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# The DrosDel Collection: A Set of *P*-Element Insertions for Generating Custom Chromosomal Aberrations in *Drosophila melanogaster*

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ABSTRACT

We describe a collection of *P*-element insertions that have considerable utility for generating custom chromosomal aberrations in *Drosophila melanogaster*. We have mobilized a pair of engineered *P* elements, *p{RS3}* and *p{RS5}*, to collect 3243 lines unambiguously mapped to the Drosophila genome sequence. The collection contains, on average, an element every 35 kb. We demonstrate the utility of the collection for generating custom chromosomal deletions that have their end points mapped, with base-pair resolution, to the genome sequence. The collection was generated in an isogenic strain, thus affording a uniform background for screens where sensitivity to genetic background is high. The entire collection, along with a computational and genetic toolbox for designing and generating custom deletions, is publicly available. Using the collection it is theoretically possible to generate >12,000 deletions between 1 bp and 1 Mb in size by simple eye color selection. In addition, a further 37,000 deletions, selectable by molecular screening, may be generated. We are now using the collection to generate a second-generation deficiency kit that is precisely mapped to the genome sequence.

GENETICALLY tractable model organisms are valuable research tools for uncovering basic biological principles that are conserved through evolution. Many molecular pathways, such as signaling cascades, gene regulatory pathways, and cell cycle control circuits, were first characterized genetically in model systems. The subsequent molecular cloning of the genes involved in such pathways has shown how evolution has utilized basic molecular building blocks to control a wide variety of biological processes. Key to the success of such approaches has been the ability to carry out genetic screens for components that function in particular pathways and characterize how individual genes participate in such pathways.

The fruit fly, *Drosophila melanogaster*, is one such tractable model that has been used extensively to elucidate many conserved genetic hierarchies. One particularly powerful approach with Drosophila is the ability to rapidly carry out focused genome-wide screens for pathway components by identifying loci that modify specific phenotypes (see ST. JOHNSTON 2002 for review). In this approach, a sensitized genetic background, most commonly exhibiting an easily scored adult phenotype such as rough eyes or a wing defect, is used to search for mutations in genes that make the phenotype more severe (enhancer) or more like wild type (suppressor). Mutation-bearing chromosomes are introduced into the

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sensitized background and the phenotype is assessed. Importantly, the mutagenized chromosome is heterozygous, allowing genetic interactions between the sensitized background and mutations that are homozygous lethal to be detected. Particularly useful tools for such approaches are characterized sets of chromosomal deletions that can be used to screen rapidly most of the genome at low resolution. As well as utility in modifier screens, chromosomal deletion sets have also been used to find, *de novo*, genes that are involved in particular biological processes, an approach exemplified by the detailed analysis of embryonic segmentation carried out by Wieschaus and colleagues (Zusman and Wieschaus 1985; Merrill *et al.* 1988). Therefore, the availability of defined and characterized sets of chromosomal deletions is of considerable importance for future genetic studies.

Since the first chromosomal deletion in Drosophila, for the genes *forked* and *Bar*, was isolated in 1914 (see Bridges 1916) the Drosophila community has isolated >5000 deletions (data from FlyBase 2003). A very useful collection of these has been organized as a core "deficiency kit," a set of 220 fly stocks that include deletions covering ~85% of the euchromatic genome (Bloomington *Drosophila* Stock Center 2003). The kit has been widely used by the Drosophila community for both classical genetic mapping and the modifier screens described above. Although this kit continues to be updated and its coverage improved, it suffers from two unavoidable drawbacks. The first is that the kit is genetically heterogeneous, including deletions uncovered between 1918 and 2003 on a wide range of genetic backgrounds. This makes the kit unusable for screens that require a homogeneous genetic background, for example, those involving complex neural phenotypes such as behavior or learning and memory. Since a variety of genomic approaches indicate that genetic background can have profound affects on gene expression (Jin *et al.* 2001; Mieklejohn *et al.* 2003) the heterogeneous background of the current deficiency kit severely limits its utility for genomics studies. The second limitation is that the current deletion kit is not firmly anchored to the genome sequence, since the limits of each deletion are known only from genetic or cytological mapping. The resolution of cytological mapping is, at very best, within 50–100 kb. To overcome these limitations we formed a consortium of European laboratories with the goal of producing a second-generation deficiency kit, DrosDel. The new kit is being generated in a uniform genetic background and uses a method that allows the end points of deletions to be determined with single-base-pair resolution.

The approach we have taken involves the use of a pair of engineered *P* elements, *RS3* and *RS5*, built by Golic and Golic (1996). The *RS* elements each carry a functional *white* gene (*mini-white*) with a recognition sequence for the *Saccharomyces cerevisiae* FLIP (FLP) site-specific recombinase (*FRT* site) placed within intron one. In the case of *RS3*, a second *FRT* site is placed upstream of the first of the *mini-white* exons; in the case of *RS5* the second *FRT* site is located downstream of the *mini-white* exons. Golic and Golic demonstrated how a pair of *RS3* and *RS5* elements can be used to generate chromosome rearrangements by design. These chromosome rearrangements include both deficiencies and duplications (Figure 6). Since the insertion site of any *P* element can be precisely mapped to the genomic sequence, the end points of any chromosome aberration derived from a pair of these *RS* elements can be determined with single-base-pair resolution.

The problem of genetic background heterogeneity is less easily overcome. Powerful genetic methods are available with *D. melanogaster* to construct "isogenic" lines and we have used these methods in our current screen (Ashburner 1989). However, in the absence of practical methods to preserve these lines cryogenically, there is no way to prevent the slow, but inevitable, divergence of these lines in subsequent years. While this may be a drawback in the long term, there can be no doubt that, in the medium term, a deficiency kit in a homogeneous genetic background will be of considerable utility in genome-scale analysis of Drosophila.

We describe here the construction of a set of isogenic lines that form the basis for a mobilization screen with *RS* elements. We describe the isolation and mapping of >3000 new *P*-element-insertion lines on this background and demonstrate their utility for generating deletions precisely mapped onto the genome sequence. This work is a prelude to an ongoing effort to generate a precisely mapped deletion kit that will cover as much of the genome of *D. melanogaster* as is possible. In addition, we have constructed a genetic and computational toolkit that allows individual researchers to design and synthesize deletions in regions of particular interest. The materials we have generated are all publicly available.

## MATERIALS AND METHODS

Genetic nomenclature is according to FlyBase (2003). The *FM7* balancer stocks were obtained from the Bloomington *Drosophila* Stock Center. The *FLP* stock *y w P{70FLP, ry*$^{+}$*}3F* and *RS3* and *RS5* plasmids were kindly provided by Kent Golic. The *w-(G)* strain was chosen for its lack of *P* elements and its use as a reference strain in some behavioral studies (Dura *et al.* 1993). *W-(G)* originated from a *w*$^{1118}$ stock outcrossed to a wild-type strain, *Cantos-S(G)*, for six generations. All other stocks were from the Cambridge stock collection.

**Construction of the isogenic lines:** Using the crossing scheme (Figure 1) described below, 40 isogenic lines of the stock *w*$^{1118}$ were constructed. These lines were isogenic for the *X*, second, and third chromosomes. The fourth chromosome was not isogenized. The six healthiest lines (1, 3, 5, 8, 15, and 31), based on homozygous viability and fertility, were then examined using two behavioral assays, along with two controls [*w(G)* and Canton-S*(G)*]. Lines were tested for regularity of circadian rhythm and learning and memory. Circadian rythmicity was tested (Hamblen *et al.* 1986) and four lines (4, 5, 15,

**1) to make $w^{1118}_{iso}$**

| | | | | |
|---|---|---|---|---|
| single ♂ $w^{1118}$ | X | ♀ *FM7/Df(1)B58* | → | |
| ♀ *FM7/*$w^{1118}$ | X | ♂ $w^{1118}$ (re-use father) | → | $w^{1118}_{iso}$ |

**2) to make $w^{1118}_{iso}$; +/*SM6a*; +/*TM3, Sb***

| | | | | |
|---|---|---|---|---|
| *SM6a/Gla* | X | *Df(3L)Pc-MK/TM3,Sb* | → | |
| ♂ *SM6a/+; TM3, Sb/+* | X | ♀ $w^{1118}_{iso}$ | → | |
| ♂ $w^{1118}_{iso}$; *SM6a/+; TM3, Sb/+* | X | ♀ $w^{1118}_{iso}$ | → | $w^{1118}_{iso}$; +/*SM6a*; +/*TM3, Sb* |

**3) to make $w^{1118}_{iso}$; +/*T(2;3)CyO-TM6B, Tb*/+**

| | | | | |
|---|---|---|---|---|
| ♀ $w^{1118}_{iso}$ | X | ♂ *Su(H)*$^{P\{w+\}1356}$*/T(2;3)CyO-TM6B, Tb* | → | |
| ♂ $w^{1118}_{iso}$; +/*T(2;3)CyO-TM6B*/+ | X | ♀ $w^{1118}_{iso}$ | → | $w^{1118}_{iso}$; +/*T(2;3)CyO-TM6B, Tb*/+ |

**4) to make $w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$**

| | | | | |
|---|---|---|---|---|
| ♀ $w^{1118}_{iso}$; +/*T(2;3)CyO-TM6B, Tb*/+ | X | single ♂ $w^{1118}_{iso}$; +/*SM6a*; +/*TM3, Sb* | → | |
| ♂ $w^{1118}_{iso}$; $2_{iso}$/*T(2;3)CyO-TM6B, Tb*/$3_{iso}$ | X | ♀ $w^{1118}_{iso}$; $2_{iso}$/*T(2;3)CyO-TM6B, Tb*/$3_{iso}$ | → | $w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$ |

FIGURE 1.—Cross 1.

and 31) showed wild-type behavior. For memory, all lines scored ~95%, using the method of PACUAL and PREAT (2001). In the case of learning, all lines were initially assayed with a 1-hr memory test using the method described by TULLY and QUINN (1985). The two lines that scored highest (1 and 31) were retested for 24-hr memory following 120 cycles of spaced training; line 31 performed best, scoring 41.5 ± 7.8. Taken together, line 31 was found to consistently score the best in all assays and was therefore used as the basis for further stock construction; it is henceforth described as $w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$.

