EDITGENE piggyBac Transposon System Reagent

Product Manual

CONTENTS

piggyBac Transposon System Kit

► Product Information

Catalog Number	Product Name	Components
EDY004-Y01	piggyBac Transposon System Kit	PB System Plasmid
		Cell

► Product Overview

This kit is a piggyBac Transposon System Kit, providing cells and PB system plasmids. The PB system plasmids include:

- **Helper Plasmid**: Encodes the transposase enzyme.
- **Transposon Plasmid**: Contains inverted terminal repeats (ITRs) specific to the transposon at both ends, with the transposable gene region in the middle, which can integrate into the host genome.

In the experiment, both the helper plasmid and transposon plasmid are co-transfected into target cells. The transposase encoded by the helper plasmid recognizes the ITR sequences at both ends of the transposon plasmid, cuts them, and integrates the transposable gene region into TTAA sites in the host genome, generating TTAA repeats at both ends of the transposed region. When the helper plasmid is re-transfected, the transposase can remove the transposon from the genome.

As a non-viral vector, the piggyBac transposon system offers several advantages over traditional viral vectors: it has high safety, is easy to use (allowing direct plasmid transfection into cells), supports large vector capacity for co-expression of multiple genes, allows adjustment of helper and transposon plasmid ratios to improve integration efficiency of exogenous genes, enables precise determination of gene insertion sites by inverse PCR, achieves precise excision of the transposon upon re-transposition, has a broad host range, high transposition efficiency, and minimal reliance on host factors.

► Application Scope

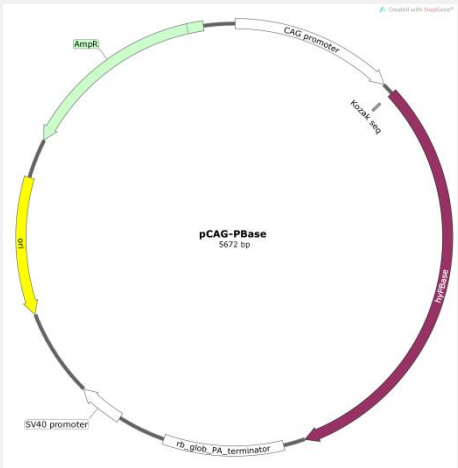
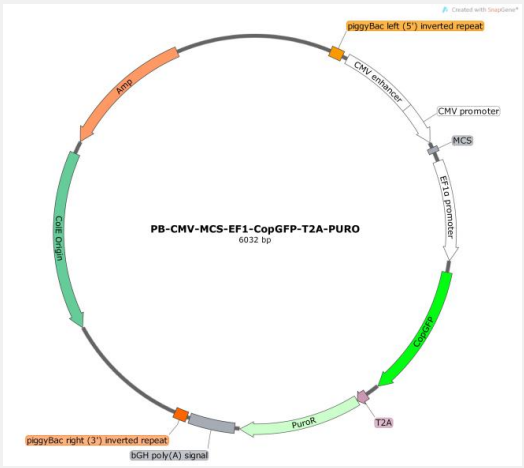
This kit is a non-viral gene delivery tool, suitable for applications including but not limited to **gene therapy**, **somatic cell reprogramming**, **cell and animal model construction**, and **gene-edited cell line development**.

► Product Components

● Kit Components

Component			Specification	Storage Temperature
PB System Plasmids	Helper Plasmid (500 ng/μL)	pCAG-PBase	4 μg	-20℃
	Transposon Plasmid (500 ng/μL)	PB-CMV-MCS-EFIα-CopGFP-T2A-Puro	10 μg	-20℃
Cells (optional)			1×10^6/tube	Liquid nitrogen

● Plasmid Map Diagram

Name	Helper Plasmid Map Diagram	Transposon Plasmid Map Diagram
Plasmid Map		
Notes		Promoters available: CMV, CAG, EF1α

Note:

1. To facilitate enrichment of successfully transposed cells post-transfection, PB Plasmids have puromycin resistance. Additionally, PB-CopGFP also has puromycin resistance, which can be used for pre-transfection and selection.
2. This kit is suitable for five transfections in a 6-well plate. The recommended transfection mass ratio of helper plasmid to transposon plasmid is adjustable from 1:2.5 to 1:5, with an optimal ratio of 1:3.
3. **It is recommended to use this kit in combination with cells provided by EDITGENE for best results.**

► Storage Conditions and Shelf Life

Storage: -20 ℃, shelf life of 1 year

► Additional Required Materials

To complete this experiment, other materials not provided in this kit may be required:

Reagents	Cell culture medium, serum, PBS, trypsin, puromycin, Opti-MEM I, cell freezing medium, transfection reagents, etc.
Consumables	6-well plates, sterile tips of various sizes, centrifuge tubes, etc.
Equipment	Biosafety cabinet/sterile workstation, cell incubator, fluorescence microscope, centrifuge, PCR machine, agarose gel electrophoresis system, gel imaging system, pipettes, etc.

