# Supporting Information

## Wang *et al.* 10.1073/pnas.0801017105

### SI Materials and Methods

**Plasmid Construction.** *CAGG-PBase* (pCyL43) was constructed as follows: *PBase* was PCR amplified from atub-pBac-K10 (*Drosophila* Genomics Resource Center, Barcode1155) with primers PBase-5′ (5′-GAT TAA GGA TCC AAA TGG GTA GTT CTT TAG ACG ATG AG-3′) and PBase-3′ (5′-CAT CTA GCG GCC GCA GTC AGA AAC AAC TTT GGC AC-3′) and cut with BamHI and NotI. The fragment was ligated into a vector under the control of CAG promoter.

*PB* transposon (pCyL50) was constructed as follows: PB-5′*TR* was PCR amplified from (with *Drosophila* Genomics Resource Center, Barcode1156) with PB-5′*TR*-5′ (5′-GTC TTA GGT ACC TCG CGC GAC TTG GTT TGC-3′) and PB-5′*TR*-3′ (5′-CCA ATT GCT AGC CAA CAA GCT CGT CAT CGC-3′) and cut with KpnI-NheI. PB-3′TR was PCR amplified with PB-3′TR-5′(5′-CGT ATG CTA GCT TAA TTA ACG AGA GCA TAA TAT TGA TAT-3′) and PB-3′TR-3′ (5′-GGA TAT GAG CTC GGT ATT CAC GAC AGC AGG-3′) and cut with NheI–SacI. The two fragments were ligated into pBlueScript and an oligo containing AscI was inserted between NheI and SacI.

*PB-PGK-Neo-bpA* was constructed by cloning a *PGK-EM7-Neo-bpA* cassette (1) into pCyL50. Construction of *PB-SB* hybrid transposon was as follows: *SB* 5′ LTR was PCR amplified with primers *SB*-5′ LTR-F (5′-GCG ATC GCC GAG CTA CAG TTG AAG TCG GAA GTT T-3′) and *SB*-5′ LTR-R (5′-GGA TCC GGC CGG CCC GGG CCT GCA GGC TAG AGT AAG CTT CTA AAG CCA TGA CAT CAT-3′) and cut with AsiSI and BamHI. An MluI–BamHI fragment containing *SB* 3′LTR was cut from pQS67 (Q. Su and P. Liu, unpublished data). The two fragments were ligated into pCyL50 digested with AscI and PacI.

PB-SB-PGK-Neo-bpA was constructed by cloning a PGK-EM7-Neo-bpA cassette into *PB-SB*. *PB-SB-SA-βgeo* was constructed by cloning a *SA-βgeo* cassette (2) into *PB-SB*. *PB-SB-SAβgeo-PGK-Bsd* was constructed by inserting a *PGK-EM7-Bsd-bpA* cassette (3) into *PB-SB-SA-βgeo*.

The *PGK-[PB-SB-SA-βgeo-PGK-Bsd]-Puro* cassette was constructed by inserting the *PB-SB-SA-βgeo-PGK-Bsd* cassette into *PGK-Puro-bpA*. The *Rosa-PGK-[PB-SB-SAβgeo-PGK-Bsd]-Puro* single transposon targeting vector was constructed by cloning the *PGK-[PB-SB-SAβgeo-PGK-Bsd]-Puro* cassette into a *Rosa26* targeting vector with a *MC1-tk* negative selection marker, pL471 (P. Liu, unpublished data). The *Rosa-PBase* transposase targeting vector was constructed by cloning a *SA-PBase-bpA* cassette and a floxed *SA-Neo-bpA* cassette into pL471.

**Cell Transfection.** AB1 and AB2.2 mouse ES cells were cultured as described in ref. 4. AB1 or AB2.2 cells ($1 \times 10^7$) were used for each electroporation. Immediately after electroporation, cells were diluted and seeded onto 6-cm feeder plates. G418 (150 μg/ml), puromycin (3 μg/ml) or blasticidine (10 μg/ml) selection was initiated after 48 h, and continued for 8 days. For each electroporation, diluted cells were also cultured without drug as a control for the plating efficiency. At the end of the selection, surviving colonies were stained by Methylene blue and counted after extensive washing with water.

