Systematic generation of high-resolution deletion coverage of the *Drosophila melanogaster* genome

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In fruit fly research, chromosomal deletions are indispensable tools for mapping mutations, characterizing alleles and identifying interacting loci. Most widely used deletions were generated by irradiation or chemical mutagenesis. These methods are labor-intensive, generate random breakpoints and result in unwanted secondary mutations that can confound phenotypic analyses. Most of the existing deletions are large, have molecularly undefined endpoints and are maintained in genetically complex stocks. Furthermore, the existence of haplolethal or haplosterile loci makes the recovery of deletions of certain regions exceedingly difficult by traditional methods, resulting in gaps in coverage. Here we describe two methods that address these problems by providing for the systematic isolation of targeted deletions in the *D. melanogaster* genome. The first strategy used a *P* element-based technique to generate deletions that closely flank haploinsufficient genes and minimize undeleted regions. This deletion set has increased overall genomic coverage by 5–7%. The second strategy used FLP recombinase and the large array of FRT-bearing insertions described in the accompanying paper¹ to generate 519 isogenic deletions with molecularly defined endpoints. This second deletion collection provides 56% genome coverage so far. The latter methodology enables the generation of small custom deletions with predictable endpoints throughout the genome and should make their isolation a simple and routine task.

Deletion screens conducted at the Bloomington *Drosophila* Stock Center are based on the observation that *P* transposase often induces chromosomal aberrations involving the sites of two *P* insertions². When two insertions are present at different sites on homologous chromosomes, deletions that remove the region between the two insertions can be recovered. In appropriately marked crosses, the deletions can be recognized from the recombination of visible, flanking markers. Our results are consistent with studies of *P* transposition showing that faulty transposition can produce deletions when the 5' end of one *P* element and the 3' end of a different *P* element are inserted in a chromosome as if they were the two ends of a single element^{3,4} (Fig. 1a). This mistake in the normal transposition process has been called hybrid element insertion (HEI), because the paired 5' and 3' ends from different *P* elements inserted at a new chromosomal site by *P* transposase can be thought of as a temporary, synthetic or 'hybrid' *P* element³. Resulting deletions are flanked by one of the starting *P* constructs (Fig. 1a). This provides a convenient molecular tag for subsequent breakpoint characterization. Because a hybrid element can potentially insert anywhere in the genome, many different chromosomal rearrangements can result in addition to simple deletions. We isolated other rearrangements (data not shown) but specifically selected for deletions among recombinant progeny by complementation tests against mutations mapping between the original *P* insertion sites.

The success of the approach depends on a strong inherent bias of hybrid elements to insert in or near one of the original *P* insertions. If HEI were random, only one deletion breakpoint would be predictable.

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Figure 1 Schematics for deficiency generation. The starting pairs of chromosomes are shown in orange and blue, with the transposon insertion elements indicated in light yellow or light blue and FRT elements in green. The order of theoretical markers A, B and C along the chromosomes is indicated. **(a)** The HEI strategy used at Bloomington. **(b)** Using FLP-FRT recombination to generate *w*- deficiencies from Exelixis transposon insertions. **(c)** Using FLP-FRT recombination to generate *w*+ deficiencies from Exelixis transposon insertions.

HEI deletions. We successfully used combinations of *P{PZ}*, *P{lacW}*, *P{SUPor-P}*, *P{EP}* and *P{GT1}* insertions.

So far, Bloomington has recovered 45 unique deletions using the HEI approach with *trans*-heterozygous *P* insertions (**Supplementary Table 1** online). **Table 2a** summarizes their contribution to genomic coverage. All the screens were designed to fill gaps in deletion coverage, and most were designed to extend from a point adjacent to a haploinsufficient locus into an existing deletion. The fact that most haploinsufficient genes in *D. melanogaster* encode protein components of ribosomes⁶ simplified the planning. By minimizing the undeleted regions to the small intervals between the two *P* insertions most closely linked to haploinsufficients, we could maximize coverage without the need for chromosomal duplications. For example, *Df(2L)BSC32* and *Df(2L)BSC36* flank the haploinsufficient locus *RpL9*, leaving only it and four other genes undeleted. So far, we have maximized deletion coverage around 14 known or potential haploinsufficients and maximized coverage to one side of an additional 10 haploinsufficients.

