Generation of an inducible and optimized piggyBac transposon system†

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ABSTRACT

Genomic studies in the mouse have been slowed by the lack of transposon-mediated mutagenesis. However, since the resurrection of Sleeping Beauty (SB), the possibility of performing forward genetics in mice has been reinforced. Recently, piggyBac (PB), a functional transposon from insects, was also described to work in mammals. As the activity of PB is higher than that of SB11 and SB12, two hyper-active SB transposases, we have characterized and improved the PB system in mouse ES cells. We have generated a mouse codon-optimized version of the PB transposase coding sequence (CDS) which provides transposition levels greater than the original. We have also found that the promoter sequence predicted in the 5'-terminal repeat of the PB transposon is active in the mammalian context. Finally, we have engineered inducible versions of the optimized piggyBac transposase fused with ERT2. One of them, when induced, provides higher levels of transposition than the native piggyBac CDS, whereas in the absence of induction its activity is indistinguishable from background. We expect that these tools, adaptable to perform mouse-germline mutagenesis, will facilitate the identification of genes involved in pathological and physiological processes, such as cancer or ES cell differentiation.

INTRODUCTION

Forward genetics, where the observation of a phenotype is followed by the identification of the responsible gene(s), provides a valuable tool to carry out functional genomics. The availability of systems to generate tagged mutations on a large scale facilitates this kind of approach, permitting the production of collections of genetically modified cells or organisms which can then be phenotypically screened (1). The molecular label conferred by the tagging mutagen allows for a ready identification of the locus responsible for the observed phenotype. Due to their ability to mobilize around genomes, DNA transposons and retrotransposons have been widely used as tools to generate mutation libraries in a variety of organisms. DNA transposons are genetic elements consisting of inverted terminal DNA repeats (TRs) which in their naturally occurring configuration flank a transposase coding sequence (CDS). This transposase follows a ‘cut and paste’ mechanism to excise the transposon from its original genomic location and insert it into a new locus (2). Retrotransposons, however, use an RNA intermediary molecule and retrotranscription to ‘copy and paste’ themselves in different locations of the genome. In mammals, the lack of efficient transposon systems has largely precluded the application of this type of mutagenesis. However, the awakening of the use of DNA transposons and the recent success with synthetic retrotransposons in the mouse is opening wide the door of forward genetics in this model organism (3,4).

In 1997, Ivics et al. resurrected a Tc1-like DNA transposon by comparing the nucleotide sequences of a number of dormant inactive elements in salmonid fishes, predicting the active sequence and repairing the inactivating mutations. This elegant and meticulous approach resulted in an active transposon termed Sleeping Beauty (SB). The SB system was soon reported to be functional in human and murine cells (3,5,6). Moreover, since its resurrection, both the inverted repeats and the transposase coding sequence have been optimized, which has yielded an SB element with increased mobilization activity (7–11). This has been used in mammals for a wide range of applications, such as gene therapy, germline mutagenesis and somatic mutagenesis (8,12–15).

The activity of the system needs to be regulated, because excessive and uncontrolled transposition results in genomic instability such as inversions, deletions and translocations (14). These large-scale genomic rearrangements mask the more subtle and informative singular transposition events, interfering with the identification of single genes responsible for specific phenotypes. Thus, the availability of a highly active, though regulatable, transposase would be desirable.

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**Table 1.** Sequences and properties of the linkers used in the fusion PB transposases engineered in this work

| Version       | Nt       | Linker       | Ct       | pI       | Charge |
|---------------|----------|--------------|----------|----------|--------|
| ERT2-L1-mPB   | PATA-    | CEFAT-       | MGSS...  | 4        |        |
| mPB-L1-ERT2   | QSCF-    | AAA-         | SAGD...  | 5.57     |        |
| ERT2-L2-mPB   | PATA-    | CELSLGAAPDAAPGSEFAAT- | MGSS... | 3.57 |        |
| mPB-L2-ERT2   | QSCF-    | AAAASLGAAPDAAPGSAGA- | SAGD... | 3.8 |        |
| ERT2-L3-mPB   | PATA-    | CEFKARLGGAPAVGGGPKAADKF- | MGSS... | 9.05 | +      |
| mPB-L3-ERT2   | QSCF-    | AAAARLGGAPAVGGGPKAADKGA- | SAGD... | 9.53 | +      |

Columns from left to right: ‘Version’ indicates the different fusion transposases. ‘Nt’ indicates the aminoacidic sequence of the N-terminal half of the fusion protein immediately proximal to the linker. ‘Linker’ corresponds to the sequence of aminoacids between both halves of fusion protein. Bold aminoacids highlight the core linker sequences. ‘Ct’ indicates the aminoacidic sequence of the C-terminal half of the fusion protein immediately proximal to the linker. ‘pI’ corresponds to the isoelectric point of the linker. ‘Charge’ indicates the predicted sign of the net electric charge of the linker at intracellular pH conditions.