For gene targeting, 20 μg of linearized *Rosa-PGK-[PB-SB-SA-βgeo-PGK-Bsd]-Puro*, *Rosa-PBase* or *Rosa-SB11* targeting vector were electroporated into $1 \times 10^7$ AB2.2 cells. *Rosa-PBase* and *Rosa-SB11* targeting were selected with 150 μg/ml G418 plus 2 μM gancyclovir. *Rosa26-PGK-[PB-SB-SA-βgeo-PGK-Bsd]-Puro* targeting was selected with 10 μg/ml blasticidine plus 2 μM gancyclovir. Correctly targeted clones were screened by long-range PCR using primers bpA-F (5′-GAC CCT GGA AGG TGC CAC TCC CAC TG-3′) and Rosa-3′-R (5′-GCA TCA GGA TAA AGT TCT TGC-3′), and by Southern analysis.

For *Rosa-PBase* and *Rosa-SB11* alleles, the *lox*P glanced selection marker was popped out by transient expression of Cre. 20 μg of supercoiled *PGK-Cre-bpA* plasmids were electroporated into $1 \times 10^7$ *Rosa-PBase* or *Rosa-SB11* cells. After electroporation, diluted cells were seeded onto feeder plates for 10 days. Single colonies were picked and confirmed by drug sib-selection and by Southern analysis.


1. Liu P, Jenkins NA, Copeland NG (2003) *Genome Res* 13:476–484.
2. Zambrowicz BP, *et al.* (1997) *Proc Natl Acad Sci USA* 94:3789–3794.
3. Liu P, Jenkins NA, Copeland NG (2002) *Nat Genet* 30:66–72.
4. Ramirez-Solis R, Davis AC, Bradley A (1993) *Methods Enzymol* 225:855–878.

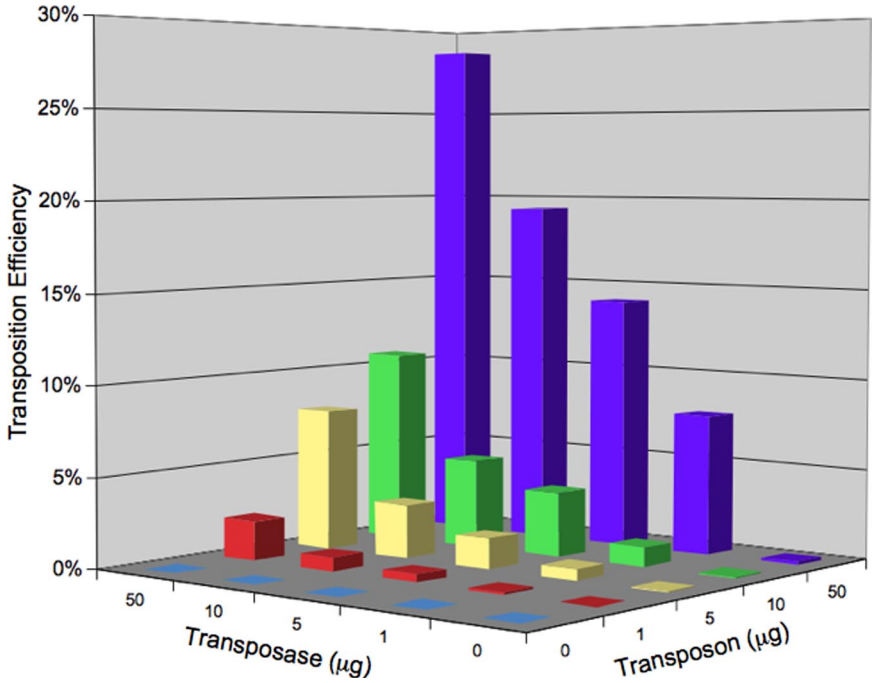


**Fig. S1.** *PB* transposition in mouse ES cells. Different amounts of *CAGG-PB*ase helper and *PB-PGK-Neo-bpA* donor plasmids were transfected into ES cells. Transposition efficiency was calculated as the percentage of $G418^R$ cells verses all of the cells surviving the electroporation. The transposition efficiency of each combination is the average obtained from three independent experiments.