**Stock construction:** Using the crossing schemes described below, the following stocks were constructed using $w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$ as the isogenic background.

Balancers:

*In(1)FM7j, y w B*; $2_{iso}$; $3_{iso}$
*In(1)FM7h, w oc ptg B/P{RS3}l(1)CB-6411-3*$^{1}$; $2_{iso}$; $3_{iso}$
$w^{1118}_{iso}$; *Sco/In(2LR)SM6a, Cy*; $3_{iso}$
$w^{1118}_{iso}$; $2_{iso}$; *In(3LR)TM2, Ubx/In(3LR)TM6C, Sb*
$w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$; *ey*$^{D}$*/l(4)P{RS3r}l(4)CB-6471-3*$^{1}$
$w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$; *ci*$^{D}$*/l(4)P{RS3r}l(4)CB-6471-3*$^{1}$

*P*-element transposase source:

$w^{1118}_{iso}$; $2_{iso}$; *In(3LR)TM6C, Sb/*Δ*2-3, Dr*

RS3 and RS5 donors:

$w^{1118}_{iso}$; *Sco/In(2LR)SM6a,Cy, P{RS}*; $3_{iso}$

FLP source:

*y w P{70FLP, ry*$^{+}$*}3F*$_{iso}$; $2_{iso}$; $3_{iso}$
*y w P{70FLP, ry*$^{+}$*}3F*$_{iso}$; *Sco/In(2LR)SM6a, Cy*; $3_{iso}$
*y w P{70FLP, ry*$^{+}$*}3F*$_{iso}$; $2_{iso}$; *In(3LR)TM2, Ubx/In(3LR)TM6C, Sb*
$w^{1118}_{iso}$; *Sco/In(2LR)SM6b, Cy amos*$^{Roi\text{-}1}$ *P{70FLP, ry*$^{+}$*}*; $3_{iso}$

*FM7h, SM6a*, and *TM6C* were used to balance inserts on the *X*, second, and third chromosomes, respectively, because of their efficiency in suppressing recombination over most of the chromosome and the fact that each is marked with an easily recognizable "user friendly" dominant mutation. *FM7h* was kept heterozygous over *P{RS3}l(1)CB-6411-3*$^{1}$, a lethal *RS3* insert. Note that *FM7h* carries a recessive female sterile mutation, *ocelliless*$^{1}$. It was also useful to have a homozygous viable and fertile *X* chromosome balancer, marked with $w^{-}$ and *B*. For this reason *In(1)FM7j, y w B* was made by selecting a *y w oc*$^{+}$ *B* recombinant from *In(1)FM7j/In(1)FM7i* females. There is no balancer for the fourth chromosome, but the lack of recombination on this chromosome meant that the two recessive lethal, but dominant visible markers, *ey*$^{D}$ and *ci*$^{D}$, could be used. *ey*$^{D}$ and *ci*$^{D}$ were kept heterozygous over the reduced ($w^{-}$) version of *P{RS3}CB-6471-3*, a lethal chromosome *4* insert, because the *ey*$^{D}$*/ci*$^{D}$ combination was too sickly in this genetic background.

The *FLP* stock *y w P{70FLP, ry*$^{+}$*}3F* was provided by Kent Golic. This *X* chromosome insertion was introduced into the line 31 autosomal isogenic background and further stocks were constructed with the appropriate balancers. To enable induction of *FLP* events on the *X* chromosome, however, it was necessary to transpose the *70FLP P* element onto an autosomal balancer (see Figures 2 and 3). The chromosome *2* balancer

**1) to make $w^{1118}/y^{+}Y_{iso}$; *Sco/SM6a*; $3_{iso}$ and $w^{1118}/y^{+}Y_{iso}$; $2_{iso}$; *TM2/TM6C, Sb***

♂ *dev*$^{1}$*/TM6C, Sb* X ♀ *SM6a/Gla* → ♂ *SM6a/+; TM6C, Sb/+* X ♀ $w^{1118}{}_{iso}$; *CS* →

♂ $w^{1118}{}_{iso}$; *SM6a/+; TM6C, Sb/+* X ♀ $w^{1118}{}_{iso}$; $2_{iso}$; $3_{iso}$ → ♀ $w^{1118}{}_{iso}$; $2_{iso}$*/SM6a*; $3_{iso}$*/TM6C, Sb*

♂ *w/y*$^{+}$*Y; Sco/CyO; TM2/TM6B* X ♀ $w^{1118}{}_{iso}$; $2_{iso}$; $3_{iso}$ → ♂ $w^{1118}/y^{+}Y_{iso}$; $2_{iso}$*/Sco*; $3_{iso}$*/TM2*

♀ $w^{1118}{}_{iso}$; $2_{iso}$*/SM6a*; $3_{iso}$*/TM6C, Sb* X ♂ $w^{1118}/y^{+}Y_{iso}$; $2_{iso}$*/Sco*; $3_{iso}$*/TM2* → $w^{1118}/y^{+}Y_{iso}$; *Sco/SM6a*; $3_{iso}$ and $w^{1118}/y^{+}Y_{iso}$; $2_{iso}$*;TM2/TM6C, Sb*

**2) to make $w^{1118}{}_{iso}$; $2_{iso}$; *TM6C, Sb/Dr* Δ*2-3***

♀ $w^{1118}{}_{iso}$; $2_{iso}/2_{iso}$; *TM2/TM6C* X ♂ *w; Sp/CyO; Dr,* Δ*2-3/TM6* →

♂ $w^{1118}{}_{iso}$; $2_{iso}$*/CyO; Dr,* Δ*2-3*/$3_{iso}$ X ♀ $w^{1118}{}_{iso}$; $2_{iso}/2_{iso}$; *TM2/TM6C* → $w^{1118}{}_{iso}$; $2_{iso}$; *TM6C, Sb/Dr,* Δ*2-3*

**3) to make $w^{1118}{}_{iso}$; *SM6B, P{70FLP, ry*$^{+}$*}/Sco*; $3_{iso}$**

♂ *SM6B, P{70FLP, ry*$^{+}$*}/If; ry*$^{8}$ X ♀ $w^{1118}{}_{iso}$; $2_{iso}$; *TM2/TM6C* →

♂ $w^{1118}{}_{iso}$; *SM6B, P{70FLP, ry*$^{+}$*}*/$2_{iso}$; *TM6C/ry*$^{8}$ X ♀ $w^{1118}{}_{iso}$; $2_{iso}$; $3_{iso}$ →

♂ $w^{1118}{}_{iso}$; *SM6B, P{70FLP, ry*$^{+}$*}*/$2_{iso}$; *TM6C*/$3_{iso}$ X ♀ $w^{1118}{}_{iso}$; *SM6a, RS w*$^{+}$*/Sco*; $3_{iso}/3_{iso}$ → $w^{1118}{}_{iso}$; *SM6B, P{70FLP, ry*$^{+}$*}/Sco*; $3_{iso}$

FIGURE 2.—Cross 2A. Construction of balancer stocks.

**1) to make *In(1)FM7j, y w B*; $2_{iso}$; $3_{iso}$ and *In(1)FM7h, w oc ptg B/l(1)CB-6411-3$^{1}$*; $2_{iso}$; $3_{iso}$**

In the following scheme, *FM7* may represent either *FM7j* or *FM7h*

♀ *FM7/+* X ♂ $w^{1118}$/$y^{+}Y_{iso}$; *Sco/SM6a*; $3_{iso}$ → ♀ *FM7/w*; *SM6a/+* X ♂ $w^{1118}{}_{iso}$; $2_{iso}$; $3_{iso}$ →

♀ *FM7/*$w^{1118}$; *SM6a/*$2_{iso}$ X ♂ $w^{1118}$/$y^{+}Y_{iso}$; $2_{iso}$; *TM2/TM6C, Sb* →

♀ *FM7/*$w^{1118}$; $2_{iso}$/$2_{iso}$; *TM6B/+* X ♂ $w^{1118}{}_{iso}$; $2_{iso}$; $3_{iso}$ →

♀ *FM7/*$w^{1118}$; $2_{iso}$/$2_{iso}$; *TM6B/*$3_{iso}$ X ♂ *FM7*; $2_{iso}$/$2_{iso}$; *TM6B/*$3_{iso}$ → ***FM7j, y w B*; $2_{iso}$; $3_{iso}$**

♂ *FM7, y w B*; $2_{iso}$/$2_{iso}$; *TM6B/*$3_{iso}$ X ♀ *l(1)CB-6411-3$^{1}$/*$w^{1118}$; $2_{iso}$; $3_{iso}$ → ***FM7h, w oc ptg B/l(1)CB-6411-3$^{1}$*; $2_{iso}$; $3_{iso}$**

**2) to make *y w P{70FLP, ry$^{+}$}3F$_{iso}$*; $2_{iso}$; $3_{iso}$ and *y w P{70FLP, ry$^{+}$}3F$_{iso}$*; $2_{iso}$; *TM2/TM6C***

♀ *y w P{70FLP, ry$^{+}$}3F* X ♂ $w^{1118}$/$y^{+}Y_{iso}$; *Sco/SM6a*; $3_{iso}$ →

♂ *y w P{70FLP, ry$^{+}$}3F* /$y^{+}Y_{iso}$; *SM6a/+* X ♀ *FM7j, y w B*; $2_{iso}$; $3_{iso}$ →