This method may be useful in certain experimental situations where *P* insertions are favorably located, but the method described below will probably prove more useful in most situations and will be used at Bloomington for further efforts to improve the Stock Center deletion collection. As described by Thibault *et al.*¹ in this issue of *Nature Genetics*, investigators at Exelixis generated a large isogenic collection of *P* and *piggyBac* insertion lines containing FRT sites. In the presence of FLP recombinase, FRT-bearing transposon insertions can be used to efficiently isolate deletions with precisely defined endpoints⁷. We used this method to generate a set of isogenic deletions as described below. The DrosDel Consortium is using a similar method (see URL).

We recovered only two deletions in all our screens in which the breakpoints differed from the sites of the original *P* insertions at the level of polytene cytology (*Df(2R)BSC25* and one not shown). The basis of this bias is unknown, but it resembles, at least superficially, the bias of single *P* elements to transpose locally⁵. Although the deletion endpoints are not entirely predictable, the method is useful because deletions can be targeted to particular genomic regions, the screens are moderately efficient and the size of deletions seems to be limited only by aneuploidy effects.

Figure 2a shows the crosses we used in HEI deletion screens and **Table 1a** gives markers and stocks we found particularly useful. When flanking markers and *P* insertions are linked (**Fig. 2a**), deletions will be recovered among progeny with both recessive marker phenotypes. With different linkage relationships, deletions will be recovered among progeny with different marker combinations. We recovered deletions with the two *P* insertions in the same and in opposite orientations. As *P* element ends are the only *cis*-acting sequences required for transposition, it seems that any two *P* constructs can be combined to produce

Table 1 Stock genotypes used in the generation of deletions

(a) Useful genotypes for generating *P*-element transposase-based deletions

Deletion in chromosome region	Useful flanking markers	Tester chromosome genotype	Transposase stock genotype	Balancer chromosome
21A–43E	<i>net¹</i> and <i>cn¹</i>	<i>net¹ b¹ cn¹ sp¹</i>	<i>T(2;3)ap^{Xa}, ap^{Xa}/CyO, H(PDelta2-3)HoP2.1, Cy¹; Sb¹</i>	<i>SM1, SM5, SM6a</i> or <i>SM6b</i> : all marked with <i>Cy</i> , <i>cn</i> and <i>sp</i> mutations
25A–43E	<i>dp^{ov1}</i> and <i>cn¹</i>	<i>dp^{ov1} cn¹ bw¹</i>	<i>T(2;3)ap^{Xa}, ap^{Xa}/CyO, H(PDelta2-3)HoP2.1, Cy¹; Sb¹</i>	<i>SM6a, Cy¹ dp^{ov1} cn^{2P} sp² bw^{k1}</i>
43E–59E	<i>cn¹</i> and <i>bw¹ sp¹</i>	<i>cn¹ bw¹</i>	<i>T(2;3)ap^{Xa}, ap^{Xa}/CyO, H(PDelta2-3)HoP2.1, Cy¹; Sb¹</i>	<i>SM6a, Cy¹ dp^{ov1} cn^{2P} sp² bw^{k1}</i>
62A–93D	<i>rho^{ve-1}</i> and <i>e¹</i>	<i>rho^{ve-1} p^p e¹</i>	<i>wg^{Sp-1}/CyO, Cy¹; TMS, P(Delta2-3)99B, Sb¹/TM6, Ubx^{P15}</i>	<i>TM2, Ubx¹³⁰ p^p e¹ or TM3, Sb¹ p^p e¹</i>
73A–99B	<i>st¹</i> and <i>ca¹</i>	<i>st¹ Sbsbd-1 e^s ro¹ ca¹</i>	<i>wg^{Sp-1}/CyO, Cy¹; TMS, P(Delta2-3)99B, Sb¹/TM6, Ubx^{P15}</i>	<i>TM2, TM3, TM6, TM6B</i> or <i>TM6C</i> : all carry <i>ebony</i> and dominant markers