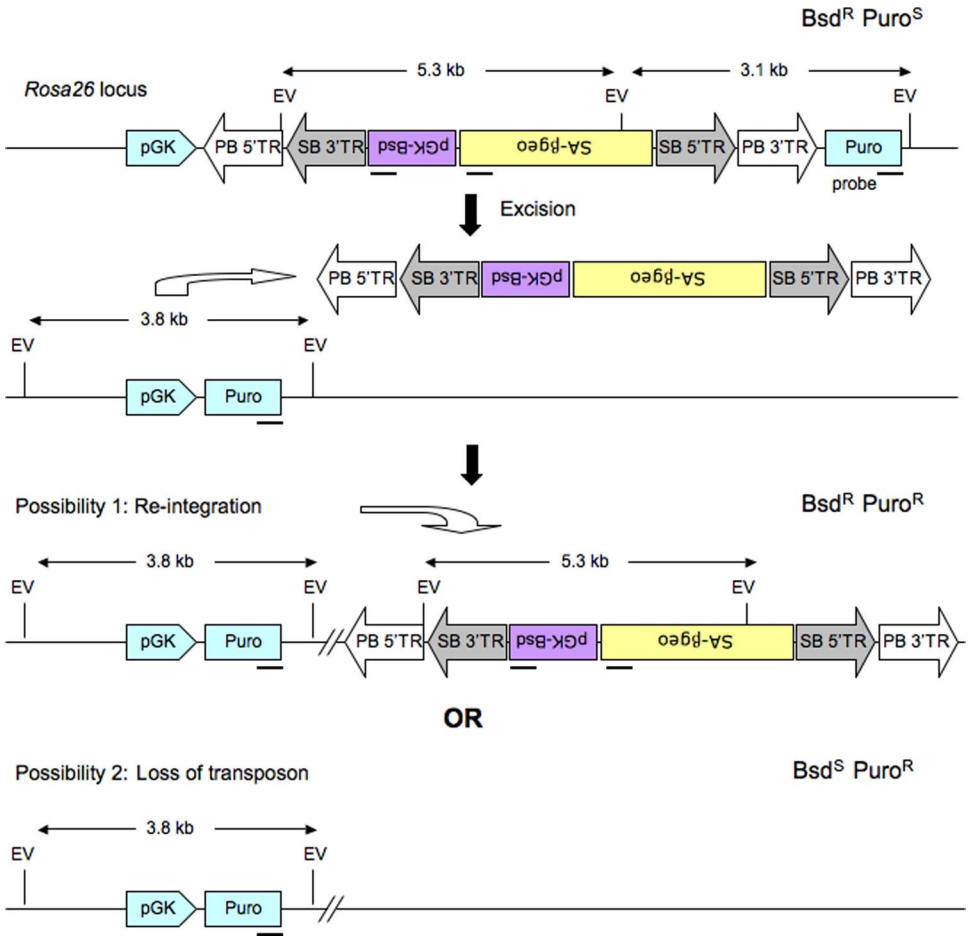


**Fig. S2.** Characterization of *PB* transposon from *Rosa26* locus by Southern analysis. A *bpA* probe was used to detect excision and reintegration of *PB* transposon. Three copies of the *bpA* sequence are present both inside and outside the transposon in the targeted cassette, which are in the *βgeo*, *Bsd* and *Puro* selection markers, respectively. EcoRV digestion identifies two restriction fragments, 3.1 kb and 5.3 kb, in the transposon targeted clones. When the transposon is mobilized, the 3.1-kb fragment changes to 3.8 kb. If the excised transposon reintegrates into the ES cell genome, the 5.3-kb EcoRV fragment will remain unchanged.

