PiggyBac™ Transposon Vector System

Cat. # PBxxx-1

User Manual

Store kit at -20°C on receipt

Contents

Transposagen's PiggyBac Vectors are Distributed by System Biosciences

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.

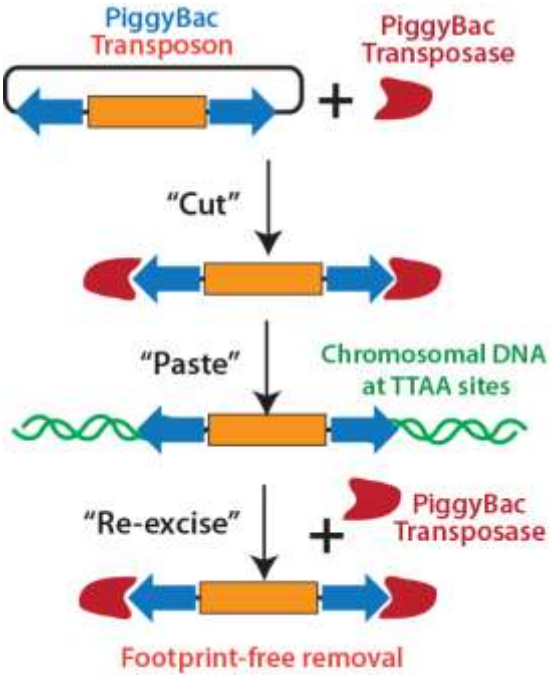
The PiggyBac Transposon System

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 transposase expression vector. The PB transposase will remove the transposons from the genome, footprint-free.

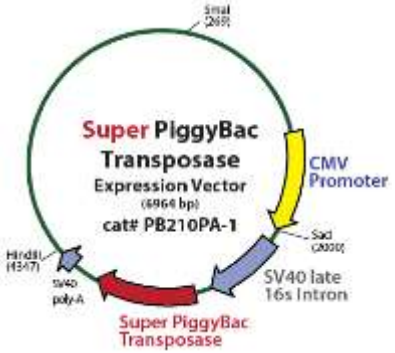


NOTE:
Grow your construct in LB+Carbenicillin (or 50 μg/ml Ampicillin) overnight, shaking at 30°C using Stbl2 or Top10 cells, do NOT use DH5α. This is important to avoid potential, undesired piggyBac vector recombination events.

B. PiggyBac transpositions

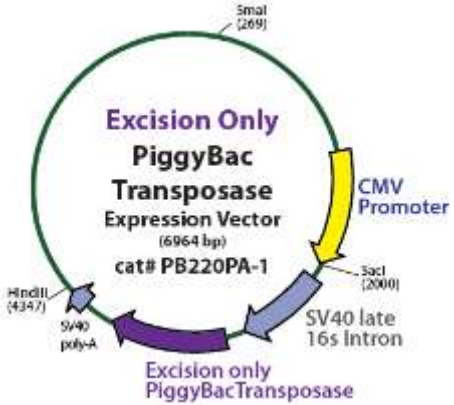
Co-transfect the Super PiggyBac transposase with your PiggyBac transposon vector for integration and then use the Excision only PiggyBac transposase for footprint-free transposon removal.

Integration PB Transposase



The Super PiggyBac transposase expression vector features a strong CMV promoter to provide robust expression of the transposase. The PiggyBac transposase coding sequence has been optimized for high expression, stability and integration activity in mammalian cells.

Excision PB Transposase



The Excision only PiggyBac transposase expression vector features a strong CMV promoter to provide robust expression of the transposase. The engineered PiggyBac Excision only transposase coding sequence has been optimized for transposon excision only activity and no transposon integration activity in mammalian cells.

NOTE: *PB210PA-1 and PB220PA-1 plasmids are designed for single use and cannot be propagated in bacteria.*

Protocol for Piggybac Integration Into Target 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 (~1 x 10^6 cells)

   Combine:

   0.5 μg PB Transposon vector clone (ex. PB511B-1)
   \+ 0.2 μg PiggyBac Transposase vector (PB210PA-1)*
   8.0μl SBI's PureFection transfection reagent
   50 μl of serum-free DMEM