♀ *y w P{70FLP, ry$^{+}$}3F/FM7j*; *SM6a/*$2_{iso}$ X ♂ *FM7j, y w B*; $2_{iso}$; $3_{iso}$/*TM6C* →

♀ *y w P{70FLP, ry$^{+}$}3F/FM7j*; $2_{iso}$; *TM6C/+* X ♂ *FM7j, y w B*; $2_{iso}$; $3_{iso}$/*TM2* → ***y w P{70FLP, ry$^{+}$}3F$_{iso}$*; $2_{iso}$; *TM2/TM6C***

♀ *y w P{70FLP, ry$^{+}$}3F/FM7j*; $2_{iso}$; *TM6C/*$3_{iso}$ X ♂ *y w P{70FLP, ry$^{+}$}3F/FM7j*; $2_{iso}$; *TM6C/*$3_{iso}$ → ***y w P{70FLP, ry$^{+}$}3F$_{iso}$*; $2_{iso}$; $3_{iso}$**

**3) to make *y w P{70FLP, ry$^{+}$}3F$_{iso}$*; *SM6a/Sco*; $3_{iso}$**

♀ *y w P{70FLP, ry$^{+}$}3F* X ♂ $w^{1118}$/$y^{+}Y_{iso}$; $2_{iso}$; *TM2/TM6C* →

♂ *y w P{70FLP, ry$^{+}$}3F* /$y^{+}Y_{iso}$; *TM6C/+* X ♀ *FM7j, y w B*; $2_{iso}$; $3_{iso}$ →

♀ *y w P{70FLP, ry$^{+}$}3F/FM7j*; *TM6C/*$3_{iso}$ X ♂ *FM7j, y w B*; *SM6a/*$2_{iso}$; $3_{iso}$ →

♀ *y w P{70FLP, ry$^{+}$}3F/FM7j*; *SM6a/+*; $3_{iso}$ X ♂ *FM7j, y w B*; *Sco/*$2_{iso}$; $3_{iso}$ → ***y w P{70FLP, ry$^{+}$}3F$_{iso}$*; *Sco/SM6a*; $3_{iso}$**

FIGURE 3.—Cross 2B. Construction of balancer stocks.

*In(2LR)SM6b, al*$^{2}$ *Cy dp*$^{lvl}$ *amos*$^{Roi\text{-}1}$ *cn*$^{2P}$ *sp*$^{2}$ was chosen, the dominant rough eye phenotype of *Roi-1* making it easy to distinguish from *SM6a*. Four independent lines of *SM6b, P{70FLP, ry*$^{+}$*}* were tested for their ability to induce somatic flip in lines carrying *RS* elements, after heat shock.

All stocks, including the $w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$ stock, were tested for the presence of extraneous *P* elements. Southern blots of genomic DNA were probed with DIG-labeled PCR products amplified from a Carnegie 2 template (Rubin and Spradling 1983) and covering either the 5′ end of the *P* element (primers P5′L and P5′R, which amplified nucleotides 24–586) or the 3′ end (primers P3′L and P3′R, which amplified nucleotides 2750–2879); coordinates refer to the wild-type *P* sequence (O'Hare and Rubin 1983). The P5′ probe hybridized to only those lines expected to carry a *P* element, but not to the isogenic stock or to stocks carrying *SM6a/Sco* or *TM6C/TM2* in the isogenic background. The P3′ probe hybridized strongly to only those lines expected to carry a *P* element, but also weakly to a DNA fragment from all lines tested; however, the same fragment was present in the transposase-producing stock and so could not come from a mobile *P* element.

**RS donor stocks:** The SM6a chromosome was chosen as the base RS donor because it can be easily identified in crosses. Using an autosomal donor meant that jumps could be recovered in both sexes and using a balancer chromosome helped maintain isogenicity. The $w^{1118}_{iso}$; *Sco/In(2LR)SM6a, Cy*; $3_{iso}$ stock was used as a host for germline transformation with the pP{RS3} and pP{RS5} plasmids (Karess 1985; Golic and Golic 1996). Using inverse-PCR, 31 *RS3* and 28 *RS5* lines were mapped to the *SM6a* chromosome. Four and six lines, respectively, were selected as potential donor candidates since they carried a single insertion, and the transposition rates for each stock were compared (Table 1).

**Mobilization:** For the mobilization screens all crosses were performed in bottles or 4-in. glass vials on cornmeal agar medium at 25°. Males of the donor stock $w^{1118}_{iso}$; *Sco/In(2LR)SM6a, P{RS}, Cy*; $3_{iso}$ were crossed to $w^{1118}_{iso}$; $2_{iso}$; *Δ2-3,Dr/In(3LR)TM6C, Sb* in bottles, 10–15 pairs per bottle.

$F_1$ dysgenic males, $w^{1118}_{iso}$; $2_{iso}$/*SM6a, P{RS}*; *Δ2-3,Dr*/$3_{iso}$, were then crossed to $w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$ virgin females. This second cross was performed under "standardized" conditions, *i.e.*, one or two males were crossed to four to six females in each vial. These conditions were repeated for a number of donor chromosomes, so that the transposition frequency (the percentage of fertile vials that show at least one transposition) of each could be compared and suitable donors selected for the large-scale screens. A single $F_2$ progeny fly with the phenotype $w^{+}$ *Cy*$^{+}$ *Dr*$^{+}$ from each vial was crossed to the $w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$ stock and an unbalanced line was established and given a unique bar code. Following successful sequencing of the insertion, these stocks were then balanced.

In an attempt to recover different insertion distributions, *RS3* and *RS5* elements were mobilized from several different *Y* chromosome insertions isolated during the course of the production screen (see Table 1). $w^{1118}_{iso}$/*Y, P{RS}*; $2_{iso}$; $3_{iso}$ males were crossed to $w^{1118}_{iso}$; $2_{iso}$; *Δ2-3,Dr/In(3LR)TM6C, Sb* virgin females. The resulting dysgenic $w^{1118}_{iso}$/*Y, P{RS}*; $2_{iso}$; *Δ2-3,Dr*/$3_{iso}$ males were crossed under standard conditions to $w^{1118}_{iso}$; $2_{iso}$; $3_{iso}$ virgin females. The first $F_2$ $w^{+}$ *Dr*$^{+}$ female progeny from each vial was used to establish a new insert line. It was found that the spectrum of insertions recovered from *Y* chromosome donors did not significantly differ from the autosomal donors used (see results).

**Data tracking:** To facilitate data tracking all stocks were bar coded at every stage so that individual lines could be easily followed from isolation through to sequencing. The unique ID for each element consists of a laboratory code (CB, Cambridge; HA, Halle; SZ, Szeged; and UM, Umeå), a four-digit serial number, and an indicator of *RS*-element type (3 or 5). All data were managed using a mySQL database (see below).

**Molecular biology:** DNA preparation and inverse PCR protocols were modified from the methods described on the Berkeley *Drosophila* Genome Project (BDGP) web site (http://www.fruitfly.org/about/methods/inverse.pcr.html); full and detailed protocols are available from the DrosDel web site (see below). At all times the coordinates of the original fly lines were preserved in a 96-well plate to facilitate data tracking. Inverse PCR sequences were determined on an ABI 3100 capillary sequencer using standard conditions and analyzed using ABI sequencing analysis 3.7 software. The following primers were used for inverse PCR and other PCR amplifications; a diagram of the location of the primers with respect to the *P*-element structure is available at http://www.drosdel.org.uk/ddelements.html:

RS3-1A: TTATGAGTTAATTCAAACCCCAC
RS3-2: TACGTACTCGCGATGAGCAC
RS5F: CGTACTTTGGAGTACGAAATGC
RS5R: CGAATCATTAAAGTGGGTATCAC
PRY1: CCTTAGCATGTCCGTGGGGTTTGAAT
PRY4: CAATCATATCGCTGTCTCACTCA
PLAC1: CACCCAAGGCTCTGCTCCCACAAT
PLAC4: ACTGTGCGTTAGGTCCTGTTCATTGTT
P5′L: TCCCGTCGATAGCCGAAG
P5′R: CTGCTGCTCTAAACGACGC
P3′L: CCCCACGGACATGCTAAGGG
P3′R: CGGCAAGAGACATCCACTTAACG
W7500D: GTCCGCCTTCAGTTGCACTT
W11678U: TCATCGCAGATCAGAAGCGG
SP1: ACACAACCTTTCCTCTCAACAA
SPEP1: GACACTCAGAATACTATTC

**Data processing:** Sequences were stripped of *P*-element sequence using custom software (http://www.flyseq.org.uk/rs_progs.htm) and aligned to Release 3.1 (Celniker *et al.* 2002) of the *D. melanogaster* genome sequence using BLASTN (Altschul *et al.* 1990). We define matches to heterochromatin as belonging to the WGS3 heterochromatin sequence available from the BDGP (http://www.fruitfly.org/sequence/sequence_db/WGS3_het_genomic_dmel_RELEASE3-0.FASTA). We define matches to natural transposable elements as being to the canonical TE sequences from the file maintained at the BDGP (http://www.fruitfly.org/p_disrupt/datasets/NATURAL_TRANSPOSABLE_ELEMENTS.embl). Alignments and additional information about the mapped *P* elements were formatted using custom Perl scripts and imported into the DrosDel mySQL database for further manipulation. The task of selecting a pair of elements in a suitable location and orientation to construct a deletion was achieved with custom Perl scripts. The scripts predict all possible $w^{+}$ deletions between 1 bp and 1 Mb in size that can theoretically be generated from the set of insertions that we have mapped (http://www.flyseq.org.uk/rs_progs.htm).

