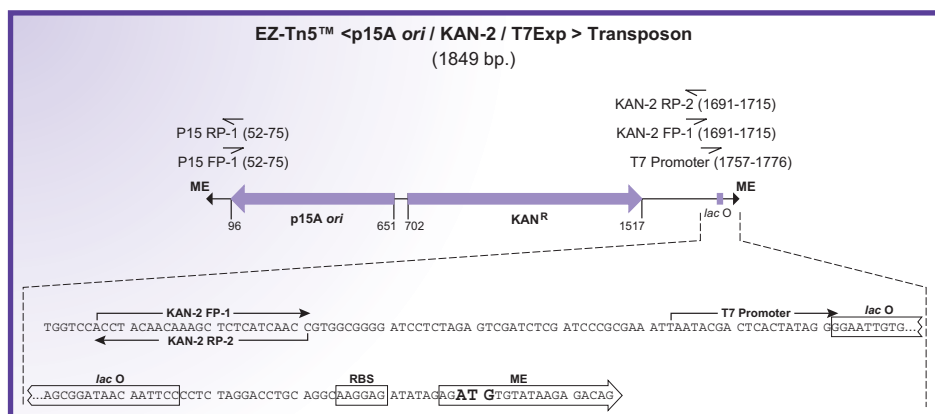


Figure 1. A schematic representation of the EZ-Tn5™ <p15Aori / KAN-2 / T7Exp> Transposon. ME refers to the 19-bp “mosaic end” sequences required for transposition.

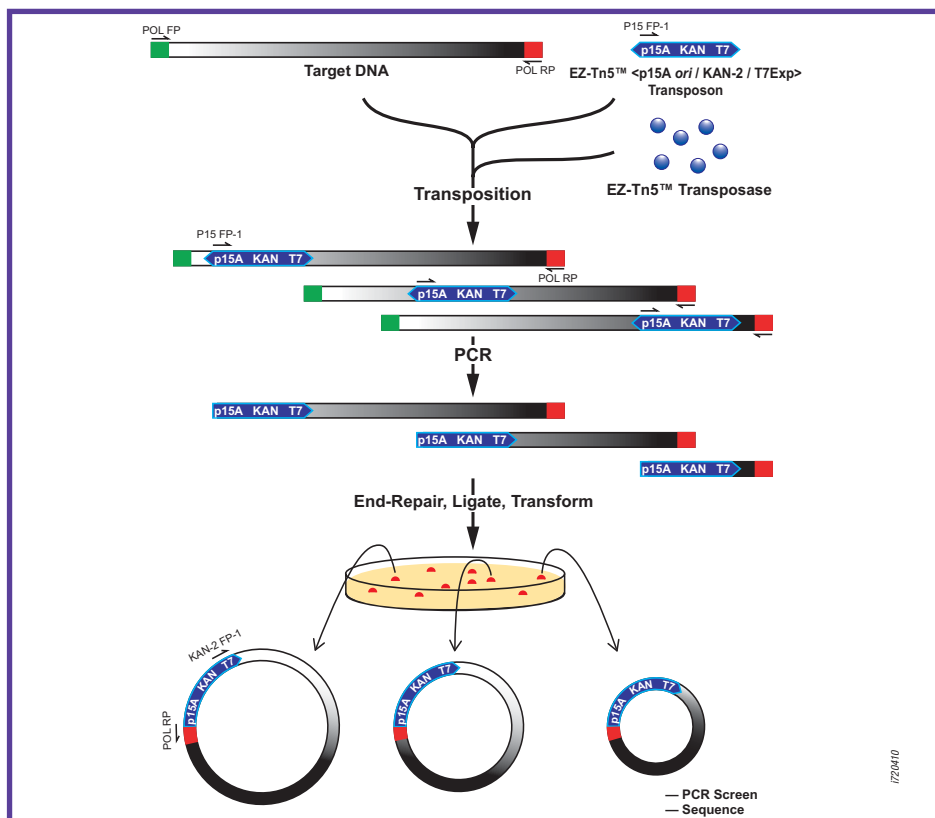


Figure 2. The EZ-Tn5™ Protein Truncation Kit can be used to create random, unidirectional deletions from the 5'-end (shown here) or 3'-end of a target sequence.

the length of the *polA* gene, amplification with this primer pair generates a library of N-terminal deletions. Likewise, amplification with the EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon primer, KAN-2 RP-2, and the 5'-end *polA* primer, POL FP, generates a library of C-terminal deletions (data not shown).