In addition to SB, piggyBac (PB), a transposable element originally isolated from the genome of the cabbage looper moth *Trichoplusia ni* has also been reported to be highly active when introduced into mammalian genomes, including human (16–18) and mouse cells (16). In a direct comparison experiment involving *in vitro* transfection, PB has been reported to be the most active of four tested transposons, including PB, SB11 (one of the hyperactive SB transposases), Tol2 and Mos1 (17). In a separate study, PB has also been shown to be more active than SB12, another hyperactive version of SB (18). Although there are other hyperactive SB transposases available, which apparently show higher activity than SB11 and SB12, they have not been directly compared to PB (19,20). Given its good performance in mammals we have characterized and optimized the PB system for its utilization in mouse ES cells and for somatic mutagenesis *in vivo*. We have generated a mouse codon-optimized version of the PB transposase CDS and we have observed that it provides levels of transposition considerably higher than those of the native PB CDS. We have analysed the differential properties of the 5′ and 3′-terminal repeats of the PB transposon, uncovering the existence of promoter activity in the 5′-terminal repeat. Finally, we have created a highly active and regulatable PB transposase, by fusing the optimized PB CDS to the modified ligand-binding domain of the oestrogen receptor, ERT2. We expect that this optimized transposon system will significantly expand the utility of transposon-based mutagenesis for the genetic characterization of the mouse genome.

**MATERIALS AND METHODS**

**Plasmids**

iPB and mPB were custom synthesized. Besides the PB transposase CDS, both sequences included a Kozak element around the start ATG at the 5′-end and two consecutive stop codons at the 3′-end. EcoRI and NotI flank the 5′ and 3′-ends, respectively, and they were used to clone both CDSs into pcDNA3 or pcDNA3-KzHA. To produce the donor plasmids, the *puro*ATK fusion gene and the bovine growth hormone polyA signal were PCR amplified from pFlexible (21). En2SA was PCR amplified from T2/Onc, kindly provided by Dr L. Collier, and the EMCV IRES sequence was sub-cloned from pPRIG, which was a gift by Dr P. Martin (12,22). The minimal 5′ and 3′-PB terminal repeats were custom synthesized and then combined by sub-cloning to produce the desired configurations. The Luc2 sequence corresponds to a HindIII/BamHI fragment from pGL4.13 (Promega). ERT2 was PCR amplified from a cDNA kindly provided by Dr P. Chambon and subcloned at the 5′ or at the 3′ of mPB. L1 linkers were created as a result of the cloning process. L2 and L3 linkers were introduced between mPB and ERT2 by sub-cloning the pairs of primers shown in Supplementary Table 1. The core aminoacidic sequence of the L3 linker was kindly provided by Dr Joseph Kaminski. The CDSs and protein sequences of mPB (GenBank accession number: EF587698) and mPB-L3-ERT2 (GenBank accession number: EF587699) are shown in Supplementary Figure 1.

**Cell culture and transfection**

AB2.2 ES cells were cultured on a layer of mitotically inactive SNL76/7 feeder cells and transfected by electroporation as described previously (23). Puromycin selection was conducted on SNLP 76/7-4 feeders (a puromycin resistant derivative of SNL76/7) with 3 μg/ml of puromycin. Puromycin resistant ES cell colonies were stained for 15 min with 1% methylene blue in 7% EtOH, washed in distilled water over night and air-dried. This protocol produced a very low background which allowed counting colonies with diameters ≥0.3 mm. COS-7 cells and HeLaS3 cells were grown in 6-well plates and with DMEM supplemented with 10% foetal bovine serum. Plasmids were transfected with Lipofectamine 2000 (Invitrogen), following manufacturer’s instructions.

