Transposagen

Better Models. Better Results.™

piggyBac™ Transposase

User Manual, Version 3.04.16

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Contents

1. Introduction and Background

A. The *piggyBac*™ Transposon/Tranposase Genetic Modification System

Transposagen's *piggyBac*™ (PB) transposon genetic modification system is a highly efficient, non-viral means of DNA integration into target genomes. To date, the *piggyBac*™ transposon has been utilized in gene therapy, regenerative medicine, cell line engineering, and animal model creation. The *piggyBac*™ genetic modification system enables you to:

- Use simple transfection to alter a variety of animal genomes
- Engineer cell lines for high level protein production
- Complete non-viral gene delivery efficiently and economically
- Revert modifications to the genome in a scarless, Footprint-Free™ manner

The *piggyBac*™ transposon is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut-and-paste" mechanism (Figure 1). During transposition, the *piggyBac*™ transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on each end of the transposon. Subsequent to ITR recognition, the *piggyBac*™ transposase excises ("Cut") the transposon from the transposon vector which is then efficiently integrated ("Paste") into random TTAA genetic locations. The *piggyBac*™ transposons have an enormous cargo limit with over 200 kb demonstrated.

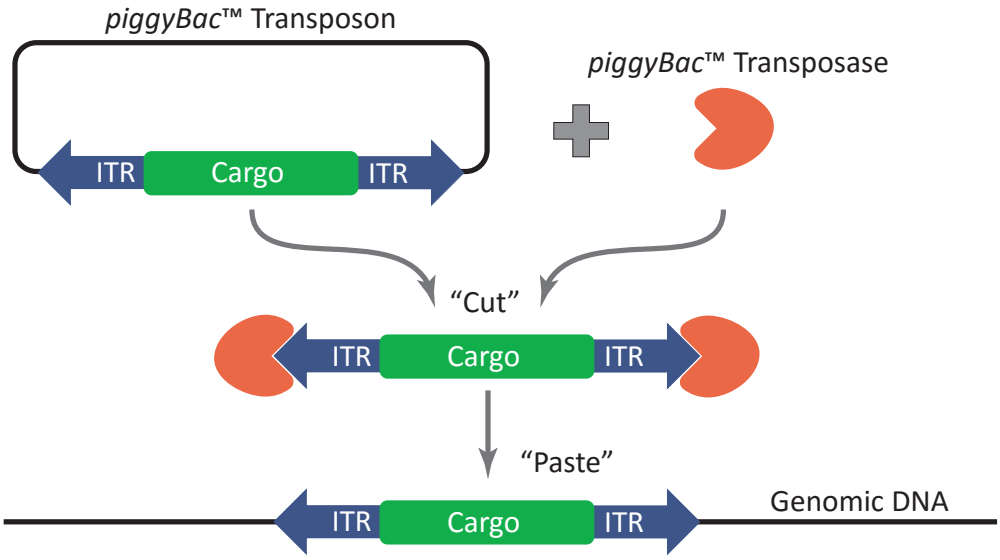

Figure 1: *Mechanism of the piggyBac™ transposase/transposon gene modification system.*

Genomes containing an inserted *piggyBac*™ cargo can be transiently re-transfected with the excision-only *piggyBac*™ (PBx) vector to remove the cargo in a scarless, or Footprint-Free™ manner (Figure 2). The powerful activity of the *piggyBac*™ transposon system enables any DNA cargo to be easily integrated into target genomes.

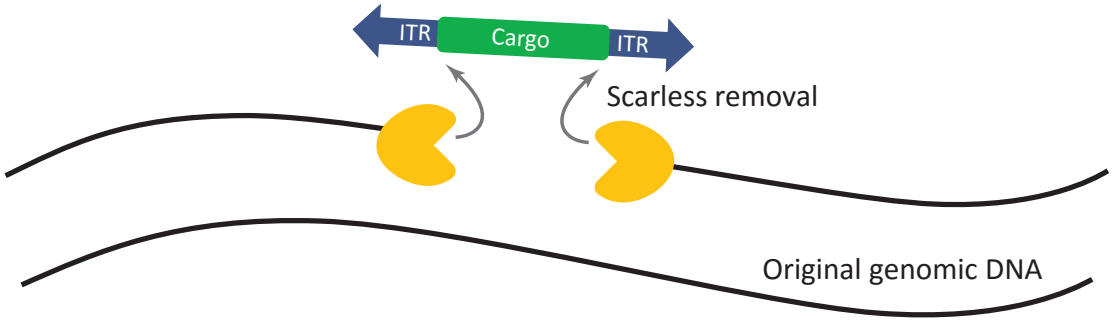

Figure 2: *Scarless, Footprint-Free™ removal of cargo with the excision-only piggyBac™ vector (PBx)*