(b) Useful genotypes for generating FLP-FRT-based deletions

	Deletions on X	Deletions on 2nd	Deletions on 3rd
A stocks	<i>w¹¹¹⁸, MKRS, M(3)76A, kar¹ ry² Sb¹, P(hsFLP)86E/TM6B, Antp^{Hu} e¹ Tb¹</i>	<i>P(hsFLP)¹, w¹¹¹⁸, Adv¹/CyO, Cy¹ dp^{ov1} pr¹ cn²</i>	<i>P(hsFLP)¹, y¹ w¹¹¹⁸, Dr^{Mio}/TM3, kni^{ri-1} p^p sep¹ [(3)89Aa¹ Ubx^{bx34e} e¹ Sb¹</i>
B stocks	<i>XPG-L³/In(1)sc^{S1L}sc^{8R}+dl-49, y^{c4} sc⁸ sc^{S1} w¹ sn^{X2} B¹; iso2; iso3</i>	<i>iso^b w¹¹¹⁸, wg^{Sp-1}/CyO, Cy¹ dp^{ov1} pr¹ cn²; Ly¹/TM6B, Antp^{Hu} e¹ Tb¹</i>	<i>iso w¹¹¹⁸, wg^{Sp-1}/CyO Cy¹ dp^{ov1} pr¹ cn²; Ly¹/TM6B, Antp^{Hu} e¹ Tb¹</i>
C stocks		<i>iso w¹¹¹⁸, wg^{Sp-1}/CyO, Cy¹ dp^{ov1} pr¹ cn²; iso3</i>	<i>iso w¹¹¹⁸; iso2; Dr¹/TM6B, Antp^{Hu} e¹ Tb¹</i>
D stock	<i>XPG-L³/FM7C, y^{31d} sc⁸ w², sn^{X2} v^{of} g⁴; +; +</i>		

^aXPG-L is an uncharacterized P lethal. ^biso refers to an Exelixis isogenized chromosome.

Figure 2 Genetic crossing schemes used to generate deletions. **(a)** Genetic scheme used for P-element transposase-based deletions. The crosses place P insertions (triangles) marked with flanking visible marker mutations ($m1^-$ and $m2^-$) in males together with a source of P transposase ($tnpase$), so that hybrid element insertions can occur in premeiotic germ cells. Deletion events accompanied by recombination of flanking visible markers are identified in crosses to females carrying appropriate tester chromosomes. Deletions are established in stocks with balancer chromosomes (Bal). Dom and $m3^-$ represent other convenient dominant and recessive markers, respectively. **(b)** Genetic scheme used for FLP-FRT-based deletions. Crosses place two FRT-bearing transposon insertions (triangles) in *trans* in the presence of heat shock-driven FLP recombinase ($hs-FLP$). Activation of FLP recombinase results in the generation of deletions that can be detected by PCR. Deletions are established in isogenized stocks with balancers (Bal). Genotypes for nontransposon stocks (A, B, C, and D stocks) are provided in **Table 1**. Dom , dominant visible marker mutation; iso , isogenized chromosome; Y, Y chromosome.