► Workflow Diagram

Figure 1. Schematic Diagram of PB Gene Transposed Cell Line Construction Workflow

► Experimental Procedures

● Cell Pre-Experiment

1. **Transfection Pre-Experiment**

The transposon plasmid in the kit contains green fluorescent CopGFP, which can be used with the helper plasmid or individually for transient transfection. It is suitable for optimizing transfection conditions to facilitate the main experiment.

Note: Efficient transfection is essential for successful editing. Common cell lines like 293T, HeLa, A549, and N2A are effectively transfected with lipofection. For therapeutically relevant cells, transfection may be challenging and may require different methods and parameters. It is advisable to optimize the transfection method before the experiment.

2. **Determining Puromycin Selection Concentration**

(1) Seed cells in the logarithmic growth phase into a 12-well plate with 1 mL of medium per well and incubate for 24 hours.

(2) When cells reach 60%-70% confluency, replace the medium with selection medium containing different concentrations of puromycin (e.g., 0, 0.5, 1, 1.5, 2, 2.5, 3, 4, 5 μg/mL).

(3) Replace with fresh puromycin-containing medium every 2 days. After 7 days, observe under a microscope and select the minimum concentration that completely kills the cells for use in future experiments (optimal selection concentration).

● Main Experiment

1. Cell Transfection

Using Lipofectamine 3000 (Lipo 3000) as an example, if electroporation is used, optimize conditions based on the electroporator's recommended parameters.

(1) Digest and count cells in the logarithmic growth phase.

(2) Seed an appropriate number of cells into a 6-well plate and incubate.

Note: Due to different growth rates, ensure approximately 80% confluency after 18-24 hours.

(3) After 18-24 hours, transfect cells when they reach 80% confluency.

(4) Prepare the plasmid mixture as shown in the table below:

Component	Amount for 6-well plate
Helper Plasmid	0.6 μg
Transposon Plasmid	1.9 μg
Add opti-MEM I to	125 μL

(5) Add 2 μL of P3000 reagent, gently mix.

(6) Prepare Lipo 3000-Opti-MEM I mixture and gently mix:

Component	Amount for 6-well plate
Lipo 3000	7.5 μL
Add opti-MEM I to	125 μL

(7) Add 125 μL of plasmid mixture to 125 μL of Lipo 3000-Opti-MEM I mixture, gently mix, incubate at room temperature for 15 minutes, then carefully add to the medium and gently shake. Incubate the plate.

(8) The next morning, transfer cells to a T25 flask and continue incubation.

Note: Lipofectamine 3000 can be cytotoxic; it is recommended to change the medium 4-24 hours post-transfection.

2. Antibiotic Selection

48 hours post-transfection, observe cell viability and fluorescence under a microscope, then replace with puromycin-containing medium for selection. Use the optimal concentration determined in the pre-experiment. Continue selection for 2 weeks, then switch to medium with half the concentration of puromycin for expansion.

3. Detection of Gene Transposed Cell Lines

After selection, the successfully transposed cells can be analyzed for gene expression levels using qPCR or Western Blot (WB).

4. Cell Expansion and Cryopreservation

Expand and cryopreserve validated cells with half-dose puromycin maintenance, or

proceed with downstream experiments.

► FAQs and Solutions

1. Does the piggyBac Transposon System Kit recommend lipofection or electroporation?

A: The PB system in this kit includes plasmids compatible with lipofection, electroporation, or other transfection methods. You can select the transfection method and parameters based on cell type. Most adherent cells are compatible with various transfection methods, while electroporation is recommended for therapeutically relevant cells.

2. What should I do if the transfection efficiency in target cells is low?

A: Before the main experiment, conduct a transfection pre-experiment to explore different transfection methods and optimize conditions. Efficient transfection is essential for successful gene transposition.

3. How can I verify the transposition activity of the piggyBac Transposon System Kit?

A: The transposon plasmid in this kit contains the CopGFP gene. Successfully transposed cells will exhibit green fluorescence under a fluorescence microscope. Due to cell heterogeneity, transfection and transposition efficiencies may vary across cell types. To minimize transposition variability, optimize transfection methods for target cells.

