GENTAUR

DATA SHEET

PiggyBac™ Transposon Vector System

Cat. # PBxxx-1

User Manual

Store kit at -20°C on receipt

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(ver. 051011-001)

Contents

The PiggyBac Genetic Modification System enables researchers to:

- Alter the genomes of numerous animal species with a simple transfection
- Reprogram somatic cells into induced pluripotent stem (iPS) cells
- Perform highly efficient and cost-effective non-viral gene delivery
- Reverse genomic modifications with footprint-free transposon removal

The piggyBac DNA transposon technology is already being utilized in multiple research areas, such as gene therapy, regenerative medicine, cell line engineering, and animal model creation.

Researchers in academia and the pharmaceutical and biotechnology industries can now purchase *piggyBac* vectors produced with SBI's **high standards of manufacturing and quality control.** Transposagen's *piggyBac* technology will be paired with SBI's existing leading-edge technologies to further broaden the utility of *piggyBac*. SBI provides *piggyBac* in custom vectors and cell lines for customers. Transposagen and SBI have agreed to collaborate in the production of custom transgenic rats. The collaboration will combine SBI's genomic tools and expertise with Transposagen's *piggyBac* and rat spermatogonial stem cell technology. Researchers will now have access to specialized transgenic rats incorporating SBI's reagents, such as species-specific RNAi, MicroRNAs or anti-MicroRNAs. Rat models with inducible gene expression can also be produced using SBI's SparQ™ cumate switch system, the first inducible system that can be induced with a small molecule that can readily cross the blood-brain and blood-testis barriers.

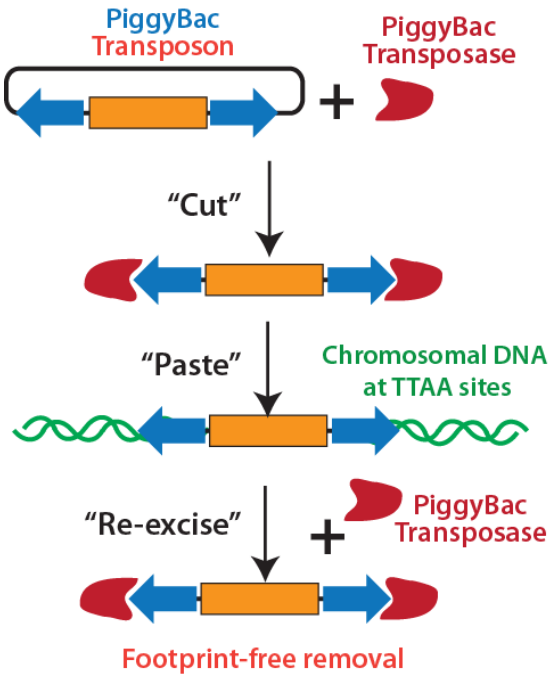
The PiggyBac Transposon System

A. Overview

The PiggyBac (PB) transposon is a mobile genetic element that efficiently transposes between vectors and chromosomes via a "cut and paste" mechanism. During transposition, the PB transposase recognizes transposon-specific inverted terminal repeat sequences (ITRs) located on both ends of the transposon vector and efficiently moves the contents from the original sites and efficiently integrates them into TTAA chromosomal sites. The powerful activity of the piggyBac transposon system enables genes of interest between the two ITRs in the PB vector to be easily mobilized into target genomes.

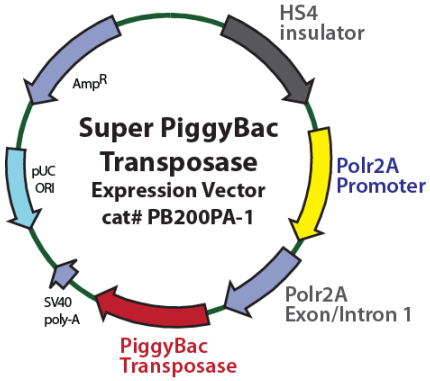
No cargo limit and is Reversible

The unique features of piggyBac transposons are that **there is NO Cargo Limit and it is also Reversible.** Genomes containing an inserted piggyBac vector can be transiently re-transfected with the PB tranposase expression vector. The PB transposase will remove the transposons from the genome, footprint-free.