The Exelixis deletion series is based on three of the four classes of transposon constructs described in the accompanying paper¹. These transposon insertions contain FRT sites of 199 bp either 5' (in XP and WH transposons) or 3' (in RB transposons) of the *white+* transgene ($w+$; **Fig. 3a**). In the presence of FLP recombinase, efficient

trans-recombination between FRT elements results in a genomic deletion with a single residual element tagging the deletion site (**Fig. 1b,c**). In deletions generated using XPs, which contain tandem FRT sites, the *cis*-recombination event occurs earlier than that in *trans*. Depending on the pair of starting insertions, their genomic orientation and the relative position of $w+$ with respect to the FRTs, some deletions can initially be detected in the progeny by a loss of the $w+$ marker (**Table 3**). The crosses outlined in **Figure 2b** and **Table 1b** allow the efficient recovery of deletions in four generations. Progeny are screened for the presence of the residual element by PCR detection of a resulting hybrid element (e.g., $XP:WH$) using element-specific primers, or by detection of residual element ends using paired element-specific and genome-specific primers (**Fig. 3b**). Final confirmation of the deletion ends can be provided by PCR or sequencing with genome-specific primers. In general, PCR screening of five $w-$ individual progeny from $w-$ deletion generation crosses, or 50 progeny from $w+$ deletion generation crosses, resulted in four confirmed deletions. In addition to deletions, tandem duplications could also be isolated using these insertions and an appropriately designed PCR strategy.

Supplementary Table 2 online provides a list of 519 deletion stocks, covering approximately 56% of CG isoforms in the genome (see **Table 2b**), which have been transferred to the Bloomington *Drosophila* Stock Center. These deletions average 140 kb in length (ranging from 4.5 to 659 kb) and delete an average of 25 CG isoforms (ranging from 1 to 83). The density of starting insertions in the Exelixis collection and the precise nature of deletion endpoints allow the design of very small customized deletions. For example, one can generate null alleles for single genes for which a null point mutant is lacking (see examples in **Supplementary Table 2** online: *Df(2L)Exel9033*, *Df(2R)Exel6062*, *Df(3L)Exel9028* and *Df(3R)Exel9056*). This targeted strategy can be also used to exclude problematic

Table 2 Genome coverage provided by *D. melanogaster* deletion sets

(a) Genome coverage at Bloomington before acquiring Exelixis collection		
	Polytene band coverage of all Bloomington deletions (%)	Polytene band coverage added by Bloomington screens (%)
X	86–93	0
2L	93–97	5–11
2R	93–95	8–13
3L	91–93	3–6
3R	90–93	6
4	42–62	0
Entire genome	90–94	5–7

(b) Genome coverage provided by Exelixis isogenic deletion collection			
	Coverage of Flybase v3.1 transcription units (CGs)* (%)	Mean number of v3.1 transcription units (CGs) per deletion*	Mean deletion size (kb)
X	27	19	124.4
2L	80	24	154.5
2R	58	28	131.6
3L	47	22	133.8
3R	65	25	140.1
4	0	0	0
Entire genome	56	25	140

*Transcription units include all splice forms given unique designations by Flybase (at the time of analysis, 18,109 CGs and CG splice forms were given unique identifiers).

Figure 3 FRT-bearing transposons and PCR strategies used in FLP-FRT-based deletions. **(a)** Schematic of the three transposon types used in FLP-FRT-based deletions. FRT sites, UAS-containing sequences and the gene *white* are indicated. See Thibault *et al.*¹ for details. FRT orientation is indicated by the direction of the arrowheads (FRT sites must be in the same orientation for deletion generation). **(b)** For transposon combinations that generate a recombinant hybrid element (composed of two different element types), transposon-specific primers were used to generate a fragment of known size across the newly formed hybrid (hybrid PCR). For transposon combinations that regenerate an intact element, a genomic primer in combination with a transposon-specific primer for each end of the transposon was used (two-sided PCR). We also carried out PCR using only genomic primers for additional confirmation (genomic PCR). **Table 3** lists the PCR confirmation method used for initial detection of the various deletion types. **Supplementary Methods** online provides transposon primer sequences and expected fragment sizes.

complex differences in the genetic backgrounds of stocks, large individual deletion sizes and imprecisely defined endpoints.