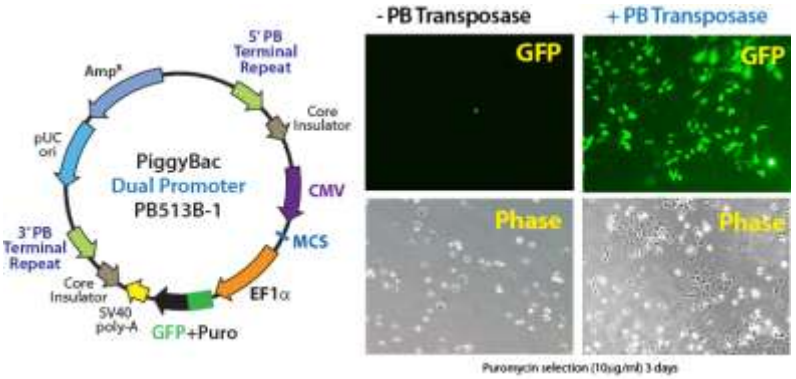
4. Vortex 15-30 seconds
5. Let stand 15 minutes at room temperature to allow PureFection/DNA complexes to form
6. Add drop-wise to cells culture and swirl to disperse
7. The PiggyBac transposase activity will terminate after 72 hours – but will integrate the transposon vector into genomes

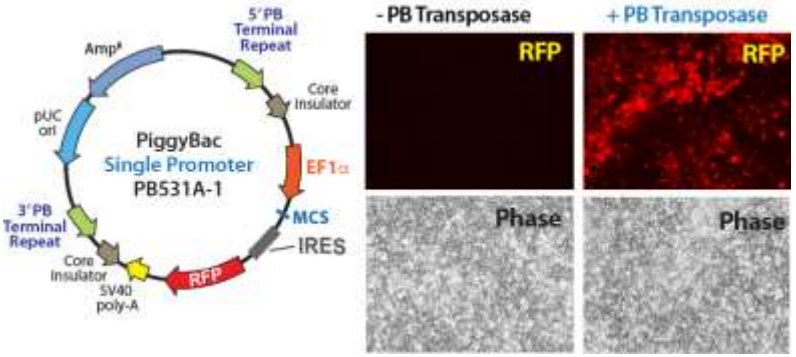
8. Check for positive integrations after 3 days

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

Single Transposition 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.

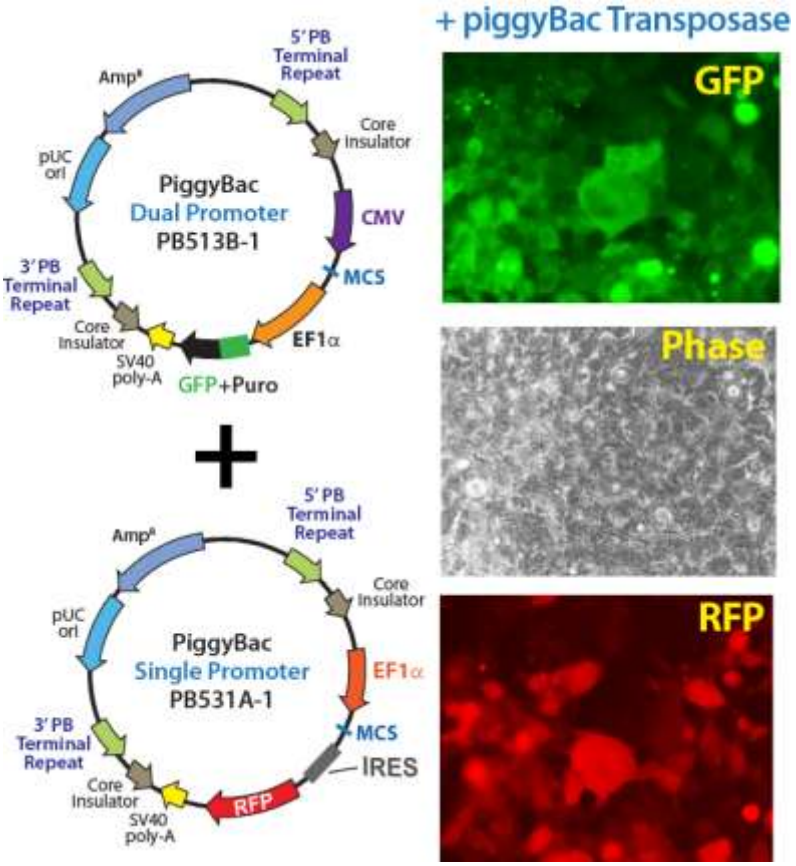




Double Transposition data

Integrate multiple PB vectors simultaneously

The Super PiggyBac transposase transient expression vector PB210PA-1 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

Integrated piggyBac transposons can be successfully removed, footprint-free using the engineered "Excision-only" piggyBac transposase expression construct (PB220PA-1). This engineered piggyBac transposase has two major advantages: 1) Integration deficiency and 2) Retains Excision capabilities. To seamlessly remove piggyBac transposons, you simply transfect your stably transposed cell line with the Excision only piggyBac transposase expression construct to mobilize and excise the integrated transposons.