B. PiggyBac transpositions

Co-transfect the Super PiggyBac transposase with your PiggyBac transposon vector



The Super PiggyBac transposase expression vector features a 5' HS4 insulator to support robust transcription from the rPolr2A promoter. The PiggyBac transposase coding sequence has been optimized for high expression, stability and activity in mammalian cells.

1. Clone the desired cDNA, microRNA or shRNA into the appropriate PB vector, sequence verify your clones.

2. Grow target cells to 60-80% confluency
3. For one well of a 6-well dish

   Combine:

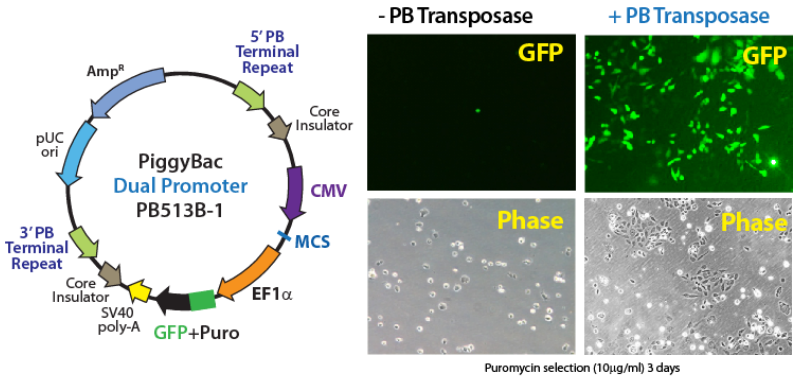
   2.0μg PB Transposon vector clone (ex. PB511B-1)
+ 0.8μg PiggyBac Transposase vector (PB200PA-1)*
   8.0μl SBI's PureFection transfection reagent
   50 μl of serum-free DMEM

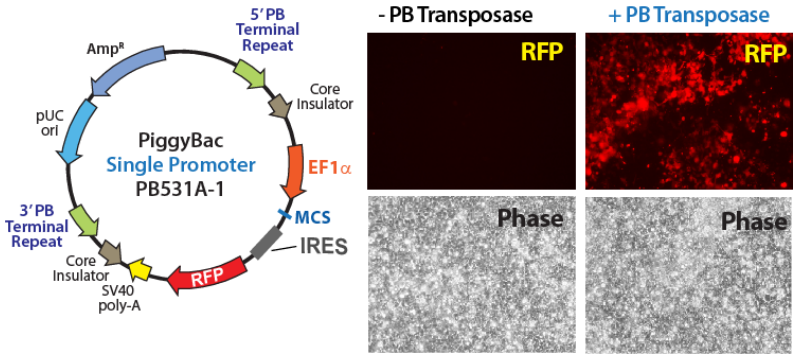
4. Vortex 15-30 seconds
5. Let stand 15 minutes at room temperature to allow PureFection/DNA complexes to form
6. Add drop-wise to cells culture and swirl to disperse
7. The PiggyBac transposase activity will terminate after 72 hours – but will integrate the transposon vector into genomes
8. Check for positive integrations after 3 days

   * We recommend using a 1:2.5 or 1:5 ratio of transposase to transposon vector ratio for transfections.

Single Tranposition data

The Super PiggyBac transposase transient expression vector and PB513B-1 were co-transfected into HeLa cells and puromycin selection applied for 10 days (10ug/ml). Cells efficiently transposed were Puro resistant and GFP positive. Human 293 cells were transfected with the Super PiggyBac transposase transient expression vector and PB531A-1. The cells were photographed after 7 days, virtually all cells were RFP positive.