**Data release and availability:** Data for this project will be periodically frozen for public release. This greatly simplifies both data analysis and stock ordering. Version 1.0 of the *RS* collection was released in March 2003 and contained data for 2527 *RS* elements. Version 2.0 is presented here and contains 3243 elements. These can be combined to produce 12,258 $w^{+}$ deletions ranging in size from 1 bp to 1 Mb. In the future we will extend the prediction software to allow the selection of elements that can be combined to synthesize other chromosome aberrations, such as $w^{-}$ deletions, inversions, and translocations. All data and software are available from the DrosDel web site (http://www.drosdel.org.uk). In addition, the sequence data have all been submitted to GenBank and to FlyBase.

**Stock ordering:** To help provide the Drosophila community

**Flip Out** ***RS w⁺ → w⁻***

Chromosome *2*

| | | | |
|---|---|---|---|
| ♂ *y w* $_{iso}$; *P{RS}*; $3_{iso}$ | X | ♀ *y w 70FLP*$_{iso}$; *Sco/SM6a*; $3_{iso}$ | → heat shock |
| ♂ *y w 70FLP*; *P{RS}/SM6a*; $3_{iso}$ | X | ♀ *y w 70FLP*; *Sco/SM6a*; $3_{iso}$ | → |
| single *w⁻* ♂ *y w 70FLP*$_{iso}$; *P{RSr}/SM6a*; $3_{iso}$ | X | ♀ *y w 70FLP*$_{iso}$; *Sco/SM6a*; $3_{iso}$ | → stock |

*Chromosome 3*

As chromosome *2*, but using *y w 70FLP*$_{iso}$; $2_{iso}$; *TM2/TM6C* to balance.

Chromosome *X*

| | | | |
|---|---|---|---|
| ♂ *w*$^{1118}$ $_{iso}$; *Sco/SM6b, 70FLP*; $3_{iso}$ | X | ♀ *w*$^{1118}$ *P{RS}*; $2_{iso}$; $3_{iso}$ | → heat shock |
| ♂ *w*$^{1118}$ *P{RS}*; *SM6b, 70FLP/*$2_{iso}$; $3_{iso}$ | X | ♀ *FM7h/l(1)CB-6411-3*$^{1}$ ; $2_{iso}$; $3_{iso}$ | → |
| single *w⁻* ♀ *FM7h/w*$^{1118}$ *P{RSr}*; $2_{iso}$; $3_{iso}$ | X | ♂ *FM7h*$_{iso}$; $2_{iso}$; $3_{iso}$ | → stock |

Chromosome *4*

| | | | |
|---|---|---|---|
| ♂ *w*$^{1118}$ $_{iso}$; $2_{iso}$; $3_{iso}$; *P{RS}* | X | ♀ *y w 70FLP*$_{iso}$; $2_{iso}$; $3_{iso}$;*+/ci*$^{D}$ | → heat shock |
| ♂ *y w 70FLP*$_{iso}$; $2_{iso}$; $3_{iso}$; *P{RS}/ci*$^{D}$ | X | ♀ *y w 70FLP*$_{iso}$; $2_{iso}$; $3_{iso}$;*+ /ey*$^{D}$ | → |
| single *w⁻* ♂ *y w 70FLP*$_{iso}$; $2_{iso}$; $3_{iso}$; *P{RSr}/ey*$^{D}$ | X | ♀ *y w 70FLP*$_{iso}$; $2_{iso}$; $3_{iso}$; *+/ci*$^{D}$ | → stock |

FIGURE 4.—Cross 3.

with stocks containing *P*-element insertions that can be used to create custom deletions outside of the core DrosDel deficiency kit we produced a web-based ordering system to interface with the web pages of the Szeged Drosophila Stock Centre. The software uses PHP and HTML to access the DrosDel mySQL database and allows users to search for, and order, insertion stocks, deletion stocks, and the stocks used for mobilization and deletion construction. The ordering system is available at http://www.drosdel.org.uk. It directly accesses the Szeged Drosophila Stock Centre (http://gen.bio.u-szeged.hu/gen/) where the stock orders are processed and dispatched.

**Construction of deletions:** Constructing chromosomal deletions using *RS* elements is a two-step process (see RESULTS for details; GOLIC and GOLIC 1996) and was carried out according to the crossing schemes in Figures 4 and 5.

**Confirmation of deletions:** Deletions constructed using this method carry a *P* element with a *mini-white* gene containing a single FRT site within intron 1. This *P* element should be stable, even in the presence of *P* transposase, since it carries two 3′ *P*-element ends (GRAY *et al.* 1996). The identity of the ends of this *P* element means that the primers usually used for *P*-element mapping are unsuitable. We therefore used an internal *P*-element primer (PRY4) together with two custom primers designed for genomic sequences expected to be ~300 bp from each side of the deletion. Unique products from each end of the deletion were then amplified and sequenced. We also verified that the genetic manipulations had indeed correctly reconstituted the predicted *mini-white* sequence by using the w7500D and w1167U PCR primers described by GOLIC and GOLIC (1996). These amplify a 1.64-kb region of $w^{hs}$ around the FRT site. In addition to verifying by sequence, each deletion described in this article has also been mapped genetically, using mutant alleles of genes that we predict should be in its vicinity.

## RESULTS

**RS-element mobilization:** Small-scale pilot experiments were performed to assess the transposition frequencies of various *RS3* and *RS5* donor elements. As pre-

**Flip In** ***RS3r, w⁻/RS5r, w⁻ → Df w⁺***

Chromosome *2*

| | | | |
|---|---|---|---|
| *y w P{70FLP, ry⁺}$_{iso}$; P{RS3r}; 3$_{iso}$* | X | *y w P{70FLP, ry⁺}$_{iso}$; P{RS5r}; 3$_{iso}$* | → heat shock |
| ♂ *y w P{70FLP, ry⁺}$_{iso}$; P{RS3r}/P{RS5r}; 3$_{iso}$* | X | ♀ *w$^{1118}_{iso}$; Sco/SM6a; 3$_{iso}$* | → |
| single *w⁺* ♂ *w$^{1118}_{iso}$; P{RS5+3}/SM6a; 3$_{iso}$* | X | ♀ *w$^{1118}_{iso}$; Sco/SM6a; 3$_{iso}$* | → stock |

Chromosome *3*

As chromosome *2*, but using *w$^{1118}_{iso}$; 2$_{iso}$; TM2/TM6C* to balance.

Chromosome *X*

| | | | |
|---|---|---|---|
| ♂ *w$^{1118}_{iso}$; Sco/SM6b, P{70FLP, ry⁺}; 3$_{iso}$* | X | ♀ *w$^{1118}$ P{RS3r}; 2$_{iso}$; 3$_{iso}$* | → |
| ♂ *w$^{1118}$ P{RS3r}; SM6b, P{70FLP, ry⁺}/2$_{iso}$; 3$_{iso}$* | X | ♀ *w$^{1118}$ P{RS5r}; 2$_{iso}$; 3$_{iso}$* | → heat shock |
| ♀ *w$^{1118}$ P{RS3r}/w$^{1118}$ P{RS5r}; SM6b, P{70FLP, ry⁺}/2$_{iso}$; 3$_{iso}$* | X | ♂ *w$^{1118}_{iso}$; 2$_{iso}$; 3$_{iso}$* | → |
| single *w⁺* ♀ *w$^{1118}$ P{RS5+3}/w$^{1118}$; 2$_{iso}$; 3$_{iso}$* | X | ♂ *w$^{1118}_{iso}$; 2$_{iso}$; 3$_{iso}$* | → |
| ♀ *w$^{1118}$ P{RS5+3}/w$^{1118}$; 2$_{iso}$; 3$_{iso}$* | X | ♂ *FM7h; 2$_{iso}$; 3$_{iso}$* | → stock |

Chromosome *4*

| | | | |
|---|---|---|---|
| *y w P{70FLP, ry⁺}$_{iso}$; 2$_{iso}$; 3$_{iso}$; P{RS3r}* | X | *y w P{70FLP, ry⁺}$_{iso}$; 2$_{iso}$; 3$_{iso}$; P{RS5r}* | → heat shock |
| *y w P{70FLP, ry⁺}$_{iso}$; 2$_{iso}$; 3$_{iso}$; P{RS3r}/P{RS5r}* | X | *w$^{1118}_{iso}$; 2$_{iso}$; 3$_{iso}$; +/+* | → |
| single *w⁺* *w$^{1118}_{iso}$; 2$_{iso}$; 3$_{iso}$; P{RS5+3}/+* | X | *w$^{1118}_{iso}$; 2$_{iso}$; 3$_{iso}$; +/+* | → |
| *w$^{1118}_{iso}$; 2$_{iso}$; 3$_{iso}$; P{RS5+3}/+* | X | *w$^{1118}_{iso}$; 2$_{iso}$; 3$_{iso}$; ci$^{D}$/+ (or ey$^{D}$)* | → stock |

FIGURE 5.—Cross 4.

sented in Table 1 the range was broad, from ~7% to >50%. The proportion of new insertions that were homozygous lethal was 12.0% for the *RS3* element ($n$ = 973) and 4.7% for the *RS5* element ($n$ = 2203). These are broadly in line with the frequencies found in other large-scale screens (SPRADLING *et al.* 1999). From these data we selected two *RS3* (*CB-0102-3* and *CB-0100-3*) and two *RS5* (*5-HA-1007* and *5-HA-2484*) donor lines to generate the majority of the new *RS* insertions. Large-scale mobilizations with *RS3* and *RS5* were carried out as described in MATERIALS AND METHODS.

