



**Transposagen**  
Better Models. Better Results.™

## **Multivector™**

User Manual

*Version 2.0*

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### 1.1 Multivector™ Overview

The Multivector™ is a flexible tool to enable generation of targeting vectors for both animal and human genome engineering projects. It contains *piggyBac*™ transposon elements, drug resistance genes, LoxP recombination sites, and unique restriction sites to accommodate a variety of research project requirements. The Multivector™ was designed primarily for making homologous recombination (HR) vectors for gene targeting or gene editing experiments. Considerations for the design of homology arms (length, genomic location, etc.) can vary greatly depending on the particular research application. One of the most compelling features of the Multivector™ is the ability to remove the selection cassette using the excision-only *piggyBac*™ transposase (PBx) without leaving behind any residual mutations in the genome once it has served its purpose. This Footprint-free™ gene editing feature, however, requires careful attention when designing the arms of homology described in the protocols.

### 1.2 Features of the Multivector™

The Multivector™ contains many unique sites and features that allow for its diversity in gene editing applications.

Feature	Use
PiggyBac (pB) Inverted Terminal Repeats (ITRs)	Footprint free removal of cassette/pBase insertion of cassette
LoxP sites	Removal of resistance cassette
PGK/EM7 promoter	Drive resistance gene expression in mammalian/bacterial cells
Puromycin resistance gene	Selection of clones
Neomycin resistance gene	Selection of clones
Truncated Thymidine Kinase gene (TK)	Selection for cassette removal using ganciclovir
Compatible sites (EcoR1/Mfe and Avr2/Nhe)	Easy removal/replacement of LoxP site(s)
Unique restriction sites	Cloning arms of homology; swap elements

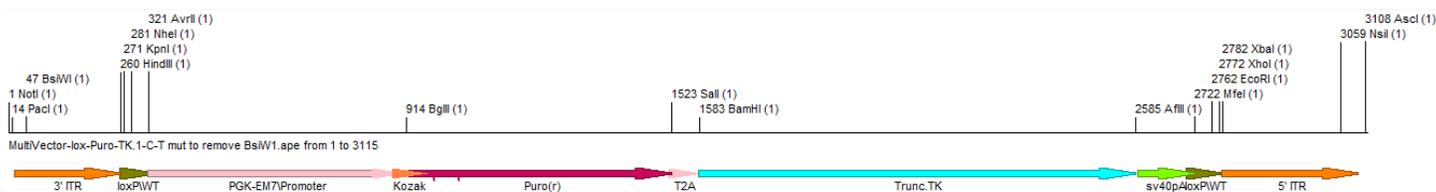
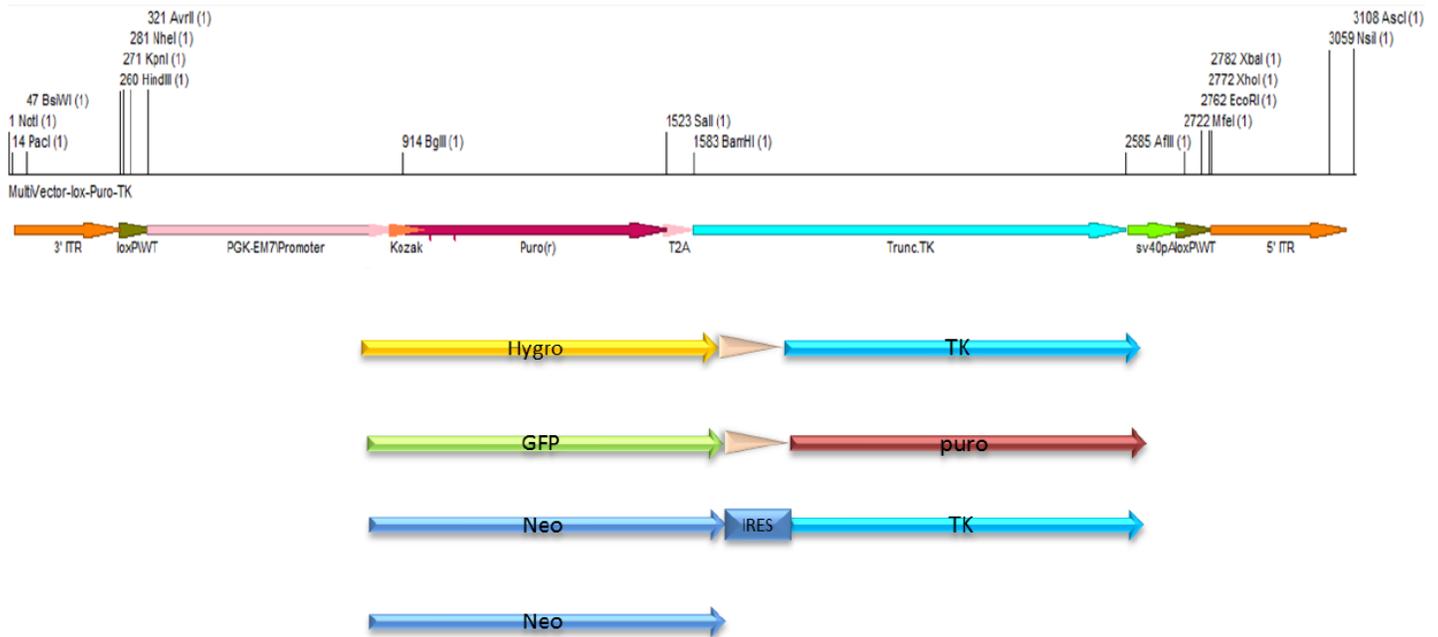


Figure 1. Description and map of Multivector™ features and use.