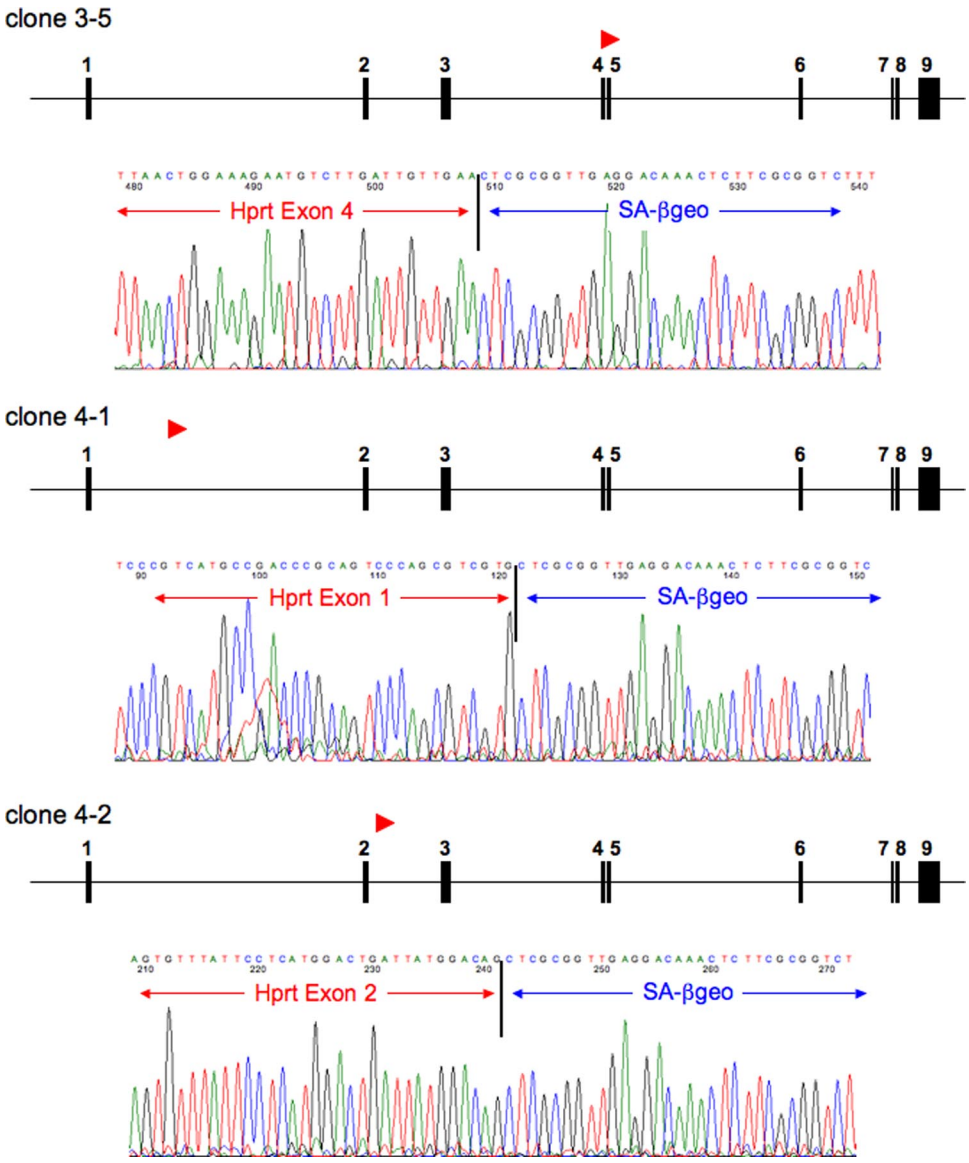


**Fig. S3.** Disruption of the *Hprt* locus by *PB* transposon integration. Transposon integration sites in three independent 6-TG$^R$ clones were mapped to the *Hprt* introns 1 (clone 4-1), 2 (clone 4-2), and 4 (clone 3-5), respectively. Analysis of the RT-PCR products from these clones identified the anticipated *Hprt*-LacZ fusion transcripts as depicted.