Using Transposagen's *piggyBac*™ transposon selection technology in conjunction with site-specific nucleases, including CRISPR/Cas9 and TALEN, provides a clean and efficient method to select for gene editing down to a single base pair (Figure 3).

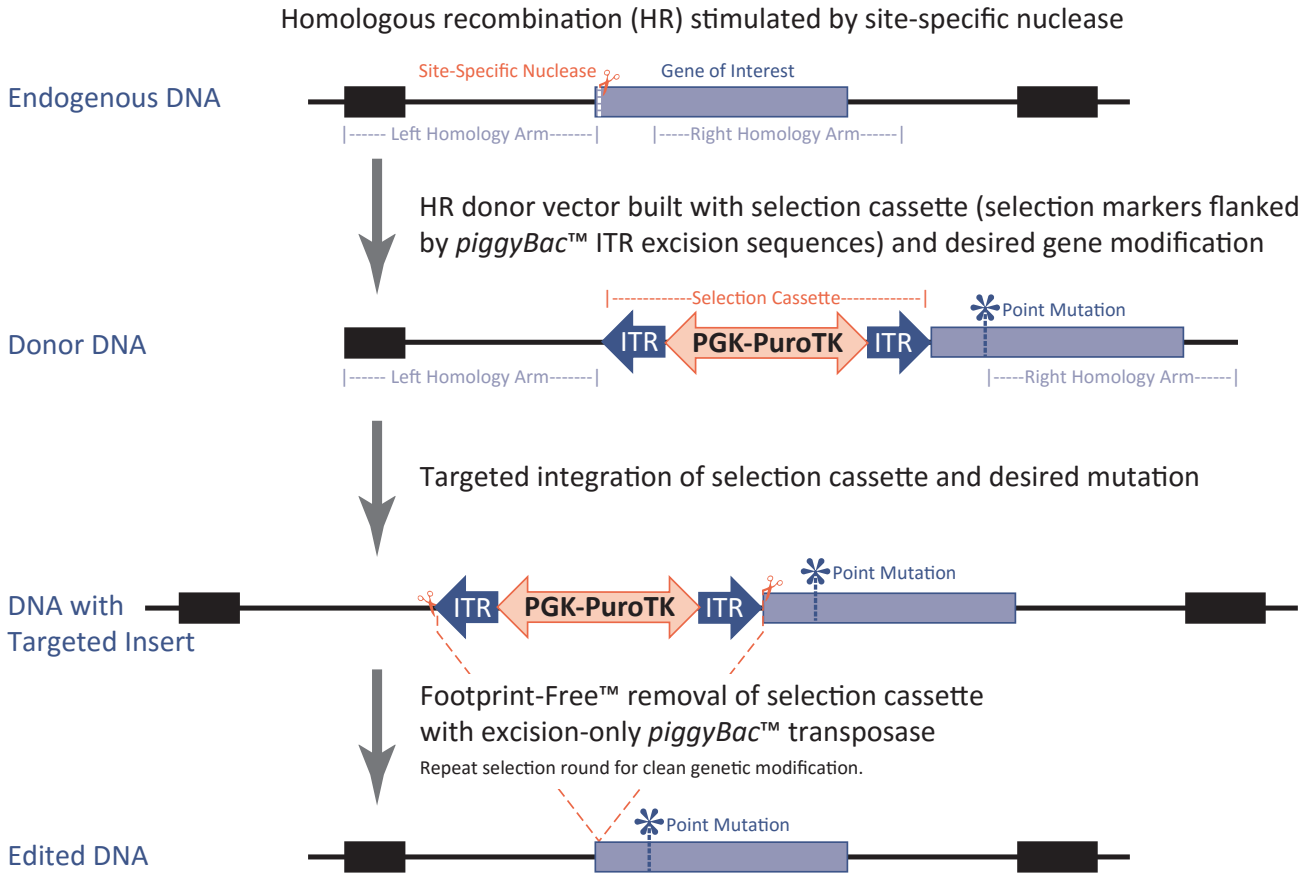

Figure 3: *Editing DNA using Transposagen's piggyBac™ transposon selection technology using a site-specific nuclease to stimulate homologous recombination, creation of donor DNA with the edited gene and Multivector selection cassette, and Footprint-Free™ removal of the cassette by excision-only piggyBac™ vector (PBx)*

Learn more about Footprint-Free™ Gene Editing at http://www.transposagenbio.com/gek-footprint-free

2. Design and Construction of *piggyBac*™ Transposon and Transposase Vectors

Transposagen sells many *piggyBac*™ transposon vectors that can be modified by customers to suit your research needs (shown below). In addition, we can provide fee-for-service cloning to create custom vectors with your gene cargo of interest (Cat # CPB-001). Contact us for instructions on how to order your custom *piggyBac*™ vector. After submission of the DNA sequence, our scientists will design and synthesize your custom vector.

A. The *piggyBac*™ transposon and transposase vectors

Transposagen has many validated, high purity *piggyBac*™ transposon and transposase vectors available for research. These include, but are not limited to:

Catalog #	Description
Transposon Vectors	
SPB-007	This customizable *piggyBac*™ transposon vector contains an insulator-flanked large multiple cloning site (MCS), allowing the flexibility to clone in your promoter and DNA cargo of choice. The core insulators protect cargo from genomic position effects and gene silencing. 10 µg size. [PB007]
SPB-008	Similar to SPB-007, but SPB-008 contains a GFP gene preceded by cloning sites that allow cloning of your promoter and gene of interest in frame to monitor expression. 10 µg size. [PB007G]
