Footprint-Free™ Cell Line Engineering Services at Transposagen

Altering the genome of cells is a powerful and increasingly necessary tool for drug discovery and research. Transposagen's custom cell line engineering service can provide precise modification of any genomic locus in a wide variety cells, including stem cells and primary cell lines. Creating isogenic cell lines and disease models for testing pharmaceutical candidates are examples of the applications of this exciting new service.

Diverse Technology Portfolio and Expertise

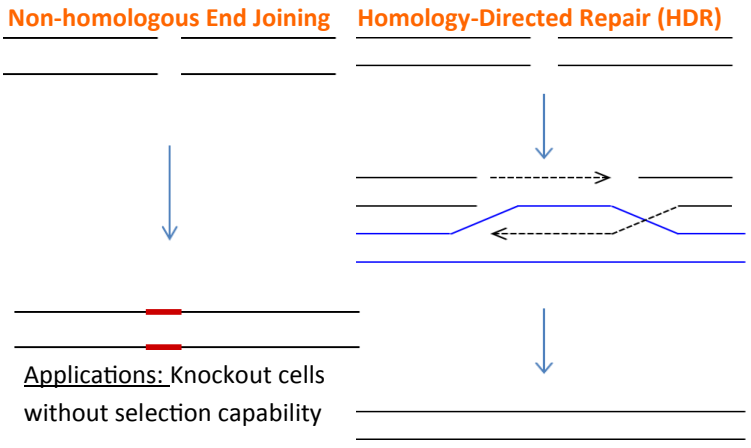
Transposagen utilizes its expertise and a diverse portfolio of gene editing tools including XTN™ TALENs, CRISPR nickases, as well as *piggyBac™* transposons, for stable cell line generation and Footprint-Free™ Gene Editing. The table below details the variety of projects available to customize a cell line to your specific research objectives, along with the gene editing technologies used for each.

Mutation type	Technology	Advantages
Knockout/Knock-in	XTN™ TALEN	Precision, efficiency & ease of design
Knockout/Knock-in	CRISPR paired nickase	Cost effective
Knockout/Knock-in with selection for rare events	XTN™ TALEN, piggyBac™	Footprint-Free™, precision & efficiency
Knockout/Knock-in with selection for rare events	CRISPR paired nickase, piggyBac™	Footprint-Free™ & cost effective
Gene editing (single nucleotide editing) with selection for rare events	XTN™ TALEN, piggyBac™	Footprint-Free™, precision & efficiency
Gene editing (single nucleotide editing) with selection for rare events	CRISPR nickase, piggyBac™	Footprint-Free™ & cost effective
Stable cell line (random integration for expression assay and protein production)	piggyBac™	Efficiency, non-viral, cargo capacity, multiplexed, identify integration sites

Transposagen's Unique Footprint-Free™ Gene Editing Methods Combine Site-Specificity and Clean Excision

Site-specific nucleases and paired nickases are designed to produce double-stranded DNA (dsDNA) breaks at a specific site in the genome. These dsDNA breaks drive non-homologous end joining (NHEJ), resulting in deletions and/or insertions for knockout cell line production. If combined with a homologous recombination (HR) donor vector, targeted knock-ins can be produced.

Targeted nucleases increase the efficiency of HR significantly, but in many cases efficiencies may not be high enough for clone isolation. The ability to select for desired mutations and rare events is made possible by the use of selection markers to render the genetically engineered cells identifiable (e.g., cells become drug resistant or express a fluorescent reporter). However, in many cases, removal of the selection marker is necessary to avoid altered endogenous gene expression and other unwanted effects.



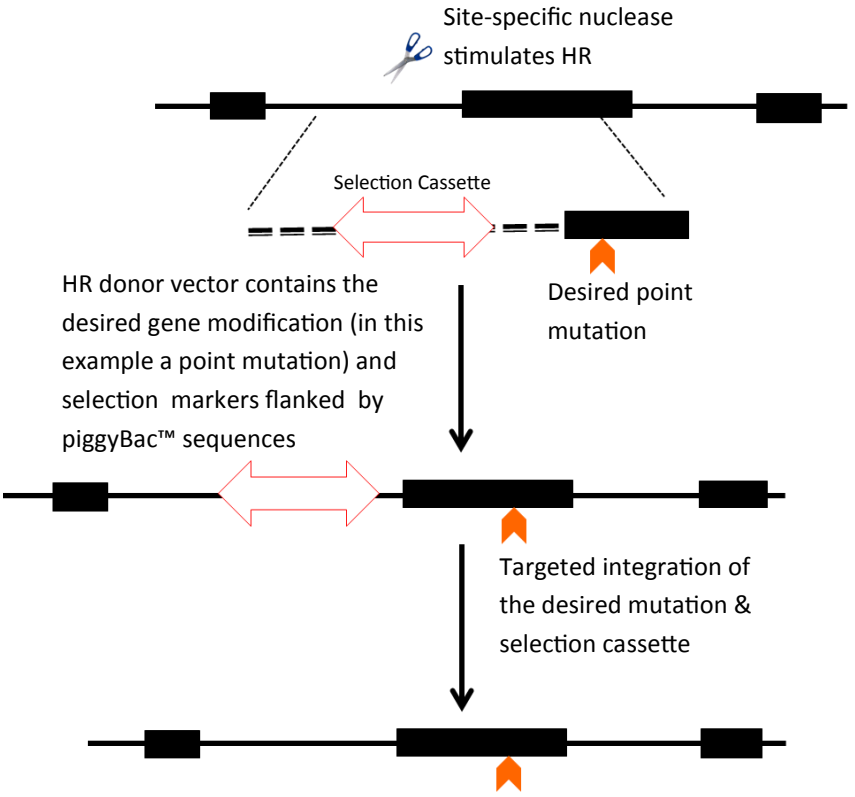
Applications: Knockout cells without selection capability