Protocol for Piggybac Excision In Target Cells

1. Transfer cells containing the piggybac transposon into a single well of a 6-well plate and grow to confluency (~1 x 10^6 cells/well).

   NOTE: If the cells have been previously growing under selection conditions (e.g. in Puro/Neo or other antibiotic markers) please passage the cells 1x or 2x in absence of selection marker prior to using the Excision-only transposase

2. For one well of a 6-well dish (~1 x 10^6 cells)

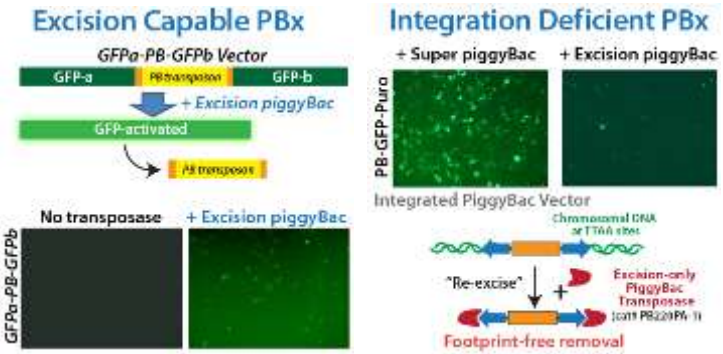
   Combine:

   0.2 μg PiggyBac Excision-Only Transposase vector (PB220PA-1)*
   8.0μl SBI's PureFection transfection reagent
   50 μl of serum-free DMEM

3. Vortex 15-30 seconds
4. Let stand 15 minutes at room temperature to allow PureFection/DNA complexes to form
5. Add drop-wise to cells culture and swirl to disperse
6. Assay for transposon removal in target cells 72hrs after transfection of the plasmid. Transposon copy number after excision in target cells can be assayed by SBI's Piggybac Copy Number Kit (Cat #PBC100A-1, Details on Section F, pg. 15)

Sample Re-excision data

Shown below is an example of the excision-only piggyBac transposase lack of integration activity and its excision capabilities. The left panel shows the comparison between the integration activities of Super PiggyBac transposase (PB210PA-1) and the inability of the Excision only piggyBac transposase (PB220PA-1) to integrate transposons. The panel to the right shows the seamless excision activity of PB220A-1. An engineered GFP expression cassette with a piggyBac transposon integrated right in the center of the GFP gene knocks out GFP expression. When the integrated piggyBac transposon is successfully excised using PB220PA-1, the reading frame is seamlessly re-joined and GFP is positively expressed. The Excision only piggyBac transposase is also not able to integrate transposons – just remove them.



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:

   0.5 μg PB Cumate switch Transposon vector clone (PBQM812A-1)
   \+ 0.2 μg PiggyBac Transposase vector (PB210PA-1)
   8.0μl SBI's PureFection transfection reagent
   50 μl of serum-free DMEM

2. After transfection of cells, split the cells into multiple wells of a 6-well plate (or a single 10cm plate) and grow them to confluency

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

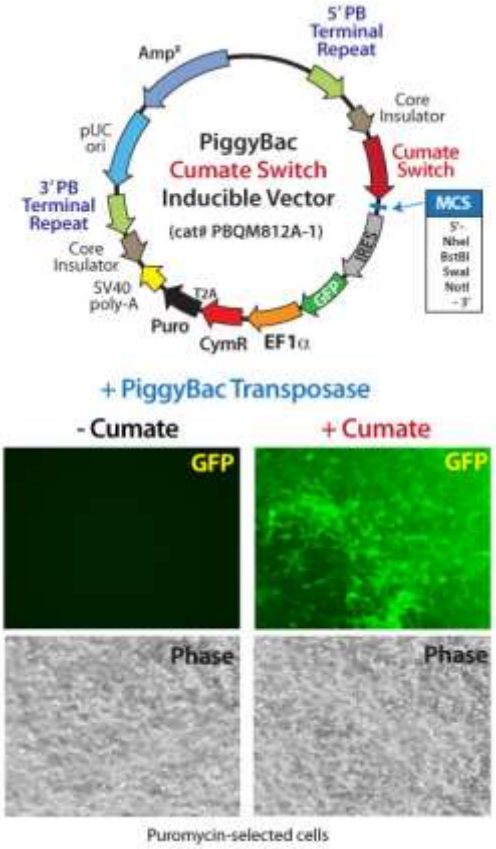
3. Change medium, maintain puromycin selection and titrate in the Cumate induction solution (10,000x high concentration, cat# PBQM100A-1). We recommend starting with a 1x concentration (equivalent to 30μg/ml cumate) and increasing the cumate up to 10x, equivalent to 300μg/ml to test the best induction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