SPB-006	This customizable *piggyBac*™ transposon vector contains a Polr2A promoter for ubiquitous gene expression. this promoter contains an intron and has demonstrated excellent expression levels *in vivo* and in transgenic animal models. 10 µg size. [PB001]
SPB-009	Similar to SPB-006, but SPB-009 provides the flexibility to clone any gene or cDNA with an in-frame GFP-fusion marker. This vector can also serve as a positive control for transposition. 10 µg size. [PB001G]
SPB-010	This customizable *piggyBac*™ transposon vector contains the strong CAG promoter driving GFP expression. This vector also features an MCS to clone an expression cassette of your choice. The GFP marker will be driven independently from your cargo. 10 µg size. [PB002G]
SPB-011	Similar to SPB-010, but SPB-011 replaces the CAG promoter with a CMV promoter to express GFP. 10 µg size. [PB003G]
SPB-005	This customizable *piggyBac*™ transposon vector contains an SV40 promoter-driven neomycin resistance gene. An insulator-flanked MCS allows for cloning in elements of interest, including user chosen genes driven by any selected promoter. The core insulators will protect cargo from genomic position effects and gene silencing. 10 µg size. [PBNeo]
Transposase Vectors	
SPB-001-10 SPB-001-25 SPB-001-50	Super *piggyBac*™ transposase (sPBo) expression vector is a high purity, transfection ready, hyperactive *piggyBac*™ transposase that facilitates stable integration of small and large genes into target genomes. This vector is non-renewable, and cannot be propagated in bacteria. Available in 10, 25, and 50 µg quantities. Also available as mRNA (Cat # SPB-003). [sPBo]
SPB-002-10 SPB-002-25 SPB-002-50	Excision-only *piggyBac*™ transposase (PBx) expression vector is a high purity, transfection ready, transposase for excision of *piggyBac*™ selection cassettes and transgenes for Footprint-Free™ gene editing or phenotype reversions. This vector is non-renewable, and cannot be propagated in bacteria. Available in 10, 25, and 50 µg quantities. [PBx]