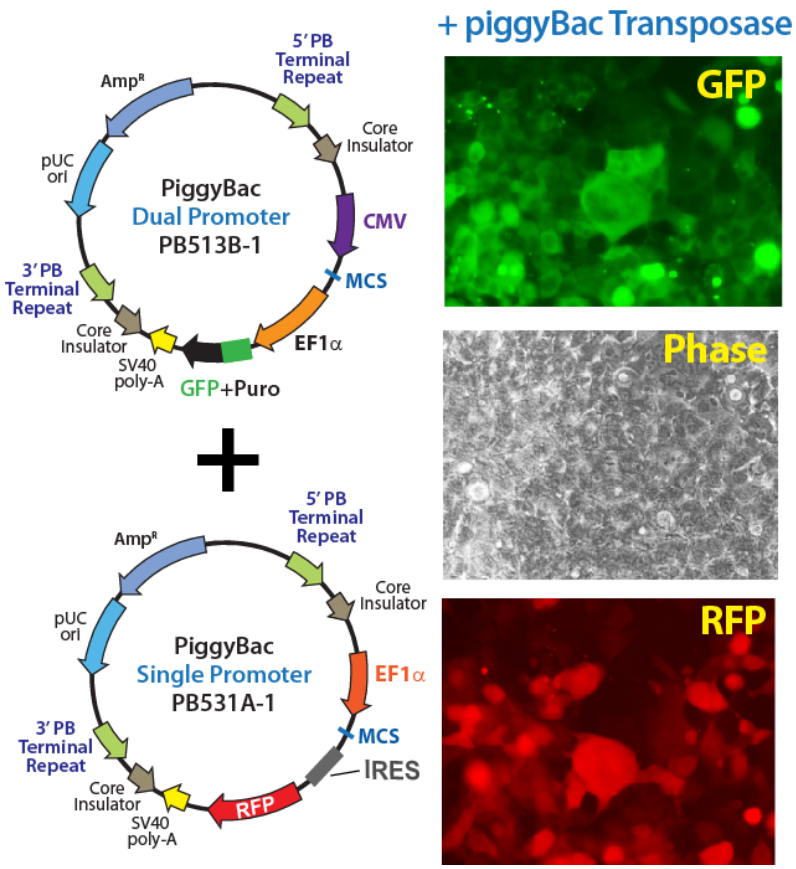




Double Tranposition data

Integrate multiple PB vectors simultaneously

The Super PiggyBac transposase transient expression vector and PB513B-1+PB531A-1 were co-transfected into Human 293 cells and Puromycin selection applied for 7 days (2μg/ml). The transposed cells were Puro resistant, GFP positive and RFP positive. Easily make novel cell lines and animal models with PiggyBac multiplexed transpositions.

