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A New Resource for Characterizing *X*-Linked Genes in *Drosophila melanogaster*: Systematic Coverage and Subdivision of the *X* Chromosome With Nested, *Y*-Linked Duplications

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ABSTRACT

Interchromosomal duplications are especially important for the study of *X*-linked genes. Males inheriting a mutation in a vital *X*-linked gene cannot survive unless there is a wild-type copy of the gene duplicated elsewhere in the genome. Rescuing the lethality of an *X*-linked mutation with a duplication allows the mutation to be used experimentally in complementation tests and other genetic crosses and it maps the mutated gene to a defined chromosomal region. Duplications can also be used to screen for dosage-dependent enhancers and suppressors of mutant phenotypes as a way to identify genes involved in the same biological process. We describe an ongoing project in *Drosophila melanogaster* to generate comprehensive coverage and extensive breakpoint subdivision of the *X* chromosome with megabase-scale *X* segments borne on *Y* chromosomes. The *in vivo* method involves the creation of *X* inversions on *attached-XY* chromosomes by FLP-FRT site-specific recombination technology followed by irradiation to induce large internal *X* deletions. The resulting chromosomes consist of the *X* tip, a medial *X* segment placed near the tip by an inversion, and a full *Y*. A nested set of medial duplicated segments is derived from each inversion precursor. We have constructed a set of inversions on *attached-XY* chromosomes that enable us to isolate nested duplicated segments from all *X* regions. To date, our screens have provided a minimum of 78% *X* coverage with duplication breakpoints spaced a median of nine genes apart. These duplication chromosomes will be valuable resources for rescuing and mapping *X*-linked mutations and identifying dosage-dependent modifiers of mutant phenotypes.

MANY eukaryotes of biomedical and agricultural importance—including humans, other mammals, birds, and Drosophila—are heterogametic. Their sex chromosomes differ drastically in size and genetic composition. In species with *X* and *Y* chromosomes, males carry only one copy of each *X*-linked gene. This poses a serious challenge for experimental geneticists, because males inheriting a mutation in a vital *X*-linked gene die before they can be used in genetic crosses. In fact, the hemizygosity of *X*-linked genes in males has been a significant barrier to the functional analysis of many *X*-linked genes and is largely responsible for the poor genetic characterization of *X* chromosomes relative to autosomes in most organisms.

The lethality of *X*-linked mutations can be rescued by providing a wild-type copy of the mutated gene elsewhere in the genome. This can be accomplished with a transgenic construct if the molecular identity of the mutated gene is known. In many cases, however, the mutated gene has not been identified and it is necessary to provide wild-type function with a multigene interchromosomal duplication, *i.e.*, a segment of the *X* inserted in another chromosome. If the proximal and distal extents of the duplicated segment are known, phenotypic rescue maps the mutated gene to the defined *X* chromosome region.

Multigene deletions can also be used to map *X*-linked mutations by complementation, but crosses between individuals carrying deletions and *X*-linked lethal mutations are impossible without rescuing the lethality of either the deletion or the lethal mutation in males. Projects at the Bloomington Drosophila Stock Center and elsewhere (Parks *et al.* 2004; Ryder *et al.* 2007) have generated large collections of deletions with molecularly defined breakpoints in *Drosophila melanogaster*, but the utility of the *X* deletions is limited without duplications of the corresponding chromosomal regions.

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Duplications are potentially important for gene discovery. Identifying sets of genes involved in the same cellular process is a major focus of functional genomics research and this can be accomplished genetically by identifying dosage-sensitive modifiers of mutant phenotypes. Often, increasing or decreasing the copy number of a gene will enhance or suppress the phenotype associated with mutating another gene involved in the same process. Screening collections of deletions is a popular way to identify interacting genes in Drosophila (for examples, see Seher *et al.* 2007; Zhao *et al.* 2008; Aerts *et al.* 2009; Salzer *et al.* 2010) and was a major impetus for the assembly of the Bloomington Stock Center "Deficiency Kit," which provides maximal coverage of the genome with the fewest deletions. Though dosage-sensitive modifiers could also be identified using increased gene dosage, the use of duplications in enhancer and suppressor screens remains largely unexplored. Assembling sets of duplications providing efficient genomic coverage would likely popularize this experimental approach.

The size of duplicated segments determines how duplication chromosomes are used experimentally. Small duplicated segments allow high resolution gene mapping, but they are not suitable for other purposes. Only large duplicated segments are capable of rescuing the lethality of sizable multigene *X* deletions. Likewise, large duplicated segments provide efficiency in initially localizing mutations and identifying dosage-dependent modifiers. Despite their usefulness, interchromosomal duplications of large segments are among the hardest chromosomal rearrangements to isolate. In Drosophila, many existing duplications were recovered fortuitously as three-breakpoint aberrations following irradiation, but such rearrangements are rare and difficult to identify in screens. Other duplications were methodically constructed from preexisting rearranged chromosomes. This approach works well when it is possible, but it can be used only when progenitor aberrations with appropriate breakpoints are available. Because of these difficulties, the selection of duplication strains generated by Drosophila workers over the past several decades is not satisfactory for many purposes. The duplications are often difficult to use experimentally, their breakpoints are sparsely distributed along the *X* chromosome and only roughly mapped, and substantial gaps in coverage exist. Obviously, improved duplication resources are needed.

