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

construct the double balancers. Another set of double balancers used CyO y^+ and TM3 Ser y^+, which most likely contain a *P* element expressing the *yellow* gene (Geyer and Corces, 1987; Mardahl *et al*., 1993). All balancers mentioned above were obtained from the Bloomington Stock Center initially. Figure 1 illustrates a scheme using the double balancers DB1 and DB2 to combine A2, the second chromosome from strain A, and B3, the third chromosome of strain B, into a single stock. By differentiating CyO from CyO {Ras}, and TM3 Sb from TM3 Sb {Ras}, the crosses were efficient and reliable.

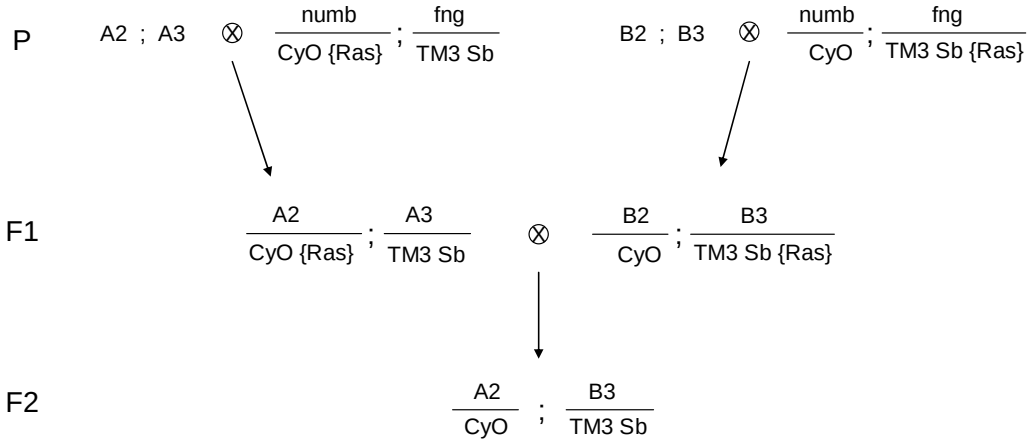

Figure 1. Combining the 2nd chromosome A2 and the 3rd chromosome B3 from strains A and B, respectively. First, cross strain A to DB1 (Table 1) and strain B to DB2. Then, mate the Cy, Sb and rough eye F1s from the two crosses with each other. Finally, select the Cy, Sb and regular eye (not rough) F2s to establish a new stock combining A2 and B3. For combining A3 with B2, the same scheme could be used except A would be crossed to DB2 and B to DB1 first.

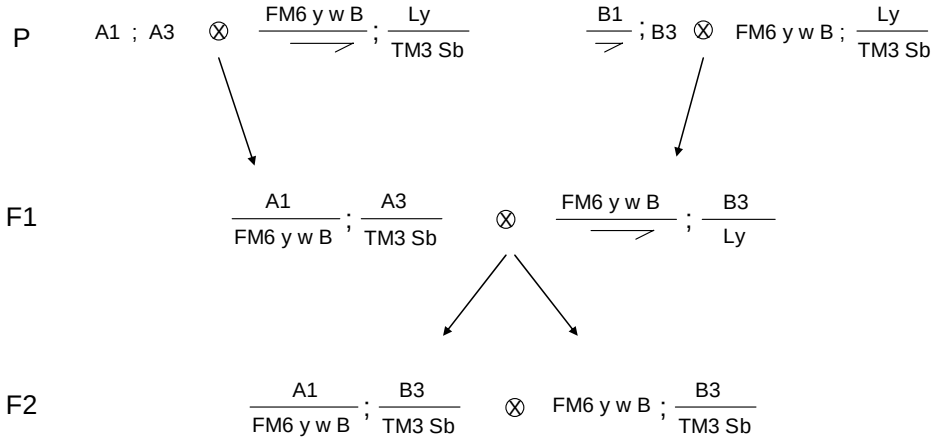

Figure 2. Combining the 1st chromosome A1 and the 3rd chromosome B3 from strains A and B, respectively. First, cross the females from strain A to the DB6 males (Table 1) and the males from strain B to the DB6 females. Then, mate the B, Sb female F1s from the first cross with the B, Ly male F1s from the second cross. Finally, select the heterozygous B and Sb females and the hemizygous B and Sb males in the F2 generation to establish a new stock combining A1 and B3. For combining A3 with B1, the same scheme could be implemented, except that the males from strain A and the females from strain B would be used in the first step.

We also constructed two stocks for double balancing the first and one of the major autosomes (Table 1). Figure 2 presents a scheme for combining A1 and B3 chromosomes into a single strain. The first and the second chromosomes from two parental strains could also be combined into one stock using a scheme similar to the one depicted in Figure 2, but employing stock DB5 instead of DB6.

All the double balancers were in *y w* background as well to further facilitate the manipulation of w^+ marked transposons. The double balancers discussed in this report are listed in Table 1 and are available from the author.

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Green, R.L., 1998, Heredity 121: 430-442.

Waters, R.L., J.T. Smith, and R.R. Brown 1990, J. Genet. 47: 123-134.

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