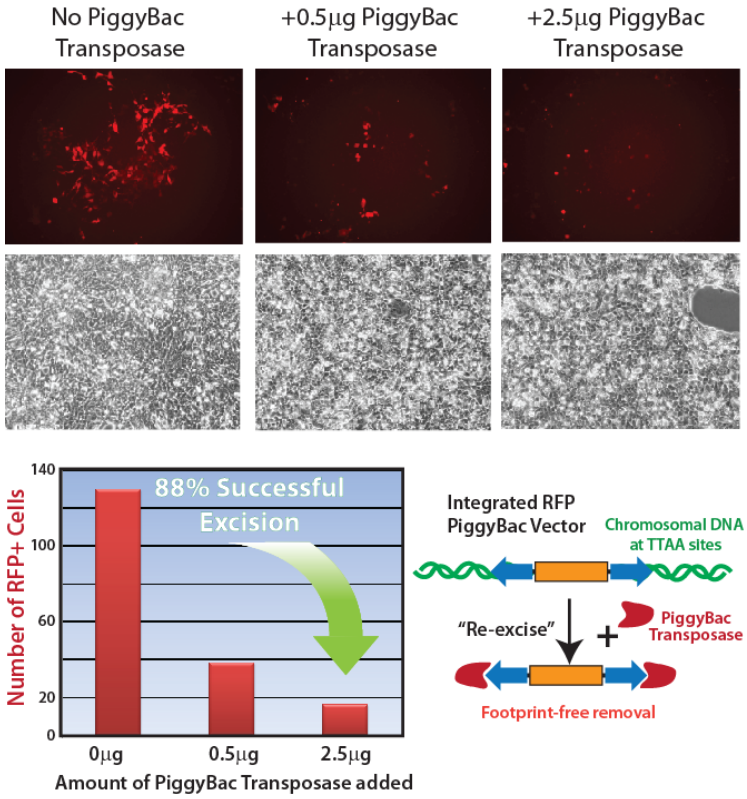


C. PiggyBac Transposon Re-excision

The integrated PiggyBac transposons can be successfully removed, footprint-free. Simply re-transfect your stably transposed cell line with increasing amounts of the Super PiggyBac transposase expression vector to mobilize and excise the integrated transposons. Shown below is an example of excising PB531A-1 RFP transposons from HEK293 cells using increasing amounts of re-transfected Super PiggyBac plasmid DNA (PB200PA-1). We recommend using increasing amounts of the Super PiggyBac transposase expression vector to achieve the desired re-excision rate.

Sample Re-excision data

We achieved greater than 88% removal of the integrated RFP transposon using 2.5ug of re-transfected Super PiggyBac transposase expression vector plasmid.



D. Inducible PiggyBac Cumate Switch

All-in-one inducible vector is leak-proof.

The inducible PiggyBac vector features the ultra-tight cumate switch combined with the EF1-CymR repressor-T2A-Puro cassette to establish stable cell lines. Expression of your cDNA or microRNA of interest can be switched on simply by adding cumate to the cells. The all-in-one single vector format offers superior control of induction with no background leakiness.

Co-transfect your target cells with the transposase and Cumate switch PiggyBac vectors. Example below is for one well of a 6-well plate.

1. Combine:

   2.0μg PB Cumate switch Transposon vector clone (PBQM531A-1)
   \+ 0.8μg PiggyBac Transposase vector (PB200PA-1)
   8.0μl SBI's PureFection transfection reagent
   50 μl of serum-free DMEM

2. Apply puromycin selection to establish positively-transposed cells (2-5μg/ml) for 3 days.

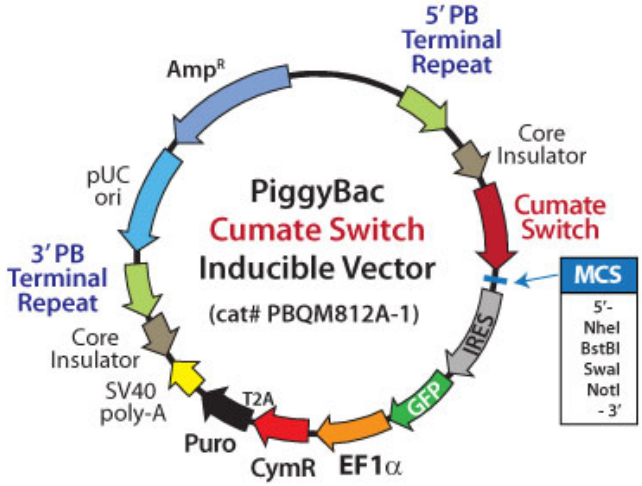
3. Change medium, maintain puromycin selection and titrate in the Cumate induction solution (10,000x high concentration, cat# PBQM100A-1). We recommend starting with a 1x concentration (equivalent to 30μg/ml cumate) and increasing the cumate up to 10x, equivalent to 300μg/ml to test the best induction in your model cell system.
   TO KEEP THE SWITCH ON, maintain the appropriate level of Cumate in the media after passages.