B. The *piggyBac*™ selection cassettes

Transposagen has many validated *piggyBac*™ Multivector plamids that you can use to make your own targeting vectors. They include a choice of the most widely used antibiotic selection genes and/or GFP, and flexible cloning options for your arms of homology to facilitate clonal isolation after gene editing using a targeted donor vector insertion strategy. Flanking *piggyBac*™ ITR excision sequences enable seamless cassette removal with Excision-only *piggyBac*™ transposase (Cat # SPB-002). These selection cassettes include, but are not limited to:

Available Multivector Options		
Catalog #	**Selection Cassette**	**Promoter**
SGK-001 [MV-CMV-GFP-Puro]	GFP/Puromycin	CMV
SGK-002 [MV-PGK-GFP-Puro]	GFP/Puromycin	PGK
SGK-003 [MV-PGK-Neo]	Neomycin	PGK
SGK-004 [MV-PGK-Neo-TK]	Neomycin/Thymidine Kinase (TK)	PGK
SGK-005 [MV-PGK-Puro-TK]	Puromycin/TK	PGK
SGK-006 [MV-Hygro-TK]	Hygromycin/TK	PGK
SGK-007 [MV-EF1α-Puro-TK]	Puromycin/TK	EF1α

▸▸ Learn more about pricing and ordering at http://www.transposagenbio.com

3. Integration of the *piggyBac*™ Transposon

Transposagen suggests following manufacturer's protocols for the introduction of DNA/RNA into your cell type of interest. The *piggyBac*™ system is titratable. More or fewer integrations can be achieved by varying the amounts of transposase and transposon, as appropriate.

A. Basic Protocol

Note: Below is an example integration protocol using Transposagen's *piggyBac*™ transposase vector and any *piggyBac*™ transposon vector you choose. Every cell line will respond differently to transfection and the introduction of foreign DNA. Be sure to determine the optimal transfection efficiency in your cell type prior to beginning the integration protocol.

Co-transfect the Super *piggyBac*™ transposase with the *piggyBac*™ transposon vector:

1. Clone the desired desired cargo into the appropriate *piggyBac*™ transposon vector.
2. Sequence verify the clones.
3. Grow target cells to 60–80% confluency.
4. Prepare the transfection mixture. For one well of a 6-well dish combine:

Amount	Item	Recommendation
1.0 µg	PB Transposon vector	Start with a 1:1 molar ratio of transposase to transposon to determine baseline integration efficiency.
1.0 µg	PB Transposase vector (or PB mRNA*)	Calculate the molar ratio using your final transposon size.
x µL	Transfection reagent of choice	Follow manufacturer's protocol for reagent amounts
50 µL	serum-free or reduced-serum DMEM	

* Note: If using pB mRNA, alter protocol to manufacterer's recommendations for RNA and/or consider electroporation.

5. Mix by pipetting.
6. Allow complex formation by incubating the mixture for 15–30 minutes at room temperature.
7. Transfer the transfection mixture drop-wise to cells in culture wells and swirl to disperse.
8. Incubate the cells and the transfection mixture at 37°C in a CO_2 incubator. If lipofection was used, change media after 24 hours.
9. Check for positive integrations after 72 hours.
   - If you can, apply antibiotic selection or isolate cells by fluorescence-activated cell sorting (FACS), if applicable, to select for positive clones and measure expression of integrated cargo by standard RT-PCR methods.
   - If you did not use antibiotic or fluorescent markers, allow appropriate time for cells to remove trace amounts of remaining episomal transposon cargo before measuring expression of cargo by standard RT-PCR methods. Use non-transfected cells as negative control in RT-PCR.

4. Excision of *piggyBac*™ Transposon Cargo

We recommend using excision-only *piggyBac*™ (Cat # SPB-002) to remove inserted cargo (see Figure 2). Increasing amounts of PBx will increase excision efficiencies. Negative selection, such as thymidine kinase/ganciclovir or loss of a fluorescent marker, can also be utilized to enhance excision efficiencies.

A. Basic Protocol

Note: Below is an example excision protocol with Transposagen's Puromycin/Thymidine Kinase (Puromycin/TK) selection cassette. The protocol assumes that you have identified puromycin-resistant clones. Before beginning any selection protocol, you need to perform kill curves using your cell line and selection method of choice. Every cell line will respond differently to drug selection.