**Sequencing results:** In total, 5699 *RS* elements were processed through our sequencing system (see Table 2). Of these, 4708 lines produced a PCR product, as judged by gel electrophoresis. Not all amplified products produced an acceptable sequence—a low percentage of sequencing reactions failed completely (6.3%). In addition, 233 amplifications (4.9%) indicated multiple *P*-element insertions, frequently visible as two or more bands by gel electrophoresis of the PCR products. To be useful for deletion construction, individual *RS* insertions must map to a single location on the Drosophila genome sequence. Of those elements successfully sequenced, 513 (12%) have a restriction site too close to the end

**TABLE 1**

***RS*-element mobilization**

| Donor | Position | Transposition frequency (%) Pilot | Production |
|---|---|---|---|
| *CB-6028-3* | *Y* | 17.9 | |
| *CB-6019-3* | *Y* | 43.0 | |
| *CB-0286-3* | *Y* | 23.9 | |
| *CB-0560-3* | *Y* | 51.3 | |
| *CB-5902-3* | *Y* | 14.9 | |
| *CB-0263-3* | *Y* | 27.8 | |
| *CB-0514-3* | *Y* | 25.5 | |
| *CB-0102-3* | *2R*:53F9 (*veg*) | 7.2 | 4.3 ($n$ = 7976) |
| *CB-0100-3* | *2L*:35D1 (*esg*) | 12.6 | 11.6 ($n$ = 15,806) |
| *5-HA-1005* | *2R*:42B | 22.7 | |
| *5-HA-1006* | *2R*:46B13 | 18.6 | |
| *5-HA-1007* | *2R*:53F9 | 23.9 | 30.0 ($n$ = 4950) |
| *5-HA-1018* | *2R*:52F7 | 11.6 | |
| *5-HA-1023* | *2L*:21E2 | 9.9 | |
| *5-HA-1026* | *2R*:41F1 | 28.5 | |
| *5-HA-1172* | *Y* | 14.4 | |
| *5-HA-2485* | *Y* | 24.9 | |
| *5-HA-2486* | *Y* | 21.1 | |
| *5-HA-2487* | *Y* | 1.2 | |
| *5-HA-2484* | *Y* | ND | 42.1 ($n$ = 2765) |
| *5-HA-3124* | *Y* | 21.0 | |
| *5-HA-2488* | X:7D (*singed*) | 22.5 | |

Results from pilot and production mobilizations using different donor sources. Transposition frequency is the fraction, as a percentage of fertile vials with at least one transposition. For the pilot experiments the number of fertile vials for each experiment was ~150; for the production runs the number of fertile vials is noted in parentheses. ND, not done.

**TABLE 2**

**Recovery and characterization of *RS* elements**

| | Total | % |
|---|---|---|
| *RS* elements processed | 5699 | — |
| Successful PCR | 4708 | 82.6 |
| Successful sequencing | 4191 | 73.5 |
| BLASTN to unambiguous site | 3380 | 59.3 |
| Multiple blast matches | 307 | 5.4 |
| Short sequence | 513 | 9 |
| Multiple insertions | 233 | 4.1 |

of the *P* element (usually below 20 bp) and were discarded. An additional 307 (7.3%) lines mapped to either heterochromatic sequences or natural transposons. In total, 3380 *RS* elements were successfully mapped to the genome sequence and can therefore be used in the deletion construction project. These results are summarized in Tables 2 and 3.

There is a large difference in the percentage of *RS3* and *RS5* elements mapping to chromosome arm *2L* (Table 3). In part, at least, this is due to the large number (196, ~11%) of *RS3* elements mapping to the *escargot* region, the site of the *RS3* donor element. Most of these elements have been discarded. As a consequence of local hopping events and also of *P*-element-insertion hotspots (see below) there is some redundancy in the collection of mapped elements; this was therefore reduced by discarding many of the *esg* insertions, reducing the collection to 3243. These 3243 insertion lines constitute version 2.0 of the DrosDel collection and can be accessed in our publicly accessible database. Using this collection we can, in theory, produce >12,000 custom $w^+$ deletions ranging in size between 1 bp and 1 Mb (see below).

To test the quality of our primary collection we assessed a set of homozygous lethal *RS3* insertions. We crossed 47 different lethal insertions with deficiencies believed to cover them. Thirty-eight (81%) of these were lethal over the appropriate deletion. Ten of these 38 were selected for reversion. All 10 were reverted to nonlethal chromosomes by *P*-element transposase.

**Donor site effects and element distribution:** Four main donors were used during the course of our *RS* mobilization screen (Table 1): three from chromosome *2* (*CB-0100-3* at scaffold location 15311845 on *2L*, *CB-0102-3* at scaffold location 12161296 on *2R*, and *5-HA-1007* at scaffold location 12161298 on *2R*) and one from the *Y* chromosome (*5-HA-2484*). We analyzed whether the site of the donor element affects the distribution of recipient elements in the genome since this information may be

**TABLE 3**

**Chromosomal distribution of *RS* elements**

| Chromosome | *RS3* | % | *RS5* | % | Total % | Average spacing (kb) |
|---|---|---|---|---|---|---|
| *2L* | 515 | 28.5 | 257 | 16.4 | 22.8 | 28 |
| *2R* | 344 | 19 | 356 | 22.6 | 20.7 | 29 |
| *3L* | 289 | 16 | 242 | 15.4 | 15.7 | 43 |
| *3R* | 354 | 19.5 | 337 | 21.4 | 20.5 | 40 |
| *4* | 14 | 0.8 | 9 | 0.6 | 0.7 | 42 |
| *X* | 292 | 16.2 | 371 | 23.6 | 19.6 | 32 |
| Total | 1808 | — | 1572 | — | — | — |

**TABLE 4**

**Predicted deletions**

| Chromosome | Deletions |
|---|---|
| *2L* | 2,305 |
| *2R* | 3,658 |
| *3L* | 1,495 |
| *3R* | 2,122 |
| *4* | 31 |
| *X* | 2,647 |
| Total | 12,258 |

useful in designing directed strategies for increasing the coverage of *P*-element insertions in future screens. The distribution of new element insertions on individual chromosome arms generated from different donors was tested using the two-sample Kolmogorov-Smirnov test (Robert and Casella 1999; data not shown). As we note above, in the case of *2L* there is a significant difference in the distribution of insertions derived from the *2L* donor and those derived from other donors. While this bias can, in part, be attributed to the fact that the site of the *2L* donor, in the *escargot* region, is a recognized hotspot for *P*-element insertions (*e.g.*, Ashburner *et al.* 1999), only 1% of insertions from the *Y* chromosome donor and 0.4% of the insertions from the *2R* donor map to this site, compared with >10% of insertions from the *2L* donor. We presume that this disparity results from synergy between local *P*-element hopping and a very permissive *P*-element acceptor site. In addition to the *escargot* locus, there are a few other hotspots for insertion. A cluster of 46 elements at 53F9, in the vicinity of the *CB-0102-3* donor, is again presumably due to local hopping. Three other sites, at 19A2, 21B, and 92B3, each have >20 independent insertions.

In general, the distribution of elements along each of the chromosome arms is uniform. On average, we have collected insertions spaced every 30–40 kb (Table 3). A relatively even distribution is important for our primary objective, to generate a set of chromosomal deletions covering the entire genome. To allow an assessment of the deletion coverage our collection can generate, we devised a custom Perl script to process the element-insertion data held in an SQL database. The script takes into account the requirement to pair *RS3r* and *RS5r* elements in the correct orientation to generate a $w^+$ deletion chromosome. In theory, our collection can generate 12,258 chromosomal deletions ranging in size from 1 bp to 1 Mb (Table 4). In terms of coverage (Table 4), we find that chromosome arms *2L*, *3R*, and *X* are equally represented with a little over 2000 potential deletions possible on each. Chromosome arm *2R* is overrepresented with >3600 deletions predicted, whereas *3L* is underrepresented with ~1500 deletions possible. These figures highlight the importance of element distribution (Table 3) for our objective. Arm *3L* is under-

**TABLE 5**

**Gene coverage of predicted deletions**

| | *2L* | | | *2R* | | | *3L* | | | *3R* | | | *4* | | | *X* | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Size range | Genes | Deletions | Average | Genes | Deletions | Average | Genes | Deletions | Average | Genes | Deletions | Average | Genes | Deletions | Average | Genes | Deletions | Average | Total |
| 1 bp to 10 kb | 11 | 287 | 0.04 | 39 | 199 | 0.20 | 8 | 98 | 0.08 | 37 | 125 | 0.30 | 0 | 7 | 0.00 | 6 | 157 | 0.04 | 873 |
| 10–100 kb | 1,775 | 225 | 7.89 | 3,208 | 344 | 9.33 | 1,435 | 183 | 7.84 | 1,639 | 219 | 7.48 | 12 | 3 | 4.00 | 1,370 | 226 | 6.06 | 1,200 |
| 100–500 kb | 32,078 | 855 | 37.52 | 58,323 | 1449 | 40.25 | 17,305 | 487 | 35.53 | 29,596 | 759 | 38.99 | 449 | 21 | 21.38 | 34,589 | 999 | 34.62 | 4,570 |
| 500 kb to 1 Mb | 85,763 | 938 | 91.43 | 176,962 | 1666 | 106.22 | 62,440 | 727 | 85.89 | 95,682 | 1019 | 93.90 | 0 | 0 | 0.00 | 103,779 | 1265 | 82.04 | 5,615 |
| Total | — | 2305 | — | — | 3658 | — | — | 1495 | — | — | 2122 | — | — | 31 | — | — | 2647 | — | 12,258 |

A summary of the number of genes uncovered by predicted deletions in a given size range is shown. Genes, the number of genes on a given chromosome arm that would be included in deletions of a particular size range. Deletions, the number of deletions that could be constructed with the current collection. Average, the average number of genes included in deletions of a given size range.