PCR products will contain a portion of the sequence of interest, the p15A origin of replication, and a kanamycin-resistance marker. Using the End-Repair Mix and Fast-Link™ DNA Ligase provided in the kit, PCR products are blunt-ended and self-ligated to create a library of “rescue” clones that can replicate after transformation into standard *E. coli* strains.

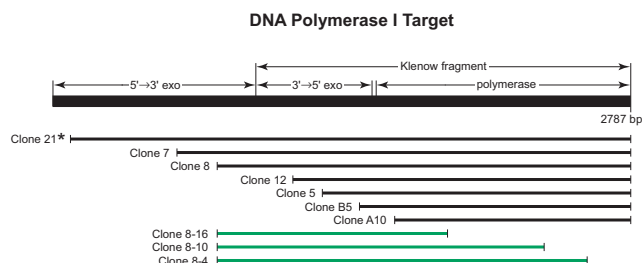


Figure 3. Unidirectional deletions in *polA* were generated using the EZ-Tn5™ Protein Truncation Kit (black lines) and the Mu-End™ Protein Truncation Kit (green lines). Asterisk indicates an insertion that is not in frame, and cannot be expressed using the T7 promoter.

Screening

Colonies can be easily screened, and the size of the target DNA deletion estimated, using the Colony Fast-Screen™ Kit (PCR Screen) and the appropriate primers. For example, the *polA* clones containing N-terminal deletions were screened with the EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon primer, KAN-2 FP-1, and the 3'-end *polA* primer, POL RP. PCR products in the size range of interest were then sequenced with the same terminal transposon primer, KAN-2 FP-1, to determine precisely where the EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon has inserted.

Expression of N-terminal deletions

Each rescue clone with an N-terminal deletion will also contain a transposon-derived T7-promoter region, which includes the lac operator (lacO), a ribosome-binding site, and an ATG start site. At least one-third of these clones will generate in-frame protein fusions that can be expressed in cells containing an inducible T7 RNA polymerase gene (e.g., *E. coli* BL21). The EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon fusion protein contains only 6 transposon-encoded amino acids.

Clone 8, for example, results in a 264-amino acid, N-terminal deletion that creates an in-frame fusion with *polA* (Figure 3). Expression in a BL21 host generates the expected 74-kD protein which is slightly larger than the Klenow fragment. An in-gel assay for DNA polymerase activity shows that the fusion is capable of DNA synthesis (Figure 4).² Clones 12 and 5 result in 387- and 435-amino acid deletions, respectively. They do not retain polymerase activity, even though both contain the entire polymerase domain (Figure 3). Presumably, more of the 3'→5' exonuclease domain is required for proper folding of the active polymerase.

Making C-terminal deletions

The Mu-End Protein Truncation Kit was developed to make unidirectional deletions from the carboxyl end of proteins generated with the EZ-Tn5 Protein

Truncation Kit. This kit features a 56-bp fragment that contains the R1 and R2 end sequence of a bacteriophage Mu transposon. An *in vitro* transposition reaction, catalyzed by EPICENTRE's HyperMu™ Transposase, randomly inserts the Mu-end into a selected clone.

PCR amplification of the resulting insertion reaction, using Mu-specific and EZ-Tn5 <p15Aori / KAN-2 / T7Exp> Transposon-specific primers, generates a library of deletions, which retain the transposon-derived origin, kanamycin marker, and T7 promoter region. These products can be "rescued" as plasmids following the end-repair and ligation steps described above. The Mu-specific primer used in the amplification contains stop codons in all three reading frames to avoid adding unwanted amino acids.