In summary, we describe the highest resolution deletion coverage of a metazoan genome so far. Improvements to genome coverage and breakpoint distribution will be continued by the Bloomington *Drosophila* Stock Center using the FRT-based deletion strategy described in this manuscript, as well as by the DrosDel Consortium. Furthermore, the establishment of these methods and the widespread distribution of FRT-bearing insertion stocks¹ and tools allows individual scientists to generate customized aberrations in the *D. melanogaster* genome in an efficient and straightforward manner.

METHODS

P transposase-based deletion screens. Visible marker mutations must first be combined in *cis* with each *P* insertion by standard meiotic crossover crosses. Once marked, the *P* insertion chromosomes may be used in the genetic scheme shown in **Figure 2a**. Many different visible markers could potentially be used, but **Table 1a** lists markers and stocks we found useful for various chromosomal intervals.

Recombinant chromosomes can arise either from HEI events involving two different *P* insertions or from events involving the two copies of a single *P* insertion present on sister chromatids. The latter yield small deletions flanking the

regions, such as haploinsufficient loci, or to generate precisely overlapping regions for high resolution coverage. Additionally, as deletions generated by this method are carried in the same isogenic background, meaningful comparisons between deletion-based modification phenotypes are relatively straightforward. We used this approach to refine regions of phenotypic interest by sequential deletion mapping (K.A.E., H.L.F.-L., M.B. Mahoney & A.L.P., unpublished data) and to carry out genome-wide screens for specific phenotypes (S. Thibault, personal communication). Previously available deletions made such analyses more difficult, owing to the

Table 3 Allowable pairwise combinations of Exelixis transposons for deletion generation

Lower coordinate tn1 (strand)	Higher coordinate tn2 (strand)	w+/-	Initial PCR confirmation method	UAS remaining?
RB (-)	RB (-)	w+	Two-sided	No
RB (-)	WH (+)	w+	Hybrid	No
RB (+)	RB (+)	w+	Two-sided	No
RB (+)	WH (-)	w+	Hybrid	Yes
RB (+)	XP (+)	w+	Two-sided*	Yes
WH (-)	RB (+)	w+	Hybrid	No
WH (-)	WH (-)	w+	Two-sided	Yes
WH (-)	XP (+)	w+	Two-sided*	Yes
WH (+)	RB (-)	w+	Hybrid	Yes
WH (+)	WH (+)	w+	Two-sided	Yes
XP (-)	RB (-)	w+	Two-sided*	Yes
XP (-)	WH (+)	w+	Two-sided*	Yes
XP (-)	XP (-)	w+	Two-sided	Yes
XP (+)	XP (+)	w+	Two-sided	Yes
XP (+)	RB (+)	w-	Hybrid	No
XP (+)	WH (-)	w-	Hybrid	Yes
RB (-)	XP (-)	w-	Hybrid	No
WH (+)	XP (-)	w-	Hybrid	Yes

*Hybrid PCR, though not tested by us, should also work for these deletion types.

original *P* insertion site⁴, which were of no interest to us. We distinguished deletions extending between different *P* insertion sites from other recombinant chromosomes by complementation tests. We characterized deletions in polytene chromosome preparations. In successful screens, an average of one in every four recombinant premeiotic clones was associated with a deletion extending from one *P* insertion site to the other, and a unique *P*-to-*P* deletion clone was detected in 1 of every 3,000 progeny. There was wide variation in the efficiency of screens: approximately one-third of the *P* insertion pairs produced no recombinant *P*-to-*P* deletions, despite repeated attempts. We attribute this variability to inherent differences in the ability of *P* insertions to participate in hybrid element insertions. *P* insertions may be poorly mobile and rarely give rise to recombinant deletions, or they may be extremely mobile, excise efficiently in the intermediate G1 generation (where a single *P* insertion is combined with a transposase source) and leave few intact insertions to participate in HEI events in the G2 generation. Consequently, we generally designed screens of ~25,000 progeny and repeated the screens twice more before switching to a different pair of *P* insertions.