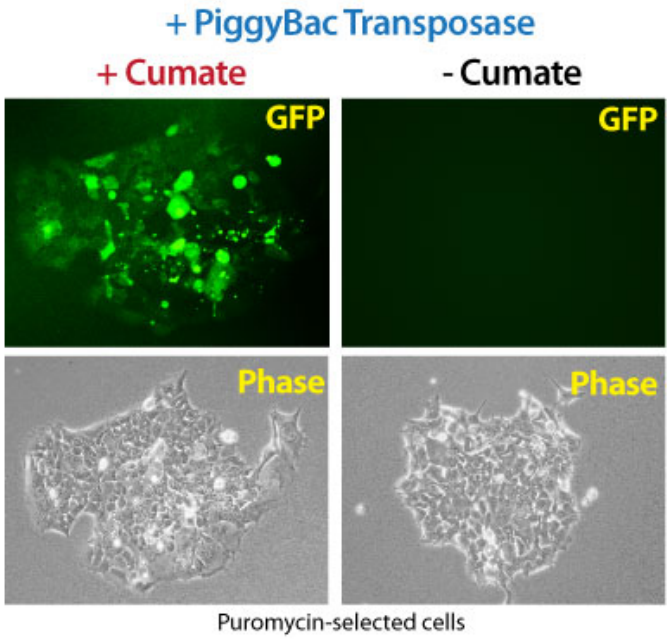
4. The induction should be immediate and you should be able to visualize induction of the GFP marker within 2-3 days.

5. **TO TURN BACK OFF** – simply rinse the cells once with fresh media and add back fresh media WITHOUT any Cumate. The cumate switch should turn off immediately and you should see the GFP levels reduce over 2-3 days.

Sample Induction data

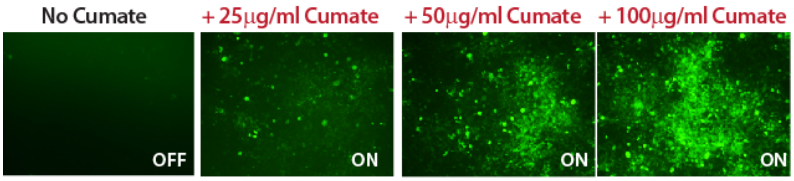
The PiggyBac Cumate Switch is absolutely leak-proof.



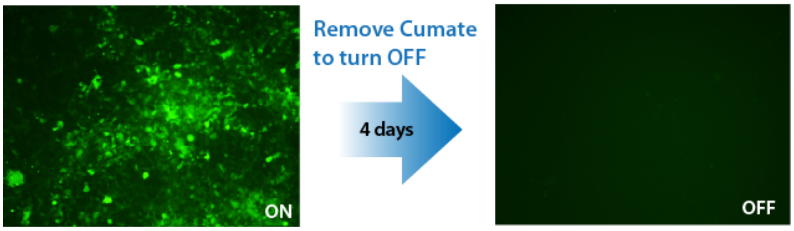


Puromycin-selected cells

PiggyBac Cumate Switch is Fully Titratable



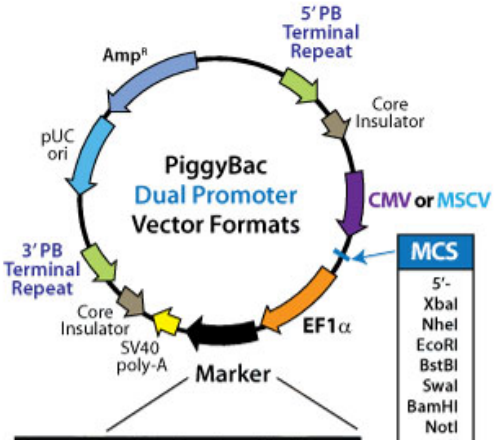
PiggyBac Cumate Switch can be Turned OFF