4. Can cells constructed with the piggyBac Transposon System Kit be stably passaged?

A: Yes. The piggyBac Transposon System Kit targets genomic DNA for gene transposition, allowing the genotype of target cells to be stably inherited.

Note: Maintain transposed cells with half-dose puromycin for stability.

► Application Example

1. Project Information

Cell Name	MC38
Gene	Cas9

2. Detection of Transposition Results

MC38 cells were selected with puromycin for 2 weeks, resulting in MC38-PB-Cas9 transposed cells. The sgRNA targeting ITGA5 demonstrated a knockout efficiency of up to 95%, confirming high-activity Cas9 protein expression post-transposition in MC38 cells.

Figure 2. Sanger Sequencing Results of ITGA5 Gene Knockout in MC38-PB-Cas9 Cells

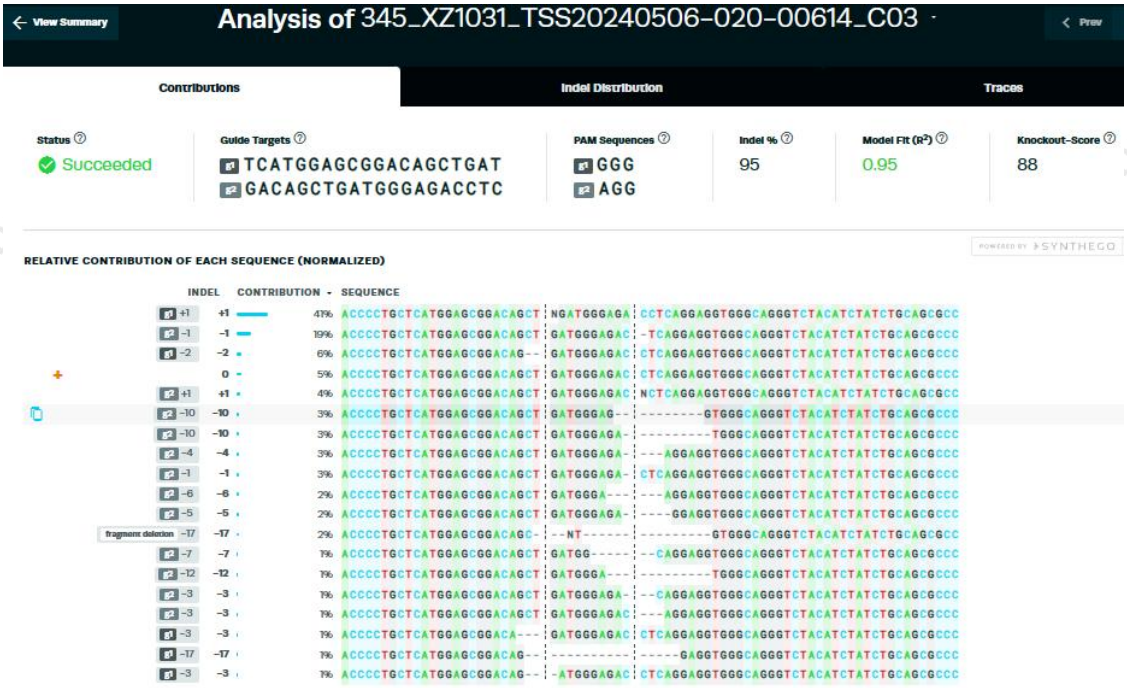


Figure 3. ICE Analysis of ITGA5 Gene Knockout Efficiency in MC38-PB-Cas9 Cells

► Notes

1. This product is intended for research use only in laboratory settings. Please comply with relevant laws, regulations, and ethical guidelines; any consequences from improper use are the sole responsibility of the user.
2. Follow the recommended guidelines for transportation, storage, and use of reagents. Avoid repeated freeze-thaw cycles unless necessary. The company is not responsible for experimental failures resulting from improper storage or handling.

► Appendix

EDITGENE provides the following transposon plasmids:

Plasmid Name	Description
PB-CMV-MCS-EF1α-CopGFP-T2A-Puro	Dual promoter transposon vector, CopGFP, Puromycin
PB-CAG-MCS-EF1α-CopGFP-T2A-Puro	Dual promoter transposon vector, CopGFP, Puromycin
PB-CMV-MCS-T2A-Puro	Single promoter transposon vector, Puromycin
PB-EF1α-MCS-T2A-Puro	Single promoter transposon vector, Puromycin
PB-CAG-MCS-T2A-Puro	Single promoter transposon vector, Puromycin