



**Gene Editing Kit
&
Footprint-Free™ Gene Editing Kit**

2022 User Manual

Version 5.0

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Gene Editing Kit (For Projects Without Selection)

1.1 Gene Editing Kit Overview

To achieve targeting of your gene edit or knock in, custom sequence specific nucleases (XTN™ TALENs, CRISPR or NextGEN™ CRISPR) are used in conjunction with a targeting vector or single-stranded oligo containing the desired changes. The nuclease will create a double strand break which, when coupled with appropriate homologous DNA, will drive repair of the break by incorporating the donor sequence and therefore the desired edit.

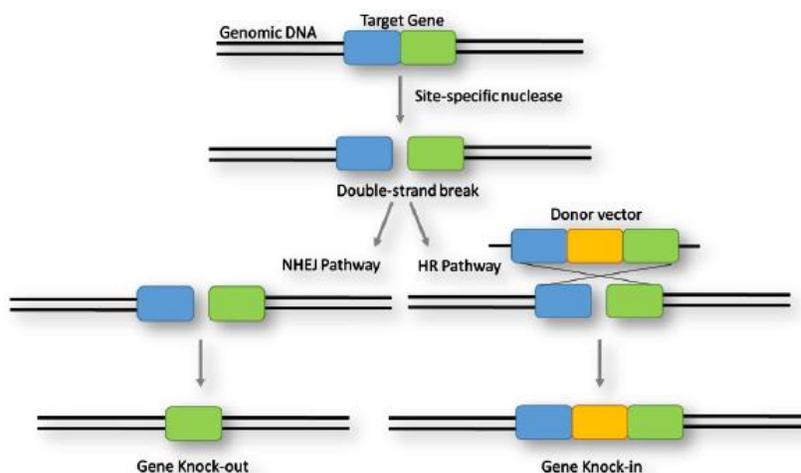


Figure 1. Cells can utilize the Homologous Recombination (HR) pathway to copy adjacent DNA to repair double strand breaks induced by site-specific nucleases. The Gene Editing Kit donor vector provides new sequences to create your gene edit of choice.

1.2 Protocol

The general workflow is to get the nuclease DNA and donor vector DNA into your cells, allow them to work for 2-4 days, then identify clones by single cell cloning or antibiotic selection.

Steps:

1. *Cell line optimization.* The method of transfection is whatever works best for your cells – lipofection, nucleofection, etc. If you do not know the upper limits of DNA your cells can handle during a transfection, it is best to perform this.
2. *Transfection.* Transfect nuclease DNA and donor plasmid into cells. We recommend equal ratios of your nuclease reagent to donor vector. For example, using XTN™

TALENs:

1 µg Left XTN™ TALEN
1 µg Right XTN™ TALEN
1 µg Donor plasmid
3 µg total

You can vary the ratios of the reagents or include additional nucleases if necessary. For a control, designate a well with donor vector only to compare the number of random integrations that were targeted. This will inform you as to how many clones may need to be screened since high vector-only background indicates random integrations that are not related to precise targeting.

More protocol information and details about conditions specific to your nuclease of choice can be found in our **XTN-TALEN** and **Cas-CLOVER® Gene Editing Systems** manuals.

3. *Gene Editing Information.* Cells should be analyzed by standard molecular methods to verify the gene edit has been made. For difficult cell lines, Hera Biolabs recommends using our Footprint-Free™ Gene Editing System to select for your gene edit. This can potentially save you valuable time and resources.

Primer design for targeting detection is important and guidance can be found in the below protocols paper, along with many other useful methods that may apply to your project.

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 β -thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac
<http://genome.cshlp.org/content/early/2014/07/30/gr.173427.114>

Footprint-Free™ Gene Editing Kit (For *piggyBac*™ selection and excision)

1.3 Footprint-Free™ Gene Editing Kit Overview

The Optimal conditions and protocols for Footprint-Free™ gene editing will depend on your cell type, selection markers, nucleases efficiency and other parameters. Below are guidelines for our commonly used Puromycin/TK selection system. The Puromycin/TK selection cassette has been designed to allow selection of both integration (using Puromycin resistance) and removal (using ganciclovir sensitivity) of the cassette. This is accomplished using a two-step process. First, clones containing the cassette are derived by selecting for Puromycin resistance. Once a Puromycin resistant clone is established, and Footprint-Free™ removal is desired, *piggyBac*™ transposase is introduced to remove the resistance markers. Ganciclovir is used to select those clones that have lost the cassette. Since cells still containing the TK gene will die in the presence of Ganciclovir, only those clones where removal is complete will survive. The resulting cells will be Puromycin sensitive and ganciclovir resistant.