**Western blot**

Transfected COS-7 cells were washed with PBS and each well was harvested with 500 μl 2 × Laemmli buffer. Fifteen microlitres of lysate were loaded per lane in a 4–12% gradient SDS–PAGE gel (Invitrogen). The gel was transferred to a Hybond-ECL™ (Amersham Biosciences) membrane, and the membrane was dried over night and blocked with 5% non-fat dry milk dissolved in PT (0.1% Tween-20 in 1 × PBS) for 1 h at room temperature.
Then, it was incubated with 12CA5 anti-HA monoclonal antibody (Abcam, cat #ab16918) or with anti-actin monoclonal antibody (Sigma, cat #A5316), both diluted 1:1000 in 3% non-fat milk dissolved in PT for 1 h at room temperature. The membrane was washed 3 times for 5 min with PT and incubated with the peroxidase-labelled anti-mouse antibody from the ECL™ Western Blot Analysis System (GE Healthcare) diluted 1/10000 in 1.5% non-fat dry milk for 1 h at room temperature. The membrane was washed again as before and specific protein bands were detected using ECL™ Western Blot Analysis System (GE Healthcare).

Luciferase assay
Transfected HeLaS3 cells were washed with PBS and each well was lysed for 15 min with 100 μl of 1× passive lysis buffer from the Dual Luciferase Reporter Assay System (Promega). Lysates were harvested and 20μl of each sample were analysed for luciferase activity following the manufacturer’s instructions in a MicroLumatPlus LB 96V luminometer (Berthold Technologies).

RESULTS
Increased transposition by codon-optimization of PB transposase
As the PB transposon was originally isolated from insect cells, we hypothesized that a mouse codon-optimization of the transposase CDS might produce higher levels of transposition in the murine ES cell context (24). To address this possibility we synthesized the native PB transposase CDS (hereafter iP) and a mouse codon optimized CDS version (hereafter mPB). iP corresponds to the original wild-type coding sequence of the PB transposase, whereas mPB is a synthetic sequence coding for the same polypeptide as iP, but where each codon has been changed for the preferred codon for translation in mouse cells. Both iP and mPB contain a Kozak sequence built around the start methionine codon. Besides this, potential cryptic splice sites generated during the codon optimization were detected and avoided in the optimized sequence.

Expression vectors were assembled by subcloning both versions of the transposase CDSs into pcDNA3. We compared their ability to promote transposition of a PB transposon carrying a promoterless puromycin resistance cassette from a transfected plasmid vector into the ES cell genome (Figure 1A). iP or mPB expression plasmids were co-electroporated with a transposon donor plasmid (5’nPTK-3’) into AB2.2 mouse ES cells and the numbers of puromycin-resistant colonies were assessed. mPB yielded considerably more puromycin-resistant colonies than iP (Figure 1B).

To address saturation effects the amounts of helper or donor plasmids were varied while keeping a constant amount of the respective donor or helper plasmid (Figure 1C and D). In both series of experiments, mPB provided higher levels of transposition than iP through-out the whole range of amounts of transfected plasmids. In these experimental conditions we did not observe overproduction inhibition, a phenomenon described in certain transposon systems in which transposition rates decline when the amount of transposase exceeds certain levels (10,14,18). Interestingly, the levels of transposition provided by iP in these series of experiments were far below those of mPB, especially in Figure 1D, where not even the highest amount of iP plasmid is able to match the levels of transposition obtained by transfection of the lowest amount of mPB. This is likely due to the stringent conditions (5 × 10^6 cells per transfection and 1 μg of donor plasmid) of this experiment, aimed at analysing saturation effects. Additional experiments performed in less-restrictive conditions showed that increasing amounts of iP provided levels of transposition in the same order of magnitude as those of mPB (Supplementary Figure 2), indicating that the differences between the performance of iP and mPB strongly depend on the experimental settings. Despite this, we must remark that mPB showed higher levels of transposition than iP in all the conditions tested.

To ascertain if the higher transposition frequency provided by the optimized sequence was caused by higher production of transposase we subcloned iP and mPB into pcDNA3-Kz-HA, a pcDNA3-based vector containing a Kozak sequence plus the nucleotide sequence encoding the hemagglutinin epitope (HA) (Figure 2A), enabling detection of protein production from HA-iP and HA-mPB by Western blot analysis using an anti-HA antibody. These two plasmids plus an empty pcDNA3-Kz-HA vector were transfected into COS-7 cells in triplicate. Analysis of protein lysates using an anti-HA monoclonal antibody revealed a clear band at the expected size (~68 kDa) in the cells transfected with transposase expression plasmids, but not in the cells transfected with the empty vector (Figure 2B). The amount of PB transposase in the cells transfected with HA-mPB was considerably and consistently higher than in the cells transfected with HA-iP, confirming that higher levels of transposase production are likely responsible for the greater transposition activity provided by mPB.