Here we present the methodology and progress of a project at the Bloomington Drosophila Stock Center to construct interchromosomal duplications of large, megabase-scale *X* segments. Our approach builds on the long history of manipulating Drosophila chromosomes *in vivo* (Novitski and Childress 1976; Ashburner *et al.* 2005), but we have eliminated the need for preexisting aberrations by generating progenitor chromosomes using the FLP-FRT system. Indeed, this site-specific recombination system has had an enormous impact on the ability of fly geneticists to engineer many kinds of novel chromosomes (Golic and Golic 1996; Parks *et al.* 2004; Ryder *et al.* 2007). We will demonstrate how we have combined FLP-mediated recombination and other chromosome manipulation techniques to produce *Y*-linked duplications of large *X* segments. As we will show, appending *X* segments to *Y* chromosomes rather than autosomes has advantages both for the synthesis and experimental use of *X* duplications.

To date, we have generated a minimum of 78% *X* coverage with duplication breakpoints spaced a median of nine genes apart. We anticipate completion of the project within the coming year. Using these duplications, mutations and genetic modifiers can be mapped first to large *X* intervals using a tiling set of the largest duplicated segments and then to small chromosome intervals using subsets of the duplications. These duplications will also facilitate deletion mapping. The creation of a set of stocks providing complete duplication coverage and extensive breakpoint subdivision of the *X* chromosome in a consistent genetic background will remove an impediment to investigating the functions of *X*-linked genes that has frustrated generations of Drosophila geneticists.

MATERIALS AND METHODS

Fly stocks: FRT-bearing *P{RS5}* and *P{RS3}* insertion stocks were obtained from the Szeged Drosophila Stock Centre. The remaining stocks were obtained from the Bloomington Drosophila Stock Center collection or the Drosophila Genetic Resource Center at the Kyoto Institute of Technology.

Genomic coordinates and cytological breakpoints: All genomic coordinates and gene counts are based on Genome Release 5.16. Except for the directly observed cytological breakpoints in Table 1, all *Dp(1;Y)* cytological breakpoints were predicted from Release 5 coordinates using FlyBase map conversion tables (http://flybase.org; Tweedie *et al.* 2009). For assessing duplication coverage, we have artificially set the euchromatin/heterochromatin boundary at sequence coordinate *X*:22420000, roughly the most proximal extent of the assembled *X* chromosome genomic contigs in Genome Release 5.16.

Mutagenesis: Adult males received 4500-R exposure to 6000 Ci of ^{137}Cs in a Shepard Mark-1 irradiator.

Cytology: Mitotic chromosomes were prepared and stained with DAPI by standard methods (Fanti and Pimpinelli 2004). Chromosomes were stained 45 min with 0.5 mg/ml chromomycin A3 (Sigma) in PBS pH 7.7 with 5 mm $MgCl_2$ and rinsed in PBS prior to mounting. Polytene chromosomes were analyzed in standard lacto-aceto-orcein preparations (Carpenter 2004).

Comparative genome hybridization microarrays: Corning CGAP slides spotted with the AROS Drosophila V1.1.1 ~70 nucleotide oligo set from Eurofins MWG Operon were hybridized and analyzed as described in Erickson and Spana (2006).

PCR: DNA was prepared from single flies as described in Engels *et al.* (1990) and amplified using Qiagen HotStarTaq master mix. The following amplification regime was used to confirm the presence of *P{RS3}* and *P{RS5}* insertions: 95° for 10 min followed by 38 rounds of 95°, 30 sec; 42°, 30 sec; and