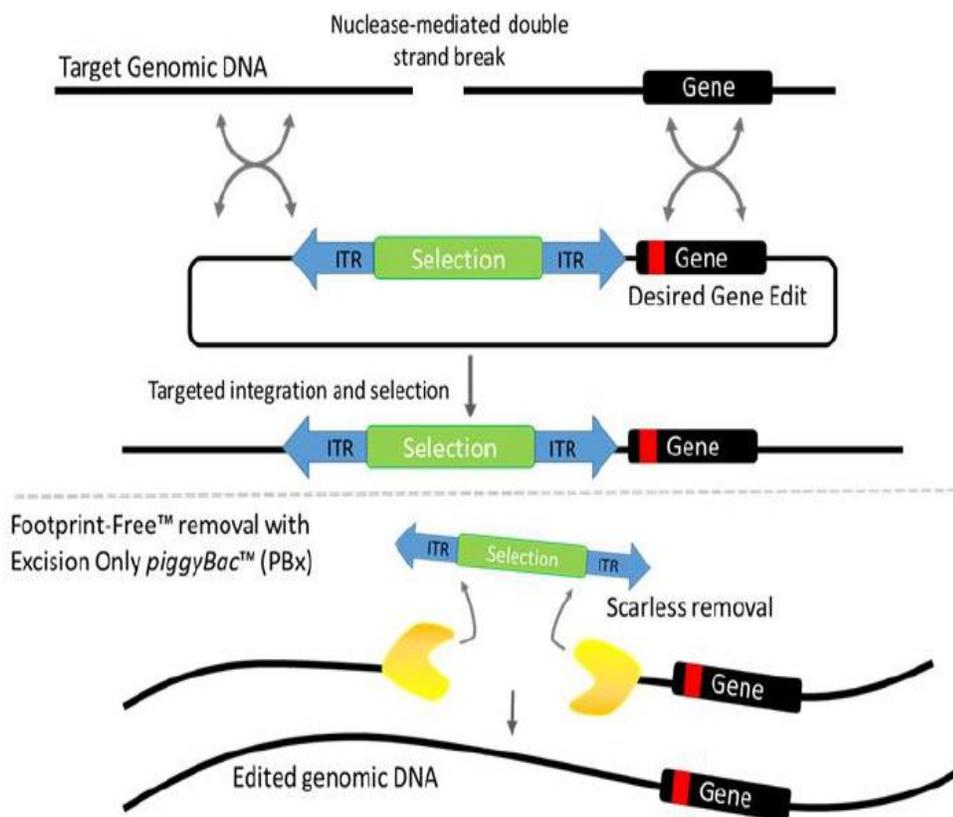


Figure 2. Brief overview of how *piggyBac*™ Footprint-Free™ gene editing works.

1.4 piggyBac™ Selection Cassettes

Hera Biolabs has many validated piggyBac™ selection cassettes available to researchers. These include, but are not limited to:

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

1.5 Protocol

The following protocol assumes Puromycin resistant clones have been identified and removal of the cassette is now desired. Before beginning any selection protocol, kill curves must be performed using your cell line of choice. Every cell line will respond differently to drug selection.

1. Mix approximately 2×10^6 cells with $10 \mu\text{g}$ *piggyBac™ plasmid and transfect according to the manufacturer's protocol using either a nucleofection device or lipofection reagents.
* $10 \mu\text{g}$ is appropriate for iPS cells and other difficult to transfect cells; for transformed cell lines use approximately $250\text{ng}/1 \times 10^6$ 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 and allow cells to remain in place.
3. On day 4, count cells and plate 1×10^4 cells onto a 10 cm dish in media containing $2 \mu\text{M}$ ganciclovir (Sigma Cat# G2536). Ganciclovir should not affect cells without the TK gene. Concentrations from $0.5 \mu\text{M}$ to $12 \mu\text{M}$ have been used. The sensitivity of your cells must be taken into consideration. Kill curves should be performed before this step.
4. Change media every other day. Ganciclovir killing takes approximately 2-4 days.
5. Surviving clones should be checked for Puromycin sensitivity and analyzed by standard molecular methods to confirm appropriate cassette removal.

Conditions for your cell type and nucleases choice is likely published. Hera Biolabs recommends a literature search 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>

Seamless gene correction of β -thalassemia mutations in patient-specific iPSCs using CRISPR/Cas9 and piggyBac

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