Applications: Knockout, Knock-in, gene edited cells with selection for rare event capability

There are three main systems for removing selectable markers in genetically engineered cells: Transposagen's exclusive Footprint-Free™ gene editing system with piggyBac™ for clean editing of as little as a single nucleotide, and the well-known CRE/lox and Flp/FRT systems, which leave behind 34 base pairs of unwanted sequence in the genome.

Footprint-Free™ Gene Editing with site-specific nucleases/nickases and the piggyBac™ DNA Modification System

Transposagen's Cell Engineering service uses the piggyBac™ transposon system - the only commercially available method capable of seamless excision of resistance or reporter genes. It restores the sequence of the DNA to its pre-insertion state, and is thus Footprint-Free™. Transposagen also uses an "excision only" piggyBac™ transposase that will excise the selectable marker but not reintegrate it into the genome for even higher efficiency and more precision.



CRE/Lox – slicing and dicing the genome leads to unwanted off-target effects and endogenous gene silencing and altered expression

Genome modification with the CRE/lox system often requires flanking a selectable marker with loxP sites ("floxed"). Following selection for targeted HR events, the selectable markers can be removed with the CRE recombinase. However, there are several disadvantages to this method: as noted previously, the excision is not footprint-free and leaves behind a single loxP site. In addition to potential interference with transcriptional regulatory elements brought about by these ectopic sequences, methylation can occur within the loxP site as well as in the adjacent DNA up to several kilobases, resulting in altered or silenced gene expression. Furthermore, the CRE recombinase can recognize "cryptic" loxP sites in the genome which results unintended off-target mutations and toxicity.

Example of Loxp methylation of endogenous genes

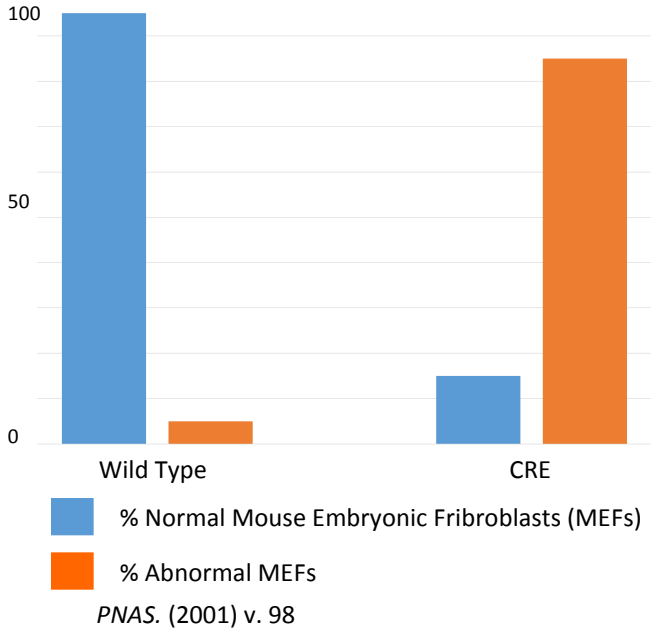
LoxP transgene UPPERCASE, Adjacent genomic DNA in lower case

GGTGGA**C**ATTAGGATGGGAAGGGTGGTTTAGttt**c**gaggaagtt**c**gg

tat**c**ggatttataactt**c**gtataatgtatgttata**c**gaagttatttagagt**c**gaga

Methylated cytosine sites underlined and result in altered gene expression and silencing (*EMBO Journal* (2002) v21)

CRE Expression Induces Chromosome Abnormalities



PNAS. (2001) v. 98

Transposagen's Cell Line Services

-Cell types: stem cells, iPS cells, primary cell lines, cancer cell line, HeLa, CHO, HEK293 and more

-Knockout, knock-in, gene editing, stable (random integration), AAVS1 safe harbor knock-in

Request More Information or a Quote