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Targeted replacement of *piggyBac* transposable element.

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Since Rubin and Spradling first developed the use of *P*-elements for transgenesis (Rubin and Spradling, 1982), the *P*-element has become an indispensable tool. Gene tagging, enhancer trapping, gene disruption, chromosome engineering, and inducible gene expression have taken advantage of the mobility of *P*-elements (Ryder and Russel, 2003). However, a *P*-element also has drawbacks, such as its strong bias in insertion sites. For reasons that are not entirely clear, it has a preference for some chromosomal locations (hot spots), but avoids others (cold spots) (Spradling *et al*., 1995).

The *piggyBac* element was discovered in the cabbage looper moth and has proven to be an efficient vector for the transformation of many insect species as well as human and mouse cells (Ding *et al.*, 2005). Because *piggyBac* has a different insertion site preference than that of a *P*-element, it has been used in several large scale gene disruption projects as a complement to *P*-elements (Hacker *et al*., 2003; Bellen *et al*., 2004; Thibault *et al*., 2004).

After a *P*-element excises from a chromosomal location, another *P*-element from a second site frequently transposes into the site of the excised *P*-element. This process is called "*P*-element replacement" (Gonzy-Treboul *et al*., 1995) or "targeted transposition" (Heslip and Hodgetts, 1994; Sepp and Auld, 1999). To date, however, similar replacement between *piggyBac* transposons has not been reported. We tested whether a *piggyBac* transposon, PB[y^+] on the 2nd chromosome (Bloomington stock ID16249, originally created by Ring and Garza) (Bellen *et al*., 2004), can efficiently transpose into the site of another *piggyBac* element, RB[w^+] e03162 (from Exelixis Collection at the Harvard Medical School), which is located in the second intron of the *slowpoke* gene (Thibault *et al*., 2004). We remobilized PB[y^+] in the presence of RB[w^+] e03162 using the constitutive {α-1 tubulin:*piggyBac* Transposase} transgene (Parks, 2004). Four out of fifteen crosses produced one or more potential replacements based on the marker phenotype. Two of those lines were molecularly characterized by PCR using one primer specific to PB[y^+] and the other from the *slowpoke* genomic region, followed by DNA sequencing. We found that in both cases, the replacement appears to be precise, leaving the local genomic DNA outside the transposon unchanged.

Since thousands of *piggyBac* insertions are widely distributed in the *Drosophila* genome and are available from stock centers (Bellen *et al.*, 2004; Thibault *et al*., 2004), the efficient targeted replacement of *piggyBac* opens up many possibilities for precise genetic manipulations and chromosome engineering. For example, a UAS containing *piggyBac* element could be placed at a desirable genomic position that harbors an existing *piggyBac* element without the UAS sequence.

Similarly, FRT or Lox-P sites can be targeted to certain genomic locations by *piggyBac* replacement. It is likely that the targeted *piggyBac* replacement we reported here could also be achieved in other organisms and mammalian cells, making this powerful genetic tool even more versatile.

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Sets of double balancers to facilitate the genetic combination of major chromosomes in *Drosophila melanogaster.*

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Drosophila melanogaster is a powerful genetic tool for basic and applied scientific research. The unique ability to quickly and precisely manipulate major chromosomes (the X, the 2nd and the 3rd) is facilitated by visible markers and ingeniously invented balancers. The ease of such genetic maneuvers contributed greatly to its early success as a model organism, and the methodology has been an essential tool ever since.

We constructed several sets of stocks (Table 1), each containing two balancers for two different chromosomes, to facilitate the combination of unmarked chromosomes from two separate parental sources. Such combination is often necessary in establishing new fly strains that harbor multiple genetic elements. These strains are used in many experiments such as genetic screens, testing genetic interactions, stable combination of UAS and Gal4 insertions, and generating mitotic mosaics.

Table 1. Double balancers for the major chromosomes.

Name	Genotype
DB1	y w; numb / CyO {Ras} ; fng / TM3 Sb
DB2	y w; numb / CyO ; fng / TM3 Sb {Ras}
DB3	y w; numb / CyO y^+ ; fng / TM3 Sb
DB4	y w; numb / CyO ; fng / TM3 Ser y^+
DB5	FM6 y w B ; Pin / CyO
DB6	FM6 y w B ; Ly / TM3 Sb

Our approach for combining the second and the third chromosome took advantage of the transgenes that confer dominant phenotypes. These phenotypes are easy to score while exerting little or no additional penalties on the strain's robustness. The balancer CyO P{sevRas1.V12}FK1 originated from Gerry Rubin's lab and contains a *P* element that expresses a hyperactive Ras protein exclusively in the eyes (Fortini *et al*., 1992; Karim *et al*., 1996). The transgene added a strong rough eye phenotype to the frequently used CyO balancer. We abbreviated this balancer as CyO {Ras} in this report. The {Ras} transgene was marked by ry^+, without obscuring the w^+ marker carried by other transposons. Similarly, {Ras} has been transposed into the third chromosome balancer TM3 Sb to make TM3 Sb {Ras} by others. We also used *numb* and *fng*, recessive lethal alleles of two randomly chosen genes on the second and the third chromosomes, respectively, to