Differential features of the minimum 5’ and 3’-PB terminal repeats
The original PB transposon is flanked by 13-bp terminal inverted repeats and has additional inverted repeats 19-bp long located asymmetrically with respect to the end of the element (24). These inverted flanking sequences, although able to permit transposition between different plasmids in insect cells, were found to be insufficient to allow transposition from a donor plasmid to genomic DNA. In fact, additional transposon DNA sequence was found to be necessary to provide this kind of transposition (25,26). A 5’-terminal repeat (5’-TR) of 313 bp and a 3’-terminal repeat (3’-TR) of 235 bp have been described as the minimum PB terminal repeats (25). Combinatorial experiments performed with the SB transposon have shown that a configuration containing two ‘left’ terminal
repeats (or 5'-TRs) provides levels of transposition 3 times higher than the original ‘left’ + ‘right’ combination (11).

In order to study the properties of the 5' and 3'-TRs of the PB element and to explore the possibility of enhancing the rates of transposition, we subcloned a promoter-less puromycin resistance cassette between different combinations of minimum 5' and 3'-PB terminal repeats (Figure 3A) (5'-5', 5'-3', 3'-5' and 3'-3') and cotransfected the resulting donor plasmids with the mPB expression cassette into AB2.2 cells. Only the cells transfected with the 5'-3' and 3'-5' configurations yielded significant numbers of resistant colonies (Figure 3A). These results indicate that the PB transposon requires one 5' and one 3'-terminal repeat in order to achieve transposition.

The number of colonies obtained with the 5'-3' transposon was 4.6 times higher than with the 3'-5' configuration (Figure 3A). As we had used a promoter-less selection cassette, one explanation of this observation is that the promoter sequence predicted in the 5'-TR, which is functional in insect cells, is also active in the mammalian genome (24,27). To address this possibility, we subcloned a luciferase expression cassette (Luc2) downstream of the 5' and 3'-PB TRs (Figure 3B) and assayed luciferase activity in transiently transfected HeLaS3 cells. The luciferase activity observed with the 5'-TR-Luc2 construct was 5 times higher than that observed in the cells transfected with the 3'-TR-Luc2 construct (Figure 3B), indicating that the promoter in the 5'-TR repeat is also active in mammalian cells.

**Figure 1.** Comparison of transposase activity of the native and codon optimized piggyBac. (A) Schematic representation of the plasmids used in this experiment. The donor plasmid, 5'-PTK-3', contains the minimal PB terminal repeats (5'-TR and 3'-TR) flanking a promoter-less puromycin resistance cassette. CMV, cytomegalovirus promoter; ATG, start codon; pA, bovine growth hormone polyA signal; En2SA, mouse engrailed-2 gene splice acceptor; IRES, internal ribosomal entry site from encephalomyocarditis virus; puTK, synthetic fusion CDS between the puromycin-resistance gene and a truncated thymidine kinase gene (21). (B) 2 μg of 5'-PTK-3' and 2 μg of the transposase plasmid were co-electroporated into 10^7 AB2.2 mouse ES cells and, after puromycin selection, the numbers or resistant colonies were counted. (C) A fixed amount (1 μg) of transposase plasmid was co-electroporated with increasing amounts (1–40 μg) of 5'-PTK-3' into 5 × 10^6 AB2.2 ES cells and puromycin-resistant colonies were counted. (D) A fixed amount (1 μg) of 5'-PTK-3' transposon plasmid was co-electroporated with increasing amounts (1–40 μg) of transposase plasmid into 5 × 10^6 AB2.2 ES cells. Numbers of puromycin-resistant colonies are indicated. n = 3, error bars: SEM.

**Generation of an inducible PB transposase**

The success of cancer-gene identification experiments performed with the SB transposase in mice has been strongly determined by the levels of transposase activity (12,13). However, an excess of transposition has been reported to create undesired genomic rearrangements in an SB-based system for mouse germline mutagenesis (14). Thus, the ability to control transposition temporally would provide an additional advantage to the PB transposase. To explore the possibility of generating an inducible transposase we created fusion proteins between mPB and the modified oestrogen receptor ligand-binding domain (ERT2), at the N or C terminus of mPB (ERT2-mPB and mPB-ERT2, respectively) (28) (Figure 4A). The ERT2 domain provides the possibility of regulating...
the activity of a protein by the presence or absence of 4-hydroxytamoxifen (4-OHT). In the absence of 4-OHT a protein containing the ERT2 domain is sequestered by heat-shock proteins, preventing it from functioning (29). In the presence of 4-OHT, the fusion protein is released and can then play its role (28).