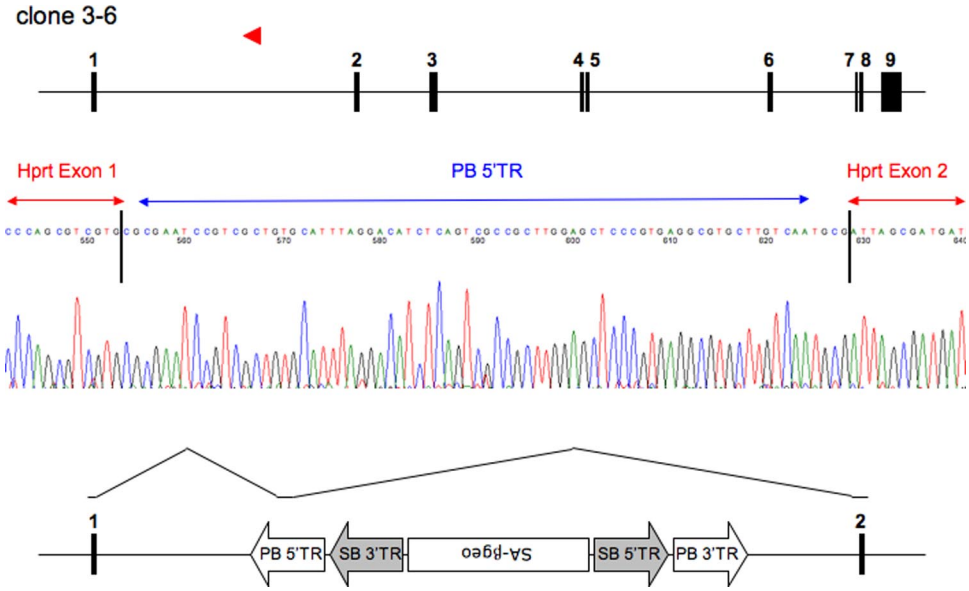


**Fig. S4.** *PB 5′TR* is mutagenic. In one of the 6-TG$^R$ clones (clone 3–6), *PB* integrates into *Hprt* intron 1 in the opposite direction to the other PB integrations shown in Fig. S3. Sequence analysis of the RT-PCR products demonstrated that a 74-bp PB 5′TR sequence was inserted in between *Hprt* exons 1 and 2 and caused a frame shift in the *Hprt* fusion transcript. This fusion transcript suggests that *PB 5′TR* contains a pair of cryptic splice acceptor and donor sequences.

**Table S1. Direct comparision of PB and SB transposon systems.**

| Cell line | Transposon | Average trapping efficiency |
|---|---|---|
| ROSA-SB11 KI | 10 $\mu$g of methylated PB-SB-SA-$\beta$geo | 0.000017 $\pm$ 0.000005 |
| ROSA-SB11 KI | 10 $\mu$g of unmethylated PB-SB-SA-$\beta$geo | 0.000002 $\pm$ 0.000000 |
| ROSA-IFP2 KI | 10 $\mu$g of methylated PB-SB-SA-$\beta$geo | 0.000278 $\pm$ 0.000013 |
| ROSA-IFP2 KI | 10 $\mu$g of unmethylated PB-SB-SA-$\beta$geo | 0.003275 $\pm$ 0.000338 |
| ROSA-SB11 KI | 1 $\mu$g of PB-SB-pGK-Neo | 0.000027 $\pm$ 0.000005 |
| ROSA-SB11 KI | 10 $\mu$g of PB-SB-pGK-Neo | 0.000253 $\pm$ 0.000020 |
| ROSA-IFP2 KI | 1 $\mu$g of PB-SB-pGK-Neo | 0.035371 $\pm$ 0.002362 |
| ROSA-IFP2 KI | 10 $\mu$g of PB-SB-pGK-Neo | 0.170960 $\pm$ 0.008113 |

## Other Supporting Information Files

Dataset S1
Dataset S2
Dataset S3
Dataset S4
Dataset S5
Dataset S6
Dataset S7
Dataset S8