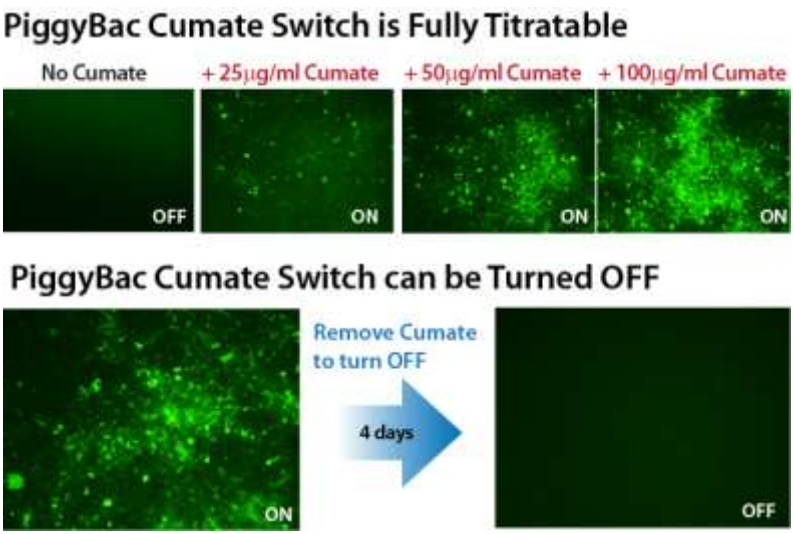
4. The induction should be immediate and you should be able to visualize induction of the GFP marker within 2-3 days.

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.

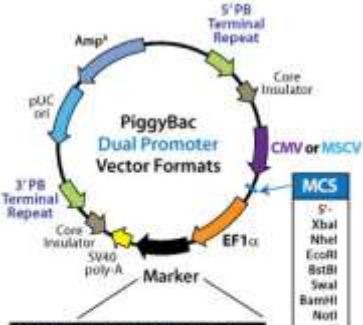



PiggyBac Cumate Switch is Fully Titratable
No Cumate
+ 25μg/ml Cumate
+ 50μg/ml Cumate
+ 100μg/ml Cumate
OFF
ON
ON
ON
PiggyBac Cumate Switch can be Turned OFF
ON
Remove Cumate to turn OFF
4 days
OFF

E. PiggyBac Transposon System Vectors

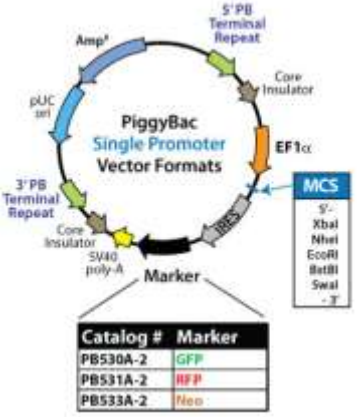
cDNA and microRNA expression vectors

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.



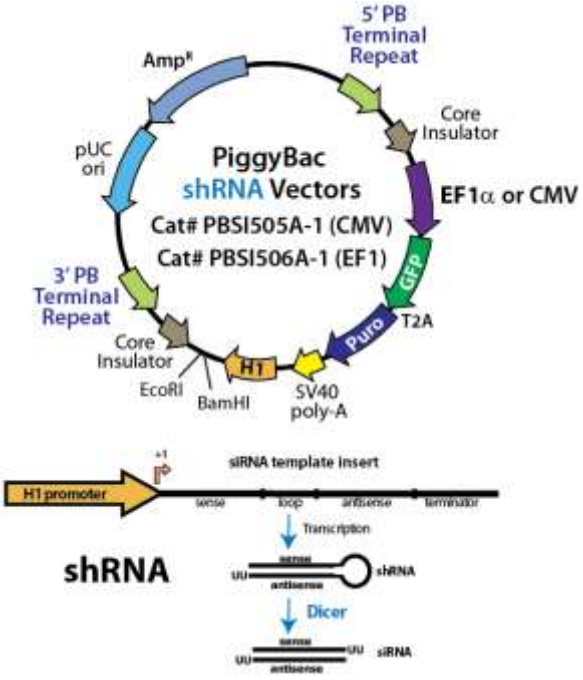
Promoter	Catalog #	Marker
CMV	PB510B-1	Puro
CMV	PB511B-1	GFP
CMV	PB512B-1	RFP
CMV	PB513B-1	GFP+Puro
CMV	PB514B-1	RFP+Puro
MSCV	PB713B-1	GFP+Puro

