USER MANUAL PIGGYBAC®

Hera BioLabs

PiggyBac® Transposase

2022 User Manual
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Introduction and Background

The piggyBac Transposon/Transposase Genetic Modification System

Hera Biolab'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. After ITR recognition, the piggyBactransposase 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.

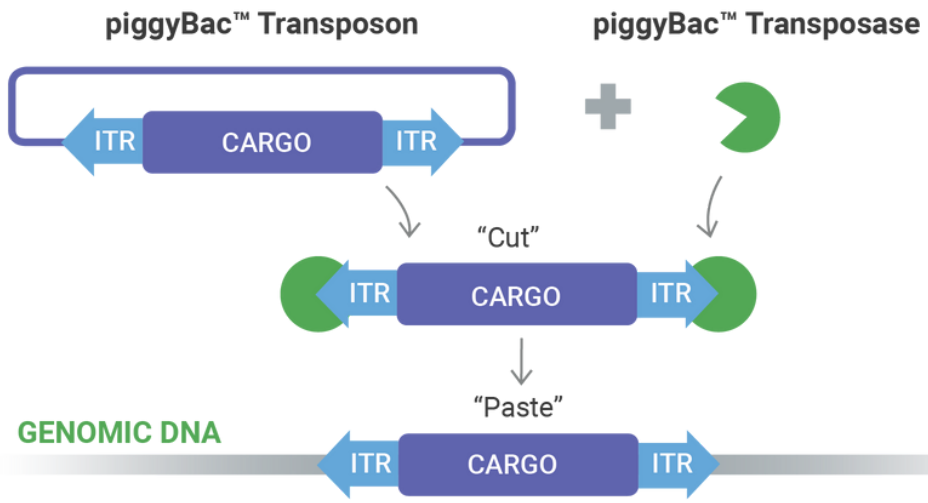

Figure 1: Mechanism of the piggyBac transposase/transposon gene modification system.

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- FreeTM manner.

Powerful activity of the piggyBac transposon system enables any DNA cargo to be easily integrated into target genomes.

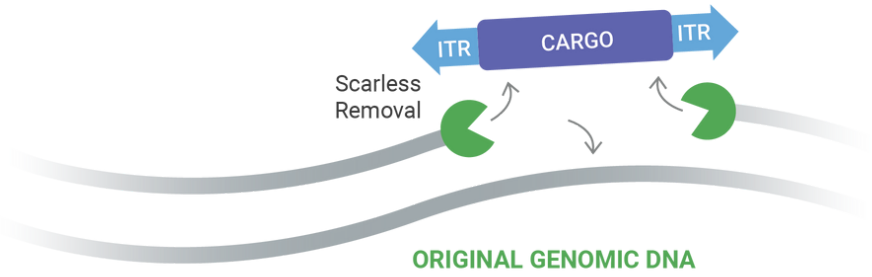

Figure 2: Scarless, Footprint-Free removal of cargo with the excision-only piggyBac vector (PBx)

Using Hera'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.

The piggyBac Transposon and Transposase Vectors

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

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.

Transposase Vectors & mRNA

SPB-DNA – Codon optimized super piggyBac transposase expression vector. 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.

SPB-100 – Improved Super piggyBac transposase mRNA. Sequence has been codon optimized (in comparison with SPB-003) to provide improved mRNA stability and protein expression in mammalian systems. The piggyBac transposase mRNA is recommended for cells that are not efficiently transfected with vector DNA.

SPB-200 – Good Manufacturing Grade super piggyBac transposase mRNA. Sequence has been codon optimized to provide improved mRNA stability and protein expression in mammalian systems. The piggyBac transposase mRNA is recommended for cells that are not efficiently transfected with vector DNA. It is ideally suited for applications where cell lines are to be used for downstream manufacturing i.e. research and master cell banks, where GMP starting materials are required.

SPB-002 – Excision-only piggyBac transposase expression vector. Excision-only piggyBac transposase (PBx) expression vector is a transposase for excision of piggyBac selection cassettes and transgenes for Footprint-Free gene editing or phenotype reversions.

2. Integration of the piggyBac Transposon

Hera BioLabs 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.

Basic Protocol

Note: Below is an example integration protocol using Hera'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 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 ration using your final transposon size
xµL	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 manufacturer'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 a t37°C in a CO2 incubator. If lipofection was used, change media after 24 hours.

9. Check for positive integrations after 72 hours.

If possible, 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.

3. 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.

Basic Protocol

Note: Below is an example excision protocol with our 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 106 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 difficult to transfect cells; for transformed cell lines use approximately 250 ng for 1 x 106 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 every 24 hours.

3. On day 4, count cells and plate 1 x 104 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 for 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

4. 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 of transposon integrations. Hera offers a fee-for-service Splinkerette Assay, or an off-the-shelf Splinkerette Kit (Cat#: KBP-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/

5. Frequently Asked Questions

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

A. No, 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 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 (SPB-DNA) is highly efficient, excision with PBx is somewhat less so. To monitor excision, we recommend inclusion of the TK gene for counterselection of unexcised cells or use of a fluorescent marker.

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 my have occurred in essential genomic locations

7. References

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

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

A high-throughput splinkerette-PCR method for the isolation and sequencing for retroviral insertion sites, Nat Protoc. 2009; 4(5): 789–798.
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