Transposagen
Better Models. Better Results.™

Edit Any Gene In Any Species

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Targeting Efficiencies & Applications
Why XTN™ TALENs
Why Transposagen
Frequently Asked Questions

Overview

Transposagen's XTN™ site-specific nucleases, also referred to as TALENs, permit researchers to specifically cut any DNA sequence, allowing for the creation of gene knockouts, knock-ins and single base pair edits.

XTN™ TALENs empower you to edit, knockout or knock-in any sequence, with applications in cells, animals and plants.

Transposagen's licensed FLASH XTN™ TALEN production system improves efficiency and reduces timelines and cost. Site-specific genetic modification is now more affordable than ever.

Validated: Transposagen's XTN™ TALENs are sequence verified and supplied in pre-tested expression vectors for optimal efficiency.

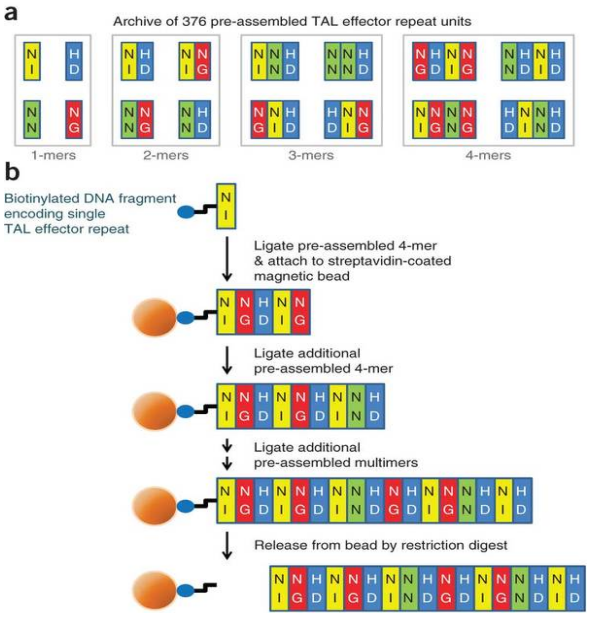
Precise and Reliable: XTN™ TALENs are guaranteed to bind and cut your target site or we will make you a new one for free.

Affordable and Flexible: Custom XTN™ TALENs are affordable and have absolutely no restrictions on use. Check our website or call you local sales rep for the latest pricing.

Speed: Fastest turnaround time in the industry.

Better Models, Better Results

Transposagen Biopharmaceuticals, Inc., is dedicated to providing better disease models through genetic modification. Transposagen specializes in custom and off the shelf XTN™ TAL Nucleases, animal models, cell lines, stem cells and cutting-edge research tools and technologies to improve drug discovery and development research.



Reyon et al. FLASH Assembly of TALENs for High Throughput Genome Editing. Nature Biotechnology. 30, 460-465 (2012).

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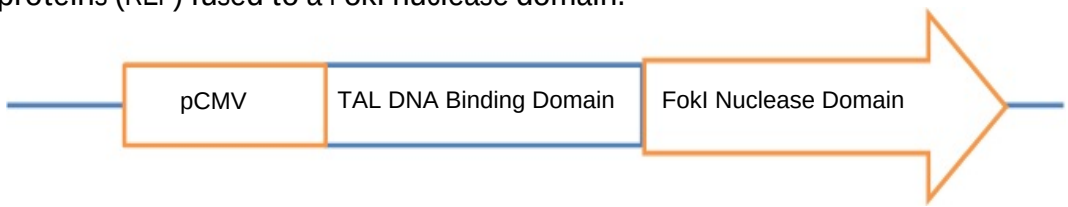
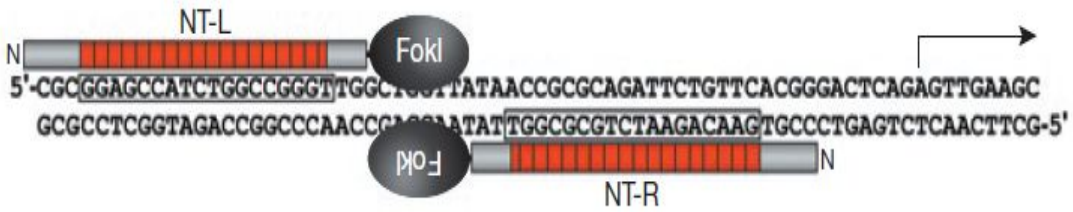
How XTN™ TALENs Work

An XTN™ (Xanthomonas TAL Nuclease), also known as a TALEN, is a hybrid protein, with the DNA binding domain from TALE proteins (REF) fused to a FokI nuclease domain.



XTN™ proteins work as a pair of heterodimers at their recognition site to induce dsDNA breaks. Subsequent NHEJ (Non-Homologous End Joining) repair can introduce deletions or insertions, which may result in a knockout mutation. Co-transfection of an XTN™ pair with a targeting vector can result in incorporation of the donor sequences through Homologous Recombination (HR) to repair cut site.
The piggyBac™ (PB) transposon system can be incorporated into HR vectors to facilitate Footprint-Free™ Genetic Engineering (Yusa et al. Nature 2011).