1. Mix approximately 2 x 10^6 cells with 10 µg excision-only *piggyBac*™ plasmid (PBx) and transfect according to the manufacturer's protocol using either a nucleofection device or lipofection reagents.

   Note: 5 µg is an appropriate starting point for induced pluripotent stem cells (iPS cells) and other difficult-to-transfect cells; for transformed cell lines use approximately 250 ng for 1 x 10^6 cells.

2. Plate the cells onto a 6-well plate in 1:2, 1:4, and 1:6 dilutions in media without puromycin. If lipofection was used, change media after 24 hours.

3. On day 4, count cells and plate 1 x 10^4 cells onto a 10-cm dish in media containing ganciclovir (Sigma Cat # G2536) at a concentration determined from your previously performed kill curve. Ganciclovir should not affect cells without the thymidine kinase (TK) gene.
4. Change media every other day. Ganciclovir killing takes approximately 2–4 days for fast growing cells, but may take up to two weeks in some cell lines.
5. Check surviving clones for puromycin sensitivity and confirm appropriate cassette removal by standard molecular analysis methods.

Note: We recommend that you perform a literature search to find the appropriate conditions for your cell type prior to undertaking any gene editing project. Below are some published examples of the Footprint-Free™ Gene Editing Kit system:

- 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 and http://www.ncbi.nlm.nih.gov/pmc/articles/PMC4559351/
- Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and *piggyBac*: http://genome.cshlp.org/content/early/2014/07/30/gr.173427.114

5. Confirmation of Integrated *piggyBac*™ Transposon Cargo by Splinkerette PCR

The Splinkerette PCR assay locates and maps the exact location of each transposition event in the genome, and provides a copy number for the number of transposon integrations. Transposagen offers a fee-for-service Splinkerette Assay, or an off-the-shelf Splinkerette kit (Cat # KPB-001) that includes the full protocol.

The full reference for the Splinkerette PCR procedure is published:

- A high-throughput splinkerette-PCR method for the isolation and sequencing for retroviral insertion sites, *Nature Protocols*, Col.4 No5. 2009, Page 789–798. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3627465/

6. Frequently Asked Questions

Q. Is excision-only *piggyBac*™ (PBx) mutagenic?

A. No, the excision-only *piggyBac*™ is not mutagenic. It will only remove cargo flanked by the specific ITR sequences inserted previously. Removal of inserts by PBx is a scarless mechanism which restores the original wildtype sequence.

Q. How many copies of my integrated gene will be present?

A. The number of integration events will largely depend on the amounts and ratios of *piggyBac*™ transposase and transposon. Titrating these amounts can yield of a majority of single integration events, or multiple events depending on your preference. Confirmation of integration events is also possible to verify the exact copy number present.

Q. How efficient are *piggyBac*™ expression vectors?

A. While integration using Super piggyBac™ transposase (sPBo) is highly efficient, excision with PBx is somewhat less so. To monitor excision, Transposagen recommends inclusion of the TK gene for counterselection of unexcised cells or use of a fluorescent marker.

7. Troubleshooting

If you see...	Then try this...
A lower-than-expected integration efficiency	Increase the amounts of transposase
A higher-than-expected integration efficiency	Decrease the amounts of transposase
Incomplete transposon excision across all cells	Multiple rounds of transfection may be required to completely remove all integrations.
Cell toxicity after transfection	Decrease DNA and transfection reagent amounts. Lower the amount of transposase used, as integration may have occurred in essential genomic locations.

8. References

1. Seamless genome editing in human pluripotent stem cells using custom endonuclease–based gene targeting and the *piggyBac* transposon. *Nat Protoc.* 2013; 8: 2061–2078. http://www.nature.com/nprot/journal/v8/n10/abs/nprot.2013.126.html
2. Seamless gene correction of β-thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and *piggyBac*. *Genome Res.* 2014; 24: 1526–1533. http://genome.cshlp.org/content/early/2014/07/30/gr.173427.114
3. A high-throughput splinkerette-PCR method for the isolation and sequencing for retroviral insertion sites, *Nat Protoc.* 2009; 4(5): 789–798. http://www.ncbi.nlm.nih.gov/pmc/articles/PMC3627465/

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