The Mu-End Protein Truncation Kit was used to create C-terminal deletions in

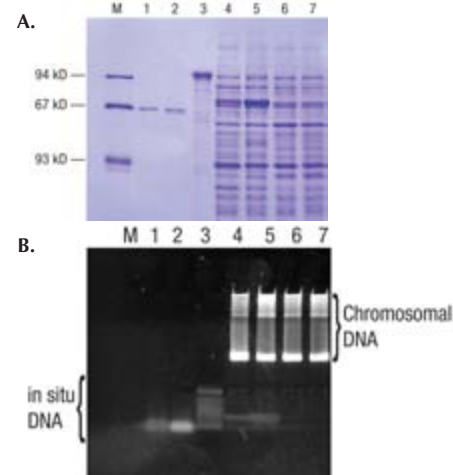


Figure 4. Analysis of *polA* deletion clones by SDS-PAGE and an in-gel DNA polymerase activity assay. A. SDS-PAGE analysis of *polA* truncations. Samples in lanes 1 to 3 are purified protein. Samples in lanes 4 to 7 are crude extracts. **Lane 1**, Klenow fragment; **Lane 2**, Klenow fragment with a point mutation in the 3'→5' exo domain; **Lane 3**, *E. coli* DNA polymerase I; **Lane 4**, Clone 8, uninduced; **Lane 5**, Clone 8, induced; **Lane 6**, Clone 8-10, uninduced; **Lane 7**, Clone 8-10, induced. **M**, MW marker. B. An SDS-PAGE gel containing a poly(dA-dT) substrate was run with the same samples as in A. After being subjected to renaturation conditions, the gel was incubated with dATP and dTTP, stained with SYBR® Gold, and bands with polymerase activity were visualized with UV light.

Clone 8. Clones were PCR screened with the Colony Fast-Screen Kit for deletions in the desired size range. Selected clones were sequenced with the transposon primer, P15 RP-1, to precisely define the Mu-end insertion site. Clone 8-10, for example, has a 139-amino acid deletion from the C-terminal end of Clone 8 (Figure 3). When the resulting 58-kD protein was expressed, DNA polymerase I activity was not detected in an in-gel assay (Figure 4).

Summary

The transposon-based EZ-Tn5 Protein Truncation Kit and the Mu-End Protein Truncation Kit provide convenient methods for generating libraries of N-terminal and/or C-terminal protein deletions that can be expressed in *E. coli*. Although not emphasized here, these kits are also useful for the functional characterization and sequencing of genes and regulatory elements, by allowing the generation of unidirectional libraries from either end of a target DNA.

References

1. Fandt, M. (2004) 30th Steenbock Sympos.; May 20-23; Madison, WI. Poster 15.
2. Spanos, A. and Hübscher, U. (1993) In: Hirs, C. Methods in Enzymology Vol. 91. San Diego; Academic Press. 263.

www.epicentre.com/truncation.asp

EZ-Tn5™ Protein Truncation Kit
EZI41110 10 Reactions
Contents:
EZ-Tn5™ Transposase
EZ-Tn5™ <p15Aori /KAN-2 /T7Exp> Transposon
EZ-Tn5™ 10X Reaction Buffer
EZ-Tn5™ 10X Stop Solution
Control Target DNA
Sterile Water
End-Repair Enzyme Mix
End-Repair 10X Buffer
ATP
dNTPs
Fast-Link™ DNA Ligase
10X Fast-Link™ Buffer
PCR Precipitation Solution
P15 RP-1
P15 FP-1
KAN-2 RP-2
KAN-2 FP-1

Mu-End™ Protein Truncation Kit
HMI41110 10 Reactions
Contents:
HyperMu™ Transposase
Mu-End™ Transposon
HyperMu™ 10X Reaction Buffer
HyperMu™ 10X Stop Solution
Sterile Water
End-Repair Enzyme Mix
End-Repair 10X Buffer
ATP
dNTPs
Fast-Link™ DNA Ligase
10X Fast-Link™ Buffer
PCR Precipitation Solution
MU-1 RP-1