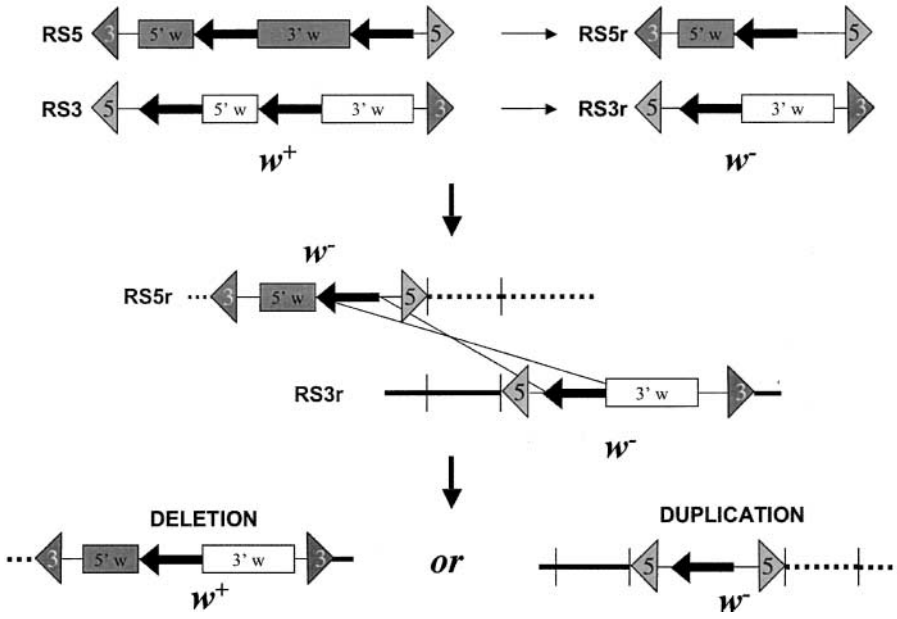



FIGURE 6.—Creating genomic deletions with *RS* elements. A stylized cartoon of the *P{RS3}* and *P{RS5}* elements shows a functional mini-white gene composed of two exons (boxes) and two *FRT* sites (shown as thick arrows in the *P* element), one of which is contained in intron 1 of the *white* gene. In reality the *w-hs* gene used in the *RS* constructs contains 6 exons, with the FRT site located in the first intron. The 5′ and 3′ ends of the *P* element are marked with triangles. The elements differ in the position of the second *FRT* site in the element and the orientation of the construct within the *P*-element ends. Internal recombination between the *FRT* sites mediated by FLP recombinase produces the remnant form of the elements, *P{RS3r}* and *P{RS5r}*, each of which has a nonfunctional *white* gene and a single *FRT* site. If a *P{RS3r}* and a *P{RS5r}* are arranged *in trans* on homologous chromosomes and they are in the orientation and relative positions shown, an FLP-mediated recombination between them produces a reconstituted *P* element with a functional *white* gene. The intervening genomic DNA in this example is deleted. The reciprocal event in this case creates a tandem duplication of the deleted segment, separated by an *FRT* site, but no *white* gene.

represented due to a lower frequency of element insertion on this arm (16% compared to ~20% for the other major arms).

The fact that our collection is precisely mapped with respect to the genome sequence allows us to accurately determine the number of genes that deletions in particular size ranges will uncover. For example, in the 500- to 700-kb range an average of ~70 genes per deletion are uncovered on each arm (Table 5). In terms of our goal to generate a new deletion kit, we have selected an average deletion size of 600 kb with, where possible, an overlap of 200 kb. This will generate a collection of ~600 deletions covering the genome. Since, on average, each 600-kb deletion will remove ~70 genes, constructing deletions overlapping by 200 kb will allow screening at a resolution of ~25 genes per unique deleted region. Overall, our predictions indicate that we can cover >95% of the euchromatic genome with deletions at this resolution. Therefore, our insert collection provides good coverage and will generate deletions at a level of resolution that will permit efficient whole-genome screens with a manageable number of stocks.

**Insertions in or near genes:** Although not our primary objective, some of the insertions we have generated are of utility for the gene disruption project since they are located in genes or regions of the genome previously not hit by *P* elements. Approximately 66% of our *RS*-element insertions map within 1 kb of a previously mapped *P* element. Thus, ~1000 elements may be used to increase the coverage of *P*-element insertions in the Drosophila genome since they are >1 kb away from a previously described *P* insert. From our collection, a total of 1970 elements are inserted within 959 annotated genes: of these, 36 are genes previously unmarked by a *P*-element insertion and 33 have no previously reported alleles in FlyBase. These data are currently being curated by FlyBase and are available from the DrosDel web site.

**Pilot deletion screen:** As a prelude to the planned deletion kit, a pilot study was performed to validate the method for constructing deletions and ensure our lines

**TABLE 6**

**Deficiencies constructed in the pilot screen**

| Df | Name | Band | Location | Name | Band | Location | Chromosome | Size (bp) |
|---|---|---|---|---|---|---|---|---|
| *Df(2R)ED1* | *CB-0140-3* | 53E10 | 12090452 | *5-HA-1118* | 53F9 | 12161047 | *2R* | 70,595 |
| *Df(3R)ED2* | *CB-0160-3* | 91A5 | 14224969 | *5-HA-1087* | 91F1 | 14922510 | *3R* | 697,541 |
| *Df(2L)ED3* | *5-HA-1150* | 35B2 | 14472727 | *CB-0183-3* | 35D1 | 15311845 | *2L* | 839,118 |
| *Df(4)ED6364* | *CB-0038-3* | 102A4 | 79870 | *5-HA-1572* | 102B1 | 213735 | *4* | 133,865 |
| *Df(4)ED6366* | *CB-5204-3* | 102A4 | 90336 | *5-HA-1572* | 102B1 | 213735 | *4* | 123,399 |

**TABLE 7**

**Deficiency results**

| Df | $F_1$ variegation | No. of $w^+$ $F_2$ | % |
|---|---|---|---|
| *Df(2R)ED1* | Strong | 81/1155 | 7 |
| *Df(3R)ED2* | Red spots | 2/1109 | 0.2 |
| *Df(2L)ED3* | Slight | 1/832 | 0.1 |
| *Df(4)ED6364* | White | 4/716 | 0.6 |
| *Df(4)ED6366* | White | 20/625 | 3.2 |

behave as expected. Constructing deletions with the *RS* elements is a three-step process (Figure 6). A pair of *RS3* and *RS5* elements in the same relative orientation is selected using the software described above. Appropriate pairs of element-bearing stocks are then individually subjected to FLP-induced recombination to make $w^-$ derivatives known as remnants (*RS3r* and *RS5r*). In the case of an *RS5* element, FLP-induced recombination will generate a remnant that carries only the first exon of the *mini-white* gene; in the case of an *RS3* element, the last five exons of *mini-white* will remain after recombination. In each case the remnant element will be flanked on one side by an FRT site. The remnant elements are then combined *in trans* in the presence of a source of FLP. Recombination induced between their FRT sites will reconstitute a functional *white* gene and generate a deletion of the sequence between the *RSr* elements. This event will happen in both the germline and the soma; consequently the heat-shocked flies will often display red sectors in the eye. The reciprocal product will be a tandem duplication of exactly the same extent, but will remain $w^-$ (Figure 6). Since all of the stocks used carry a nonfunctional endogenous *white* allele, each step can be followed simply by eye color. Flies that carry the deletion can be identified as the only red-eyed progeny from heat-shocked parents. The size of the deletions and the elements used are shown in Table 6. Deletions between 70 and 839 kb were chosen to test the robustness of the system.

**Flip-out and flip-in frequencies:** The internal recombination of the *RS* elements to produce the $w^-$ remnants (flip-out) was extremely efficient, with rates of conversion well over 98% in all lines tested ($n = 378$). Rates of FLP-mediated recombination to produce reconstituted *white* genes and, hence, deficiencies, varied with the size of the deletion produced, the smaller 70-kb deletion [*Df(2R)ED1*] occurring much more readily than the larger ones (Table 7). The appearance of mosaic-eyed flies in the heat-shocked generation is indicative of the success of the flip-in. The frequency and size of red sectors is often reflected in the frequency of red-eyed progeny in the next generation and there is some correlation between the degree of $F_1$ variegation and the size of the deletion.

**Verification of elements:** The pilot deficiencies were examined genetically by complementation testing with existing mutations and deletions. In addition to the genetic tests, the reconstituted *RS* element in each deficiency line was analyzed molecularly to confirm that it contained the predicted deletion end points. To this end, specific PCR primers were designed for genomic DNA flanking the end points of each deletion and PCR-amplified products generated from heterozygous deletion-bearing lines were sequenced. In all cases the genetic and molecular data were in agreement with the predicted results.

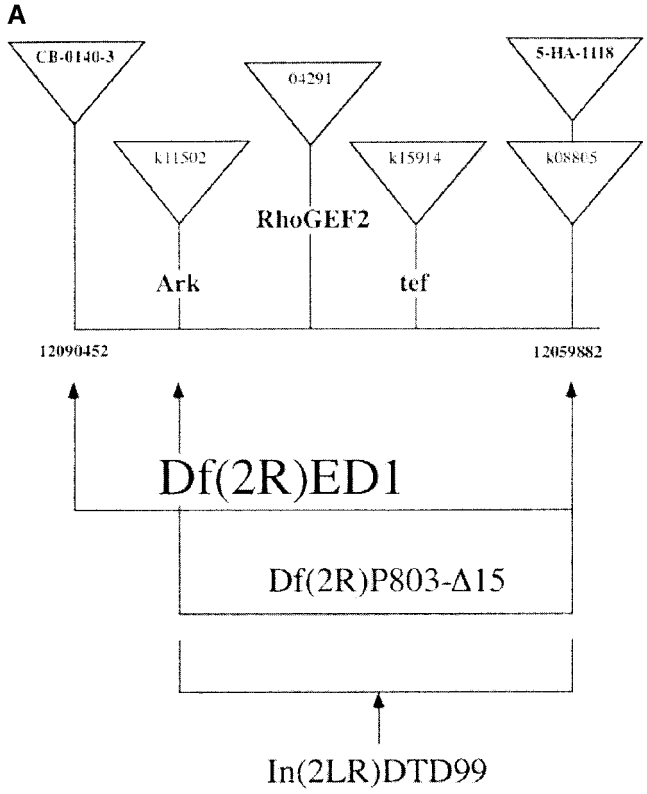



FIGURE 7.—Genetic analysis of deletions generated with the *RS* system. The diagrams show the genetic extent of known deletions relative to a set of known gene mutations and *P*-element insertions (triangles above the line). The end points of the *ED* deficiencies with respect to the scaffold sequence location are indicated below the line. See text for details. (A) *Df(2R)ED1* (53F1;53F11, 70,776 bp). (B) *Df(3R)ED2* (91A5;91F1, 697,541 bp). (C) *Df(2L)ED3* (35A;35D, 839,118 bp).