Introducing Transposagen's GENOME Discovery Database

XTN™ site-specific nucleases targeting each gene in the genome are now available from Transposagen. The XTN™ site-specific nucleases permit researchers to specifically cut any DNA sequence, allowing for the creation of gene knockouts, knock-ins and single base pair edits. Researchers can now search Transposagen's GENOME Discovery Database for the availability of a XTN™ targeting any gene in the human genome. Custom XTNTM pairs for other species are also available.

Targeting Efficiencies & Applications

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Plate__A11_NHEJ-R1.seq     TTGCTGCTGACCCTAGCCTGAGTTCTCTTTGCG-----------ACGG TCCCGTCTAGCATGGTTGAGCACA 61
Plate__E02_NHEJ-R1.seq     TTGCTGCTGACCCTAGCCTGAGTTCTCTTTGCG-----------ACGG TCCCGTCTAGCATGGTTGAGCACA 61
Plate__A04_NHEJ-R1.seq     TTGCTGCTGACCCTAGCCTGAGTTCTCTT-GCGCGGCCGCTTGCGACGG TCCCGTCTAGCATGGTTGAGCACA 72
Plate__H11_NHEJ-R1.seq     TTGCTGCTGACCCTAGCCTGAGTTCTCTT-GC-----------ACGG TCCCGTCTAGCATGGTTGAGCACA 59
Plate__E05_NHEJ-R1.seq     TTGCTGCTGACCCTAGCCTGAGTTCTCTT-GCGCGGCCGCTTGCGACGG TCCCGTCTAGCATGGTTGAGCACA 72
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Plate__E03_NHEJ-R1.seq     TTGCTGCTGACCCTAGCCTGAGTTCTCTTTGCG-----------ACGG TCCCGTCTAGCATGGTTGAGCACA 61
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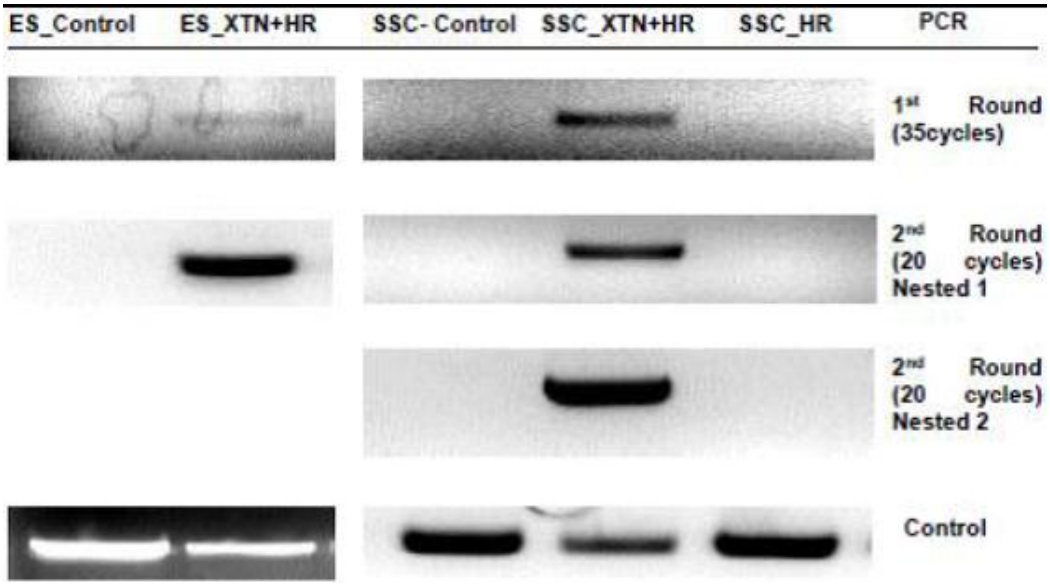
XTN™ site-specific nucleases demonstrate over 15% mutagenesis efficiency when targeting the Rag1 locus in stem cells. XTN™ mutagenesis efficiencies averaged 22.2% and was as high as 95% when disrupting human cancer genes in U2OS human cells. Reyon et al. Nature Biotech 2012.



XTNs™ Facilitate Enhanced Targeting in Stem Cells

A targeting vector (HR) with homology to the Rag1 locus near the XTN™ cut site was nucleofected into stem cells with and without XTNs™. PCR using primers designed to detect correct targeting of the locus indicates incorporation of the vector only when XTNs™ are present.

The XTN™ technology can be used in conjunction with Transposagen's other core technology, piggyBac™, as the only system in the world that allows for Footprint-FreeTM Genetic Engineering down to a single base pair (Yusa et al. Nature 2011).