The two key requirements of inducible proteins are high activity under induced conditions and low/no activity in the absence of induction. To achieve the maximum activity and inducibility we evaluated a series of linkers between mPB and ERT2 of different lengths, charges and flexibilities (Table 1). Most of the fusion proteins appeared to have reduced activity. The L1 fusions show 100-fold reductions compared with mPB. The L2 fusions also exhibited reduced activity although one of them (ERT2-L2-mPB) was not leaky when not induced. However, the fusion proteins containing L3 linkers provided high levels of transposition (Figure 4B). The L3 linker was based on a sequence which has previously been used between the GAL4-DNA-binding domain and the native PB transposase, which resulted in levels of activity of the fusion protein close to 100% with respect to the wild-type enzyme (17). The number of colonies obtained in the presence of 4-OHT using mPB-L3-ERT2 was 6 times higher than those obtained with iP7, though it was less efficient than mPB. Importantly, the activity was highly 4-OHT-dependent, since in the absence of 4-OHT transposition was reduced more than 800-fold to background levels. To our knowledge, mPB-L3-ERT2 is the first PB transposase inducible by 4-OHT, it provides higher activity than iP7 and it shows undetectable levels of leakiness.

**DISCUSSION**

The recent availability of DNA transposons which are active in the mammalian genome has widened the set of tools to carry out functional genomics and transgenesis. SB, a transposon reconstructed from inactive sequences present in salmonid fishes, has already shown great utility, having been successfully used in cancer-gene identification, germline mutagenesis and human cell transgenesis experiments (3,8,12–14). More recently, PB, an active transposon from the cabbage looper moth *Trichoplusia ni*, has also been shown to work in mammalian cells, providing transposition levels higher than those displayed by certain hyperactive SB forms (16–18). These two transposable elements have distinctive properties, for instance target sequence preference, which suggests that they will be used as complementary tools to modify and study the mammalian genome (8,18,30,31).

We have characterized, optimized and further enhanced the functionality of the PB transposon/transposase system. We have synthesized a mouse-codon-optimized PB transposase CDS, which provides levels of transposition significantly higher than the native PB transposase CDS over a wide range of transposon/transposase ratios. Remarkably, the difference in performance between iP7 and mPB strongly varied with the experimental conditions, although mPB always provided higher levels of transposition. Notably, overproduction inhibition, the decrease of transposition produced by excessive transposase previously observed in SB, was not detected for PB in the experimental conditions we used (10,17,18). Intriguingly, two previous reports have tried to address the existence of this phenomenon in PB with disparate results. On one hand, Wu et al. (17) found overproduction inhibition of PB in HEK293 cells. On the other hand, Wilson et al. (18) concluded that PB lacks overproduction inhibition in HEK293 cells. Interestingly, in the same study, Wilson et al. transfected equivalent amounts of a pCMV-based SB12 helper and donor plasmids in HEK293 cells, and observed the previously reported overproduction inhibition. Although our results support the lack of overproduction inhibition in PB, further work is needed to clarify this issue.

We have also shown that the higher transposition provided by mPB correlates with increased protein production. This result highlights the usefulness of codon optimization to increase protein levels. Besides this, the availability of two different sequences encoding PB transposase offers the opportunity to obtain different levels of protein and transposition activity.

We have analysed the differential properties of the 5’ and 3’-PB terminal repeats. In contrast with the previous results obtained with different combinations of the SB terminal repeats we have only detected transposition activity when the naturally occurring combination of PB
5'‑TR and 3'‑TR was used (11). Interestingly, we obtained higher numbers of colonies when the resistance cassette was oriented from the 5'‑TR to the 3'‑TR than in the opposite orientation. In these experiments the transposon contained a gene-trap selection cassette. As the 5'‑TR has been described to contain a promoter active in insect cells, we hypothesized that the observed differences in colony numbers could derive from transcriptional activity from the 5'‑TR activating the selection cassette (27). In a promoter analysis experiment using luciferase as a reporter, we observed that the 5'‑TR does act as a promoter which is 5-fold stronger than the 3'‑TR. This observation should be considered in the design of PB-based promoter-less transposons, as the expression of the promoter-less cassette from the 5'‑TR repeat may produce unwanted results. In our view, the simplest solution would be to rearrange the transposon so that the 3'‑TR is upstream of the 5'‑end of the promoter-less sequence. Remarkably, recent work has detected enhancer activity in the 3'‑TR of the piggyBac transposon (32). In our opinion, the presence of promoter and enhancer sequences in the piggyBac 5'‑TR and 3'‑TR, respectively should also be considered in gene-therapy applications, so as to avoid the negative experiences of generating activating mutations previously obtained with retroviruses (33).