*Df(2R)ED1* is the smallest deficiency we constructed at 70 kb (Figure 7A). As expected, it is lethal in combination with $RhoGEF2^{04291}$ (53E4-F2) and *Df(2R)P803-Δ15* (53E;53F11). Sequencing confirms the presence of the *CB-0140-3* and *5-HA-1118* insertion end points and the PCR product from the reconstituted *w* gene is also as expected. *Df(3R)ED2* is ~10-fold larger at 697 kb (Figure 7B). The deletion chromosome is lethal in combination with *P{lacW}l(3)S007302* (91B1-6) and *Df(3R)Cha9* (91C7-D1; 92A2); again the molecular mapping confirms the predicted end points and the correct *w* gene structure. *Df(2L)ED3* is the largest deletion we attempted at 839 kb, just under 0.7% of the euchromatic genome (Figure 7C). It is genetically deleted for *noc* (*no-ocelli*) and *nht* (*no-hitter*), but not *el* (*elbow*) or *esg* (*escargot*), as

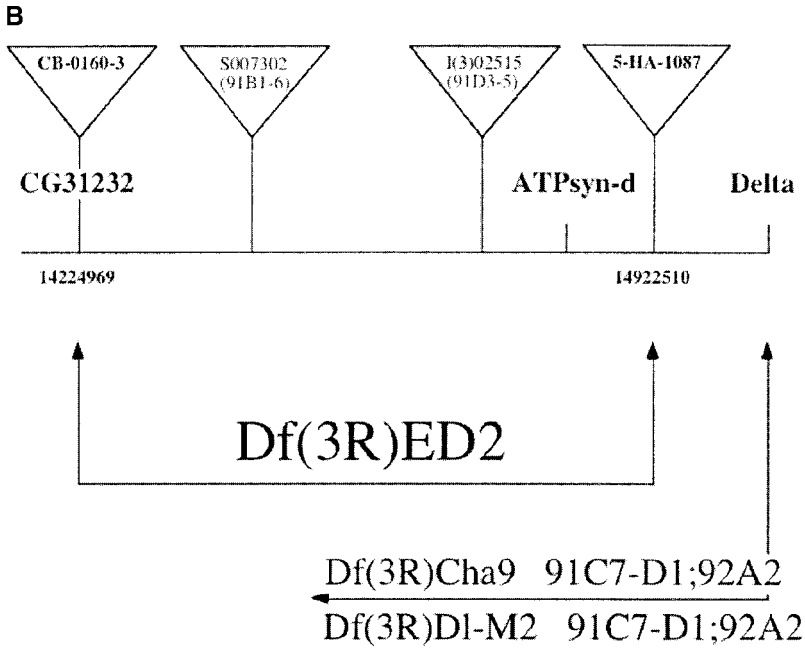
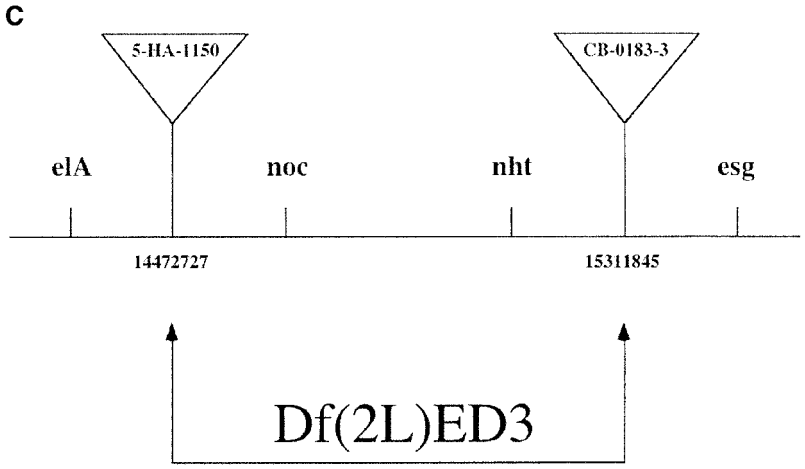



FIGURE 7.—*Continued.*




predicted from the *RS*-element-insertion sites. Molecular mapping confirmed the predicted end points. Finally, to demonstrate the resolution and utility of the system, we attempted to construct two fourth chromosome deletions differing by a single, predicted, haploinsufficient gene (Figure 8). We began with a common *RS5* element (*5-HA-1572*) inserted in *CG2316* and generated a 123-kb deletion [*Df(4)ED6366*] ending in the 5′ end of *pangolin* by recombination with *CB-5204-3*. A second deletion [*Df(4)ED6364*] extended a further 10 kb proximal (*CB-0038-3*, 2 kb distal to *ci*) and removes the predicted ribosomal protein-encoding gene, *RpS3A* (*CG2168*). Molecular mapping confirmed the predicted breakpoints and a genetic analysis confirmed that both deletions fail to complement *pan* mutations. Since *Df(4)ED6364* removes *RpS3A* we would expect it to show a dominant Minute phenotype and this is indeed what we observe.

**Element stability:** A potential problem with using deletions based on *P* elements for subsequent genetic experiments could be that the reconstituted *RS* element would mobilize in the presence of a source of transposase. However, deletions constructed with this method generate a recombinant *P* element that is flanked by two 3′-*P*-element ends. This element should be stable in the presence of *P*-element transposase since transposition requires intact 5′ and 3′ ends (MULLINS *et al.* 1989). To confirm the stability of the double 3′ *P* elements we screened for the loss of the $w^+$ marker in three different deletion lines in the presence of the Δ*2-3* transposase. No $w^-$ progeny were observed from *Df(2R)ED1* (18,305 progeny), *Df(3R)ED2* (7368 progeny), or *Df(2L)ED3* (16,743 progeny).

## DISCUSSION

We describe here the results of *P*-element-mobilization screens using a pair of transposons that facilitate the generation of custom chromosomal aberrations using the methods developed by GOLIC and GOLIC (1996).

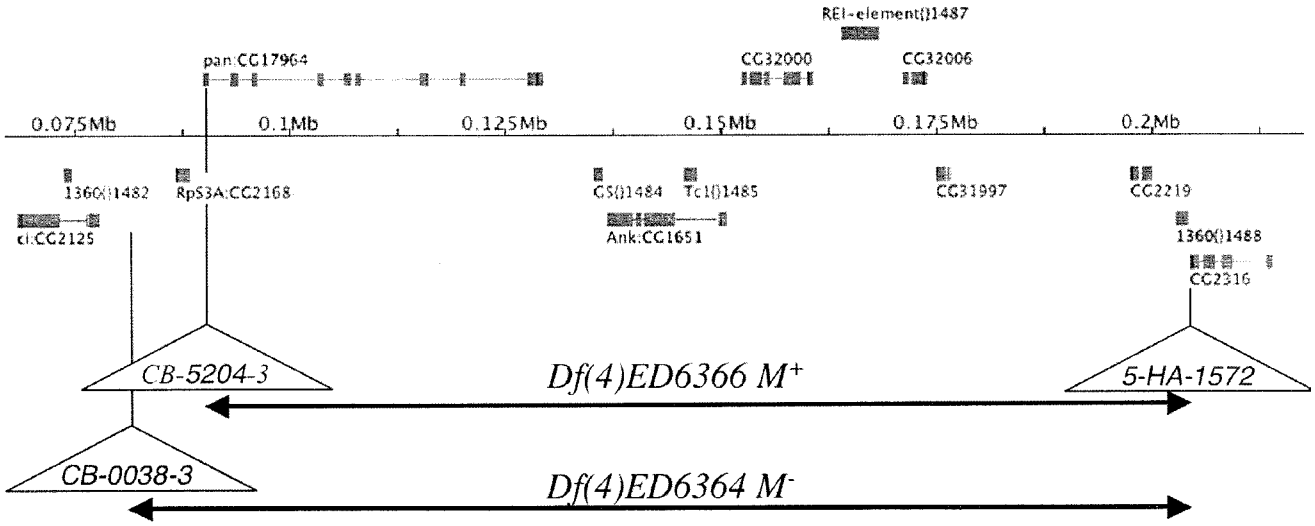



FIGURE 8.—Generating deletions with single-gene resolution: *Df(4)ED6364* and *Df(4)ED6366* both start at the distal insertion, *5-HA-1572*. *Df(4)ED6366* extends to *CB-5204-3*, which is distal to the ribosomal protein gene *RpS3A*. As a heterozygote *Df(4)ED6366* is phenotypically normal (*Minute*$^+$). *Df(4)ED6364* extends a further 10 kb proximal to *CB-0038-3* and deletes the *RpS3A* gene. Individuals heterozygous for this deletion show a strong *Minute* phenotype. The molecular map is a representation of the genome annotation from Apollo.