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Why XTN™ TALENs, Why Transposagen

Why XTN™ TALENs vs. Zinc Finger Nucleases (ZFNs)

Each XTN™ DNA binding module binds a single specific DNA nucleotide. The XTN™ binding units are typically created in an array that is designed to bind 18 base pairs on each side of the target site.
Each ZFN DNA binding module binds 3 specific DNA nucleotides. ZFN binding units typically bind just 12 base pairs on each side of the target site. Some ZFN DNA binding modules do not to work well with each other. Therefore, XTNs™ can be designed to target nearly any site in any genome, whereas ZFNs are much more limited. The longer binding sites for XTN™ pairs should also confer increased specificity, with less off-site cutting, than ZFNs.

Why Transposagen vs. The Competition? Faster, Cheaper, Better

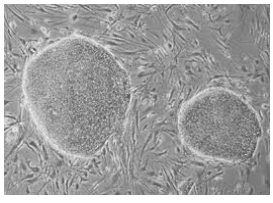
Transposagen's licensed "FLASH" XTNTM assembly system provides the fastest turn-around time in the industry.

Transposagen is also able to create the targeting vector for knock-in and gene editing studies.
The XTN™ technology can be used in conjunction with Transposagen's other core technology, piggyBac™, as the only system in the world that allows for Footprint-Free™ Genetic Engineering down to a single base pair.

Transposagen does not restrict the use of XTN™ TALENs, whereas competitors require additional licensing for projects such as animal model and cell line creation, as well as material transfer agreements for use with collaborators.

If the first custom XTN™ TALEN does not bind and cut the first target we will design an additional XTN™ targeting the same locus free of charge.

In addition, Transposagen offers custom cell line and animal model production. When a custom service order is received we always provide the XTN™ TALEN to the customer for in-house use as the first milestone for the project. Therefore, these services provide you with a free custom XTN™ site-specific nuclease, as well as the cell line or animal model.


Cell Lines
Stem Cells


Rodents


Zebrafish


Xenopus


Arabidopsis

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Frequently Asked Questions

Q. How do XTNs (TALENs) work to cut DNA?

A. Xanthamonas TALE Nucleases (XTNs or TALENs) consist of highly conserved 33-35 amino acid repeat sequences from TALEs (Transcription Activator-Like Effectors), which can be joined together into more extended arrays capable of recognizing novel target DNA sequences. Joining TALEs to a dimer-dependent ,non-specific nuclease enables double-stranded DNA cutting at specific sequences when both the forward and reverse XTNs bind to their target sequenceWhen cutting occurs, the DNA can be repaired by Non-Homologous End Joining (NHEJ), resulting in insertions or deletions of varying sizes, or by homology-directed repair using donor DNA.

Q. How do I know where to cut my DNA?

A. The answer depends on your particular application. However, a general design parameter is to BLAST your sequence to be sure it isn't repetitive. Also, if you plan to knockout a gene by cutting with an XTN, a common strategy is to cut in the first coding exon, usually 50-100 bases from the start codon. Please note that this strategy does not necessarily result in completely non-functional gene product(s) and you should do the proper bioinformatic analysis before ordering.

Q. Can I make RNA of the XTN from these plasmid backbones?

A. Yes. There is a T7 promoter for in vitro RNA synthesis using kits such as mMESSAGE mMACHINE T7 Ultra kit from Ambion/Invitrogen. The vector can be linearized using Bgl2, Stu1, or Avr2.

Q. Do the XTNs employ Fok1 nucleases that can homodimerize?

A. Our current XTNs use the WT Fok1 nuclease and it can form homodimers. However, the long recognition sequences employed (generally 16-18 bp per XTN) help minimize any concerns about off-site cutting.

Q. Is the DNA prep I receive suitable for immediate transfection of my cells?

A. Yes, it is endotoxin free and ready to use.

Q. If I need to make more DNA, are there any special instructions?

A. We recommend using recombination-deficient cells, STBL3 or the equivalent, to minimize the risk of recombination of the repetitive elements inherent in XTN–encoding DNA.

Q. Do you BLAST or otherwise analyze the requested cut sequence for specificity within the genome?

A. No. It is the responsibility of the end user to determine the suitability of the sequence for his/her own research purposes. We make no attempt to discern the intended use of the XTN you are ordering.

Q. How should I transfect my cells?

A. There is not one "correct way" to transfect cells. You should use the method shown to work for your cell type. Either lipofection or electroporation methods are suitable. In general you should transfect 3 ug of each XTN (6 ug total for the pair). You may experiment with more or less for your particular application.

Q. How are these XTNs verified?

A. We design each XTN pair to specifically cut the sequence we provide to you. We sequence verify that they are assembled correctly. We do not perform cleavage assays before shipping.

Q. What if my XTN doesn't cut?

A. There are many factors that can affect the cleavage efficiency of any nuclease, such as transfection efficiency into your cells and the chromatin structure and accessibility of the DNA sequence targeted. While we can be just about certain that any given XTN will cut its target on "naked" DNA, it is difficult to predict whether they will have access to their target in vivo. Some loci are simply not amenable to nuclease cleavage even though the XTN was properly constructed. However, we do offer a limited guarantee.. If, after repeated good faith attempts with proper transfection controls, you do not see any evidence of cleavage activity, we will make another XTN to a nearby site from the original sequence you provided to us in your order form free of charge. You will only be responsible to pay for shipping.