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.

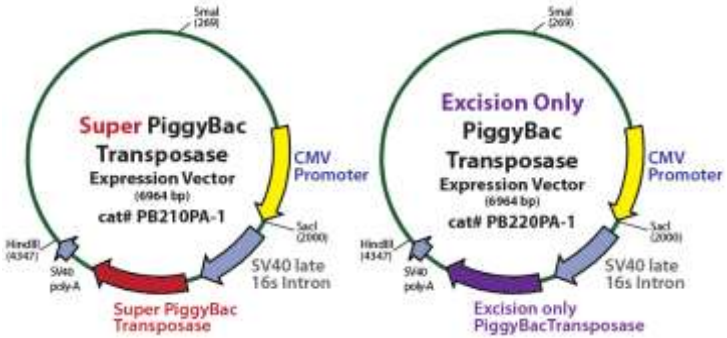


Catalog #	Marker
PB530A-2	GFP
PB531A-2	RFP
PB533A-2	Neo

shRNA expression vectors



PiggyBac Transposase expression vectors



F. PiggyBac copy number kit

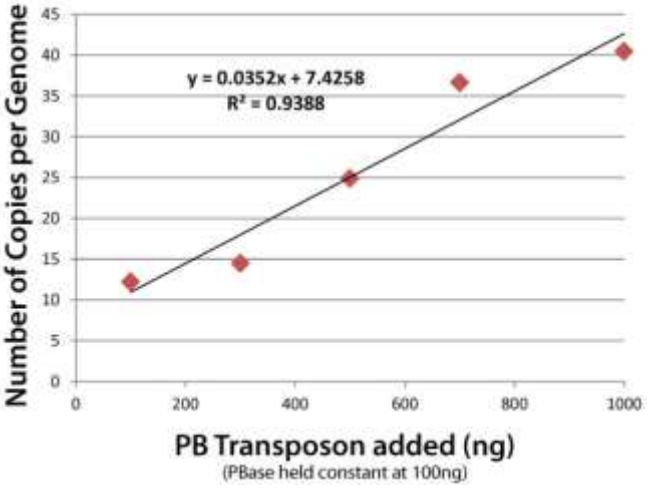
To determine the number of piggyBac transposon integrations, SBI has developed a qPCR-based system to measure the copy number of transposons in genomes relative to a genomic counting primer set (cat# PBC100A-1). The UCR1 primer mix counts the number of genomes and the PBcopy primer set measures the number of piggyBac transposons present in the transgenic genome. The PBcopy primer set is compatible with all of SBI's piggyBac transposon vectors. The copy number of piggyBac transposons is calculated using the cycle threshold (Ct) values of the UCR1 signals relative to the PBcopy signals. The PBC100A-1 copy number kit comes with primers and cell lysis buffer reagents enough for 20 copy number determinations.

NOTE: Your cells must be passaged at least once before performing this copy number measurement to ensure that residual, non-integrated piggyBac transposon plasmid will not interfere with the qPCR.

Ratios of PB transposon to PB transposase can be done to adjust the copy number of integrations. First, average the Ct values for the UCR1 and PBcopy measurements. The Delta-delta ($\Delta\Delta$) Ct calculation = 2^-(avg PBcopy Ct - avg UCR1 Ct)

The copy number is determined by dividing the $\Delta\Delta$ Ct by 2, as there are 2 copies of the UCR sequence per genome.