E. PiggyBac Transposon System Vectors

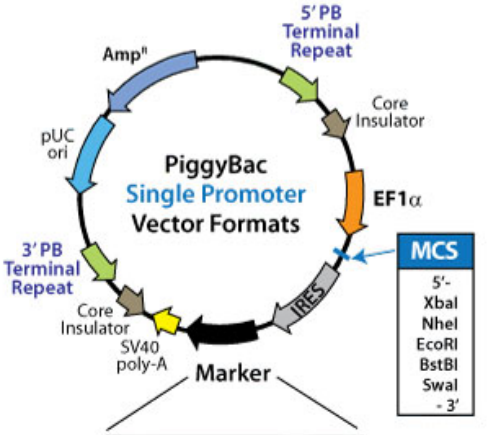
cDNA and microRNA expression vectors

The PB51x Dual Promoter Series.
High levels of expression from the CMV promoter (most cell types) and PB713B-1 features the MSCV promoter (active in stem cells). The multiple cloning site (MCS) located downstream of a promoter allows for convenient cloning of your gene or microRNA of interest. Downstream of your expression cassette is an EF1alpha promoter driving the expression of either the Puro, GFP, RFP, GFP+Puro or RFP+Puro markers. The entire cassette is flanked by genomic insulator elements for stabilized expression and PiggyBac Inverted Terminal repeats for mobilization and integration.



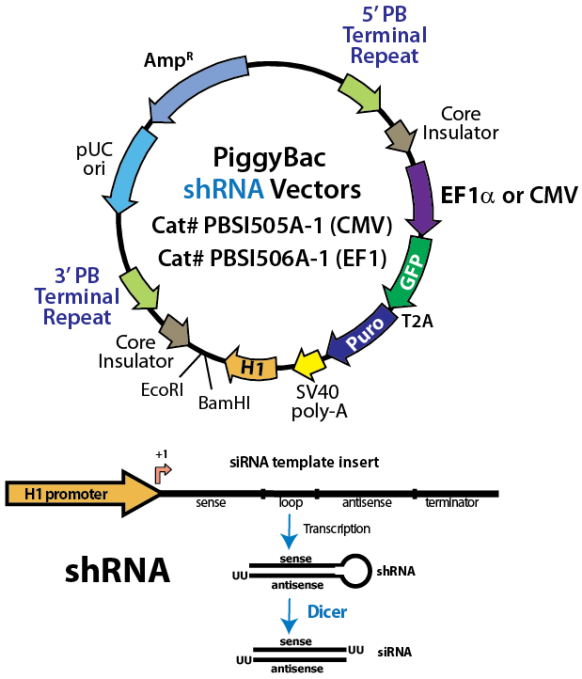
Promoter	Catalog #	Marker
CMV	PB510B-1	Puro
CMV	PB511B-1	GFP
CMV	PB512B-1	RFP
CMV	PB513B-1	GFP+Puro
CMV	PB514B-1	RFP+Puro
MSCV	PB713B-1	GFP+Puro

The PB53x EF1 Series with IRES Co-expressed markers.
SBI's PiggyBac IRES expression vectors use the EF1alpha promoter to drive expression of your cDNA or microRNA cloned into the MCS along with IRES-mediated co-expression of the marker. The markers available for this PiggyBac vector series include GFP, RFP and Neo.

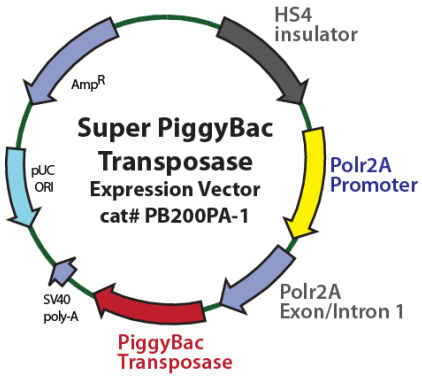


Catalog #	Marker
PB530A-2	GFP
PB531A-2	RFP
PB533A-2	Neo

shRNA expression vectors



PiggyBac Transposase expression vector



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