### 1.3 General Protocol Notes

There are many options for using the Multivector™ for gene editing. Below are a few examples in section 1.4, 1.5, 1.6 and 1.7. Transposagen has many validated Multivector™ variations available to researchers to suit most project needs. These include, but are not limited to:

Puromycin/TK	Multivector™ Catalog# PB-MV1Puro-TK
Neomycin/TK	Multivector™ Catalog# PB-MV1Neo-TK
GFP/Puromycin	Multivector™ Catalog# PB-MV1CMV-GFP-Puro
Neomycin	Multivector™ Catalog# PB-MV1Neo
Hygromycin/TK	Multivector™ Catalog# PB-MV1Hygro-TK



**Figure 2.** All of the major elements of the Multivector™ are flanked by unique restriction sites and can therefore be removed and exchanged to suit various research needs.

### 1.4 Protocol for Footprint-Free™ excision

In addition to the rare restriction sites on each end of the cassette region (Not1 and Asc1), the *piggyBac*™ Inverted Terminal Repeats (ITRs) each contain unique restriction sites (BsiW1 and Nsi1) near their ends, which can be used for cloning homology arms. These sites are close enough to the ends to be incorporated into a PCR primer which includes the remainder of the ITR and which primes extension of your genomic region of interest. In this way, a homology arm that is seamless with the ITR can be amplified and cloned. **Important: no BsiW1 or Nsi1 restriction sites can be present in the homology arms being amplified.**

The *piggyBac*™ transposase absolutely requires “TTAA” sequences flanking the ITRs to work. To achieve seamless excision, the insertion site must be directly after an endogenous “TTAA” and a second “TTAA” must be added directly after the 3’ ITR (in this example, incorporated in your PCR

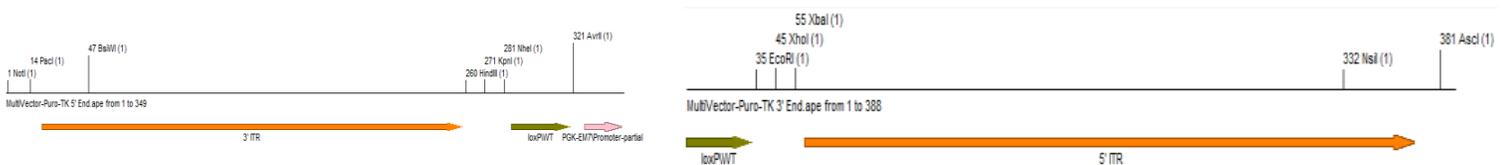
primer). An alternative is to gene synthesize the ends of the ITRs along with a portion or all of the homology arm. Another is Gibson cloning of the arms.

Many applications call for the removal of the selection cassette once a targeted clone is selected. In addition to the correctly inserted HR vector, there is a possibility the vector could have been randomly inserted elsewhere in the genome. It should be noted that whether by PBx or Cre/Lox, incomplete removal from all cells is a possibility. The thymidine kinase gene, which is co-expressed using a viral T2A sequence, is included in the Puromycin Multivector™ to enable selection for complete cassette removal using ganciclovir. Cells expressing TK will not survive growth in medium containing ganciclovir, therefore using this selection after excision should yield clones that are free of the cassette.

Additional details are covered in the **Gene Editing Kit Gene Editing Kit & Footprint-Free™ Gene Editing Kit Manual**.

### 1.5 Protocol for minimal footprint

Restriction sites that include “TTAA” in their recognition sites are present flanking the ITRs. However, using them will not achieve completely footprint free excision, since 2-3 extra bases (depending of the sequence following the endogenous “TTAA”) will remain upon excision with PBx. This is still far fewer than the 34 bases left behind with Cre/LoxP and therefore much less likely to disrupt any sequences involved in important regulatory functions.



**Figure 3.** *piggyBac™* nomenclature denotes the ITR at the 5' end of the Multivector™ as 3' and the ITR at 3' end as 5'.

### 1.6 Protocol for LoxP excision

As noted, the Multivector™ also includes loxP sites flanking the resistance cassette. If a researcher is not interested in using *piggyBac™* excision, the choice of three unique sites flanking each side of the loxP sites offers convenient cloning options. If one or more of the loxP sites need to be removed (i.e. for conditional targeting considerations) compatible sites flank each loxP to allow for easy removal.

### 1.7 Examples and published protocols

Conditions for your cell type and nucleases choice is likely published. Transposagen recommends a literature search prior to undertaking any gene editing project. Below are some published examples of the Footprint-Free™ Gene Editing Kit system that utilizes the Multivector™ (or similar derivative):

*Seamless genome editing in human pluripotent stem cells using custom endonuclease-based gene targeting and the piggyBac transposon*

<http://www.nature.com/nprot/journal/v8/n10/abs/nprot.2013.126.html>

*Seamless gene correction of  $\beta$ -thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac*

<http://genome.cshlp.org/content/early/2014/07/30/gr.173427.114>