In total, we have mapped 3243 insertions by DNA sequencing onto the current Drosophila genomic sequence scaffold. Our analysis indicates that we can use these insertions to produce, in theory, 12,258 different deletions between 1 bp and 1 Mb. With the current element distribution the collection can potentially generate deletion coverage for ~95% of the euchromatic genome. The materials to synthesize all of these deletions are available from public stock centers. The pilot experiments presented here indicate that molecularly defined deletions of >800 kb can be efficiently generated. Whether or not any particular deletion can be recovered will, of course, depend upon the phenotypic consequences of haploidy for its chromosome region. In very general terms deletions that are >1 Mb in *D. melanogaster* are inviable as heterozygotes (ASHBURNER 1989). We are, therefore, aiming to generate a set of 600-kb deletions overlapping by an average of 200 kb. This will cover the majority of the euchromatic genome with deletions at a resolution of ~25 genes per deletion. In practice, this means that the whole genome can be screened at high resolution with ~600 stocks.

Our collection is of particular utility because all of the insertion lines and the deletions that are derived from them are in a uniform genetic background. The importance of this fact cannot be overstated since many biological processes, particularly complex multigenic traits, are very sensitive to genetic background. In constructing our collection we were careful to assay several isogenic lines to select a genetic background that scored as close to wild type as possible in different behavioral assays. This selection alone will make our collection of particular utility in genetic screens for behavioral phenotypes. Along with complex genetic traits, it is becoming clear from data generated in microarray experiments in both *Saccharomyces cerevisiae* and Drosophila that genetic background can have profound effects on gene expression. A genome-wide genetic resource generated on a single genetic background will be of considerable utility in pursuing genomics approaches to Drosophila biology. It might be argued that the genetic background for this resource should be that sequenced by the Drosophila genome project (ADAMS *et al.* 2000). However, this background carries four visible mutations ($y^1$, $cn^1$, $bw^1$, and $sp^1$), each of which could interfere with a phenotypic assay. For this reason we chose to use a genetic background that was as free of known mutations as possible and was also consistent with the genetic methods needed to attain our end. Of course the isogenic background of the stocks will not be maintained forever since the natural accumulation of mutations in the stocks will, over time, generate second-site lethals, in particular, deletion or element stocks. While this is a difficulty that affects all Drosophila stocks, our collection is unique in that it starts from a uniform genetic background. In addition, since our collection can be used to generate custom deletions, any suspect genetic interaction detected with a DrosDel deletion can, in many cases, be rapidly checked by simply selecting an alternative pair of elements and generating a new deletion. Individual *RS* stocks are unlikely to accumulate the same mutations.

We demonstrate the utility of our *P*-element collection for generating molecularly defined deficiencies by constructing five deletions, ranging in size from 70 to 839 kb, which delete 14 (*ED1*), 52 (*ED2*), 51 (*ED3*), 8 (*ED6364*), and 7 (*ED6366*) predicted genes. Both ge-

netic and molecular mapping of each deletion indicates that they are all of the expected structure. In addition to this we show how the collection can be used to generate deletions with single-gene resolution. This aspect of the collection may be useful for studies with previously intractable regions of the genome and suggests that interested researchers may be able to rapidly construct a set of defined deletions that can provide a fine-structure genetic map in regions of particular interest.

We note that other researchers have recognized the need for generating custom chromosomal deficiencies on a genome-wide scale. A recently described method, using a hybrid *P*-element – *hobo* transposon, demonstrates that it is possible to generate deletions of at least 0.5 Mb and that by using a molecular screen one can recover many nested deletions in a particular region (Huet *et al* 2002; Mohr and Gelbart 2002). While this system is clearly very flexible, the advantage of our collection is that deletions may be generated from stocks already mapped to the genome sequence and further molecular characterization is limited to confirming the deletion end points.

The process of generating deletions once appropriate pairs of elements have been identified is straightforward. We have produced a "genetic toolbox" of 14 fly strains, all based on our isogenic background, that researchers can use to construct custom deletions. These are available from public stock centers and detailed instructions for crossing schemes are on the DrosDel web site. In addition, we provide a range of informatics tools on the web site that allow researchers to easily select pairs of elements to generate deletions in regions of interest. The initial flip-out to generate *RSr* remnant elements is extremely efficient and the easily identified $w^-$ individuals are recovered after a single heat-shock pulse during embryogenesis. In the case of the flip-in to generate the deletion, efficiency varies with the size of the deleted genomic segment. For small (<100 kb) deletions, almost 10% of the progeny are $w^+$ and have therefore undergone the appropriate recombination. With larger deletions the efficiency drops off to 0.1% or less; however, $w^+$ individuals are easily spotted in a population, even at a level of 1/10,000. We therefore believe that, within the limitations of the deletion size that flies will tolerate, we can efficiently construct a tile of genomic deletions spanning much of the euchromatic portion of the genome.

Any researcher can order a particular pair of *RS* elements and, from them, construct a deletion. If more than one laboratory did so for a particular pair of elements and named the resulting deletion differently, then recording and annotating information from the literature could become difficult. For this reason we have prenamed all theoretically possible $w^+$ deletions up to 1 Mb in size and appeal to the community to use these names. When researchers order stocks from the DrosDel web site they are automatically informed of the correct name for each potential deletion they could generate. The names of the deletions follow the current nomenclatural guidelines (http://flybase.bio.indiana.edu/docs/nomenclature/lk/nomenclature.html) with the generic symbol *Df(n)*, where *n* is the chromosome or chromosome arm containing the deletion, followed by the suffix *EDN*, where *N* is a preassigned integer, *e.g.*, *Df(3R)ED2*. Data on all $w^+$ DrosDel deletions that can theoretically be generated have been submitted to FlyBase. Those that have not been made are listed under a new class of aberration: potential deficiency.

In addition to the $w^+$ deletions we describe here, the *RS* elements can generate other aberrations. For example, if we remove the limitation that deletions produced by recombining *RSr* elements need to generate $w^+$ progeny, the coverage of the collection is substantially increased. With these criteria we estimate that an additional 37,000 chromosomal deletions could be constructed with our collection. Such deletions may be generated from any two elements that are a suitable distance apart and in the correct orientation; there is no longer a requirement to use one *RS3* and one *RS5*. Although these deletions cannot be selected on the basis of eye color they may be isolated using selection strategies combined with molecular methods such as PCR (*i.e.*, Kaiser and Goodwin 1990) or by the classical genetic method of uncovering a recessive visible marker. Alternatively, $w^-$ deletions or duplications can be selected by scoring for male recombination; however, in this case the isogenicity of the genetic background would be lost.

As with the current, widely used deficiency kit, a barrier to any attempt to cover the entire Drosophila genome with deletions or mutations is the presence of haplo-insufficient regions. Approximately 76 haplo-insufficient loci are known in *D. melanogaster* (Lindsley *et al.* 1972; Ashburner 1989; K. Cook, personal communication). These may have a visible, lethal, or sterile phenotype. Of these, at least 55 are known or are predicted to be due to the haplo-insufficiency of genes encoding ribosomal proteins (Lambertsson 1998; K. Cook, personal communication). Deletions for most of these will have a *Minute* phenotype (Lambertsson 1998) and should be recoverable if suitable precautions are taken (*i.e.*, by allowing slowly developing genotypes to hatch). In some regions more than one ribosomal protein maps close together; however, in general, *Minutes* are not additive in their phenotypic effects (Schultz 1929). When we take into account the locations of known haplo-insufficient loci, we calculate that our insertions will enable the construction of deficiencies that will cover 86% of the euchromatic genome. This is virtually identical to the 85% coverage attained with the current, genetically heterogeneous deficiency kit.

Several stratagems are available to obviate the problems of haplo-insufficient loci. If these loci have been cloned, one is transformation rescue. Another would

be to balance the deletion with a suitable duplication. Such duplications can be constructed as the reciprocal products of the deletions described here (Figure 6); however, in this case it is not clear how stable such tandem duplications will be in isolation when they do not balance a haplo-insufficient region. An alternative approach is to generate duplications as recombinants between similar pericentric inversions, either directly or via autosynaptic intermediates (Gubb 1988; D. Gubb and J. Roote, unpublished observations). In their article describing the system, Golic and Golic (1996) show how the *RS* elements can be used to generate other chromosomal aberrations by design. For example, inversions and duplications can be produced with similar ease from *RS* insertions, considerably increasing the utility of the collection we have produced. We confirm their observations by generating both inversions and autosynaptics from our *RS* collection in pilot experiments (D. Coulson, unpublished observations). We are currently exploring the possibility of generating a genome-wide duplication kit to complement the deficiency kit. A final approach to increasing deletion coverage is to attempt to construct individual deletions that flank the haplo-insufficient locus. We demonstrate here the possibility of this approach by generating two fourth chromosome deletions that differ by a single gene, *RpS3A*. Animals haploid for *RpS3A* can be recovered; they do, however, display a strong *Minute* phenotype.

In summary, we have generated a collection of *P*-element insertions on an isogenic genetic background that permit the construction of molecularly defined chromosomal aberrations in Drosophila. Using our collection, >12,000 precisely mapped deletions can theoretically be generated. We demonstrate the feasibility of using the collection to generate genetically and molecularly verified deletions and further show how the collection can be employed to delete genomic regions with single-gene resolution. The collection, along with computational tools for mapping, is available to the wider research community, giving researchers the capability of generating defined chromosomal deletions in regions of interest. We are now proceeding with the construction of a core deficiency kit.

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*Note added in proof*: Subsequent to the acceptance of this manuscript, two articles describing a collection of *piggyBac*-based insertions and a set of deletions created by an FRT-based approach similar to the one described here have been published, supporting the utility of the method we propose (S. T. Thibault, M. A. Singer, W. Y. Miyazaki, B. Milash, N. A. Dompe *et al.*, 2004, A complementary transposon tool kit for *Drosophila melanogaster* using *P* and *piggyBac*. Nat. Genet. **36:** 283–287; A. L. Parks, K. R. Cook, M. Belvin, N. A. Dompe, R. Fawcett *et al.*, 2004, Systematic generation of high-resolution deletion coverage of the *Drosophila melanogaster* genome. Nat. Genet. **36:** 288–297).

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