Most deletions generated by *P* transposase in the presence of *trans*-heterozygous *P* insertions will be tagged with one of the original *P* constructs (Fig. 1a). There is, however, a class of *P*-to-*P* deletions that will not be flanked by a *P* construct. When the two original *P* insertions are present in the same relative orientation, deletions may also form by a DNA repair process called hybrid excision and repair, the rejoining of the broken chromatid ends left behind when the 5' and 3' ends of two different *P* elements excise and participate in a HEI event³. No *P* construct will be present at the junction point. Hybrid excision and repair deletions have been recovered in *trans*-heterozygous *P* element screens, but they seem to be generated less often than HEI deletions (A. Paré and J. Ewer, personal communication).

Two variations on the HEI deletion method have been successful. First, one can eliminate the process of marking *P* insertions with flanking visible markers and scoring recombinants by testing for deletions among progeny that have lost the internal marker carried by one of the *P* constructs if one has an efficient assay for distinguishing deletions from the larger number of simple excisions occurring in the intermediate G1 generation. Such an approach was previously reported⁸. Second, one can recover deletions between the sites of *P* insertions if two insertions are first recombined in *cis* by meiotic crossover⁹.

Genetic crosses for generation of FLP-FRT deletions. Figure 2b provides a schematic of genetic crosses we used for FLP-FRT deletion generation; genotypes used can be found in Table 1b. The following describes autosomal deletion screens (X-chromosome deletion crosses are similar; Fig. 2b). We carried out all crosses at 25 °C unless otherwise stated. We selected transposon elements flanking the region to be deleted (Table 3). Males carrying one element were mated with females carrying a FLP recombinase transgene. Progeny males carrying both the element and FLP recombinase were then mated to females carrying the second element. After 2 d, we subjected parents and progeny (progeny contain both the FRT-bearing elements in *trans* and FLP recombinase) to a 1-h heat shock by placing the bottles into a 37 °C water bath. We removed parents after 72 h of total egg-laying time and subjected the bottles to daily 1-h heat shocks for 4 d more. We raised progeny to adulthood, collected virgin females and crossed them to males containing marked balancer chromosomes. Individual progeny males (five *w*- males for a *w*- deletion; 50 males for a *w*+ deletion) were then crossed pairwise to virgin females to generate additional progeny for PCR confirmation analysis and to balance the stocks in an isogenic background.

As we were unable to maintain healthy balanced *FM7c* stocks in our isogenic background, most X chromosome deletions were stocked in a nonisogenized *FM7c* background (D stock, Table 1b). Because the X chromosomes are derived from the same isogenized X and the backgrounds of the X deletion stocks are similar, phenotypic comparisons between X chromosome deletions should still be

valid. Alternatively, one could balance X chromosome deletions with the isogenized B stock for X deletions (a *Binsinsy* balancer in an isogenized background).

PCR confirmation of FLP-FRT-based deletions. We carried out all PCR reactions using purified genomic DNA from five homogenized flies obtained from each isolate line (for genomic DNA preparation, see **Supplementary Methods** online). In general, it was sufficient to test 5 putative lines for each *w*- deletion and 50 putative lines for each *w*+ deletion. We used three different PCR strategies for deletion confirmation. Figure 3b provides a schematic and description of these strategies. Table 3 lists the PCR confirmation strategies used for initial detection of the various deletion types. **Supplementary Methods** online provide transposon primer sequences and expected fragment sizes for these combinations, as well as additional primer pair sequences for 'two-sided' PCR that may be used for secondary confirmation.

For products longer than 3 kb (see **Supplementary Methods** online), we carried out PCR using TaKaRa La *Taq* (Takara Bio). For products less than 3 kb, we used AmpliTaq Gold (Applied Biosystems). We used a standard touchdown program with both systems. Genomic primers were chosen using an in-house primer design tool and made by Bioscience.

URL. DrosDel Consortium is available at <http://www.drosdel.org.uk/>.

Note: Supplementary information is available on the Nature Genetics website.

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COMPETING INTERESTS STATEMENT

The authors declare competing financial interests (see the *Nature Genetics* website for details).

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