Transposon (ng)	Transposase (ng)	Pbcopy avg Ct	UCR1 avg Ct	ΔΔCt: 2^-(Pbcopy-UCR1)	copy# (ΔΔCt/2)
100	100	23.82	28.43	24.47	12
300	100	19.88	24.74	29.00	15
500	100	19.05	24.69	49.76	25
700	100	18.77	24.97	73.29	37
1000	100	18.15	24.49	80.92	40



Materials provided in kit

- 25x UCR1 primer mix
- 25x PBcopy primer mix
- 1X cell Lysis buffer

Materials required but not included

- 2X SYBR green mastermix
- qPCR instrument
- 1x PBS

PROTOCOL

Genomic DNA preparation from cells in a 12 well culture plate

1. Remove media and wash cells with 1mL, 1x PBS.
2. Remove all of PBS and add 250uL Lysis buffer to each well. You may freeze cells at -80°C until ready. It is recommended to perform one freeze-thaw cycle with the Lysis buffer to ensure complete cellular lysis, then thaw the plate at room temperature.

3. Pipet cells up and down to detach them, and remove cell clumps.
4. Transfer cell lysates in a 1.5ml eppendorf tube.
5. Heat lysates at 95°C, 2 minutes.
6. Centrifuge at 13,000 rpm, 2 minutes in a standard microcentrifuge to pellet debris.
7. Transfer supernatant to a new tube and place tubes on ice or store at -20°C until ready to proceed.

qPCR set-up for copy number quantitation, 384 well plate

per reaction for PB detection

water	4.75 uL
2x SYBR Green	6.25 uL
PB copy primer mix (5uM)	0.5 uL
lysate (≤500ng DNA)	0.5 uL

per reaction for genomic DNA detection

water	4.75 uL
2x SYBR Green	6.25 uL
25x UCR1 primer mix	0.5 uL
lysate (≤500ng DNA)	0.5 uL

1. Prepare 2 mastermixes of water, 2x SYBR green, and primers (one for UCR1 and one for PBcopy primers) for all samples including duplicates. (e.g. double all reaction ingredients and multiply by the number of samples).
2. Aliquot master mixes in each well in 96-well plate
3. Add 1uL of lysate, and mix by pipetting up and down.
4. Transfer 10uL of lysate/SYBR mix into duplicate wells in a 384-well plate.
5. Place seal cover and briefly spin to collect all the liquid.
6. Use standard qPCR programs.

G. Technical Support

For more information about SBI products and to download manuals in PDF format, please visit our web site:

http://www.systembio.com

For additional information or technical assistance, please call or email us at:

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General Information: info@systembio.com
Technical Support: tech@systembio.com
Ordering Information: orders@systembio.com

II. Licensing and Warranty Statement

Limited Use License

Use of the PiggyBac™ Transposon Vector System (*i.e.*, the "Product") is subject to the following terms and conditions. If the terms and conditions are not acceptable, return all components of the Product to System Biosciences (SBI) within 7 calendar days. Purchase and use of any part of the Product constitutes acceptance of the above terms.

The purchaser of the Product is granted a limited license to use the Product under the following terms and conditions:

The Product shall be used by the purchaser for internal research purposes only. The Product is expressly not designed, intended, or warranted for use in humans or for therapeutic or diagnostic use.

The Product may not be resold, modified for resale, or used to manufacture commercial products without prior written consent of SBI.

This Product should be used in accordance with the NIH guidelines developed for recombinant DNA and genetic research.

SBI has pending patent applications related to the Product. For information concerning licenses for commercial use, contact SBI.

Purchase of the product does not grant any rights or license for use other than those explicitly listed in this Licensing and Warranty Statement. Use of the Product for any use other than described expressly herein may be covered by patents or subject to rights other than those mentioned. SBI disclaims any and all responsibility for injury or damage which may be caused by the failure of the buyer or any other person to use the Product in accordance with the terms and conditions outlined herein.

Limited Warranty

SBI warrants that the Product meets the specifications described in this manual. If it is proven to the satisfaction of SBI that the Product fails to meet these specifications, SBI will replace the Product or provide the purchaser with a refund. This limited warranty shall not extend to anyone other than the original purchaser of the Product. Notice of nonconforming products must be made to SBI within 30 days of receipt of the Product.

SBI's liability is expressly limited to replacement of Product or a refund limited to the actual purchase price. SBI's liability does not extend to any damages arising from use or improper use of the Product, or losses associated with the use of additional materials or reagents. This limited warranty is the sole and exclusive warranty. SBI does not provide any other warranties of any kind, expressed or implied, including the merchantability or fitness of the Product for a particular purpose.
SBI is committed to providing our customers with high-quality products. If you should have any questions or concerns about any SBI products, please contact us at (888) 266-5066.