Finally, we have engineered an inducible, optimized PB transposase. We have made and assessed six different combinations of mPB with ERT2 (28). The six versions have two different arrangements of ERT2 either at the C-terminus or at the N-terminus of the PB transposase coupled with three different linkers (L1, L2 or L3) between ERT2 and mPB. Four of these fusion proteins show extremely reduced levels of transposition when compared to the parental mPB, even in the presence of the inducer, 4-OHT. However, the other two fusion proteins, ERT2-L3-mPB and mPB-L3-ERT2, produce high levels of induced transposition. Both of them contain a linker which was based on an aminoacidic sequence previously used to fuse the native PB transposase to the GAL4-DNA-binding domain (17). The length and the net positive charge of this linker compared with the ones which were less successful are likely to underlie the huge differences in activity. Remarkably, mPB-L3-ERT2 yields levels of transposition 6 times higher than iPB in the presence of 4-OHT, whereas no detectable transposition was observed without induction. In the absence of 4-OHT the transposition activity provided by mPB-L3-ERT2 is reduced 800-fold, returning back to the low experimental noise levels. Thus mPB-L3-ERT2 is not only an improved version of the PB transposase, but it can also be temporally regulated. This newly engineered enzyme will be useful for diverse applications, ranging from gene therapy, where the possibility of switching on and off the enzyme would contribute to making it safer, to forward genetics cancer screens in mice, where the

**Figure 3.** Analysis of the properties of the piggyBac terminal repeats. (A) Left, schematic representation of transposon plasmids used in this experiment. Each plasmid contains the same gene trap puromycin resistance cassette flanked by different combinations of 5'-TR (blue arrows) and 3'-TR (red arrows). Right, 2 µg of transposon plasmids were co-electroporated into 10^7 AB2.2 mouse ES cells with 2 µg of mPB helper plasmid. The numbers of puromycin-resistant colonies were counted. (B) Left, schematic representation of the firefly luciferase expression plasmids used in this experiment. 5'-TR-Luc2 contains the minimal left terminal PB repeat followed by the firefly luciferase CDS (Luc2). 3'-TR-Luc2 contains the minimal right terminal PB repeat followed by Luc2. pA, SV40 late polyA region. Right, 5'-TR-Luc2 or 3'-TR-Luc2 were transfected in HeLaS3 cells together with pGL4.74, a transfection control plasmid containing the renilla luciferase gene under the thymidine kinase promoter. Twenty-four hours after transfection firefly and renilla luciferase activities were measured. n = 3, error bars: SEM.
temporal regulation of the transposition activity would facilitate the adjustment of the dynamics of the tumourigenic processes to prevent undesired genomic rearrangements.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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Figure 4. Activity and inducibility of piggyBac transposases regulatable by 4-hydroxytamoxifen. (A) Schematic representation of the plasmids used. A modified version of the oestrogen receptor ligand-binding domain, ERT2 (orange oval), was cloned at the 5′ or 3′ of the codon-optimized PB transposase CDS, to create ERT2-mPB and mPB-ERT2 plasmids. To facilitate domain folding and function, three different types of sequences encoding linker peptides were alternatively used (L1, green box; L2, blue box and L3, red box), creating six different types of sequences encoding linker peptides were alternatively used (L1, green box; L2, blue box and L3, red box), creating six different fusion proteins. (B) 5µg of 5′-PTK-3′ and 5µg of the indicated transposase expressing plasmid were co-electroporated into 1010 AB2.2 mouse ES cells. Puromycin-resistant clones were counted following selection in the presence (+) or absence (−) of 1µM 4-OHT. ipB and mPB were used as positive controls and pcDNA3 was used as negative control. A representative picture of plates with puromycin-resistant colonies obtained using mPB-L3-ERT2 as helper plasmid in the absence (left plate) or presence (right plate) of 4-OHT is shown. n = 3, error bars: SEM.
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