TABLE 1

***Dp(1;Y)* chromosomes derived from *C(1;Y)6, In(1)sc*$^{260\text{-}14}$**

Duplication	Cytology[a]	Rescues
Dp(1;Y)BSC1	10C1,2;11D3-8	*l(1)G0241*^{G0241} (10D1), *l(1)G0102*^{G0102} (10E3-4), *m*^{38c} (10E1-2), *dy*^{1} (10E1-2), *Hsc70-3*^{G0111} (10E3-4), *qs*^{1} (10F1-7), *fw*^{34e} (11A1), *tsg*^{2} (11A1), *l(1)G0060*^{G0060} (11B14-C2)
Dp(1;Y)BSC2	10E1,2;11D3-8	*l(1)G0102*^{G0102} (10E3-4), *m*^{38c} (10E1-2), *dy*^{1} (10E1-2), *Hsc70-3*^{G0111} (10E3-4), *qs*^{1} (10F1-7), *fw*^{34e} (11A1), *tsg*^{2} (11A1), *l(1)G0060*^{G0060} (11B14-C2)
Dp(1;Y)BSC3	11B1,2;11D3-8	*l(1)G0060*^{G0060} (11B14-C2)
Dp(1;Y)BSC4	11B1,2;11D3-8	*l(1)G0060*^{G0060} (11B14-C2)
Dp(1;Y)BSC5	11B18;11D3-8	*l(1)G0060*^{G0060} (11B14-C2)
Dp(1;Y)BSC6	11B18;11D3-8	*l(1)G0060*^{G0060} (11B14-C2)
Dp(1;Y)BSC7	11B18;11D3-8	*l(1)G0060*^{G0060} (11B14-C2)
Dp(1;Y)BSC8	11C3-4;11D3-8	*l(1)G0060*^{G0060} (11B14-C2)
Dp(1;Y)BSC9	Undetectable	None of the tested mutations
Dp(1;Y)BSC10	Undetectable	None of the tested mutations

[a] These cytologically observed breakpoints may not represent the full extents of the duplicated segments, because euchromatic bands juxtaposed to centric heterochromatin may not be visible.

72°, 5 min. Primer sequences are given in supporting information, File S1. For mapping of duplication endpoints, DNA from *Dp(1;Y)* males carrying an *X* chromosome transposon insertion was amplified as follows: 95° for 15 min followed by 35 rounds of 95°, 30 sec; 53°, 30 sec; and 72°, 60 sec. The transposons and primers in the mapping panel (Table S1) were chosen to be spaced 10 protein-coding genes apart, but the spacing varied occasionally on the basis of the availability of insertions or the presence of large genes.

Genetic crosses: Extensive details are provided in File S1.

RESULTS

Generating *Y*-linked duplications of *X* chromosome segments: Our goal is to generate comprehensive duplication coverage and extensive breakpoint subdivision of the *X* chromosome. The approach we have taken is to replace the tips of *Y* chromosomes with large segments of the *X* chromosome. These chromosomes are denoted "*Dp(1;Y)*" to indicate that a segment of the first chromosome (the *X*) is duplicated on the *Y*. In crosses, *Dp(1;Y)* chromosomes behave like normal *Y* chromosomes. They show typical *Y*-linked inheritance. While it is convenient for *Dp(1;Y)* chromosomes to carry dominant marker mutations for following them in crosses, it is not absolutely necessary. The segregation pattern of the *Y* is usually sufficient to track *Dp(1;Y)* chromosomes in experiments. This is a distinct advantage over duplications carried on autosomes, where dominant marker mutations are usually essential for following duplicated segments in crosses. Also, in the context of modifier screens, *Y* linkage provides flexibility with the easiest way to assay interactions of duplicated *X* segments with recessive mutations on the autosomes.

Y linkage does not, however, restrict the use of *Dp(1;Y)* chromosomes to males. Because the *Y* plays no role in Drosophila sex determination and carries only genes necessary for spermatogenesis, *Dp(1;Y)* chromosomes may be introduced into females where they can be used to rescue the female-specific phenotypes of *X*-linked mutations, such as ovarian defects caused by female sterile mutations. (Methods for placing *Dp(1;Y)* chromosomes into females are described in a later section.) While duplicating large *X* segments can cause lethality, sterility, and other phenotypes associated with excess hyperploidy, sex determination is unaffected by duplications of sizes compatible with the viability of hyperploid flies (Patterson *et al.* 1937).

Extensive chromosome manipulations were needed to create the progenitor chromosomes used in screens isolating *Dp(1;Y)* chromosomes. In this section, we will provide a general overview of the steps. For background, we will first describe the recovery of simple *Dp(1;Y)* chromosomes—those carrying segments from the tip of the *X* appended to an intact *Y*. Then we will present the variation on this method that we used. It employs inversions to duplicate segments from the entire *X*. In subsequent sections, we will describe how we generated the inversions and how we conducted the final *Dp(1;Y)* screens. In the overview, we will also show how a single progenitor chromosome gives rise to a set of *Dp(1;Y)* chromosomes with duplicated *X* segments of different sizes.

Our approach to isolating *Dp(1;Y)* chromosomes utilizes an *attached-XY* chromosome, a single chromosome carrying all *X*- and *Y*-linked genes. It was generated by a translocation event (Figure 1) involving an *X* chromosome break in centric heterochromatin and a *Y* chromosome break near the telomere. *Attached-XY* chromosomes are denoted "*C(1;Y)*" to indicate a compound chromosome formed by a first (*X*) chromosome and a *Y*. An *attached-XY* can substitute for a regular *X* in crosses and, in most situations, its segregation behavior is indistinguishable from a regular *X*. If a male carries an *attached-XY*, there is no need for a regular *Y*, because all *Y*-linked spermatogenesis genes are provided by the *Y* portion of the *attached-XY*.

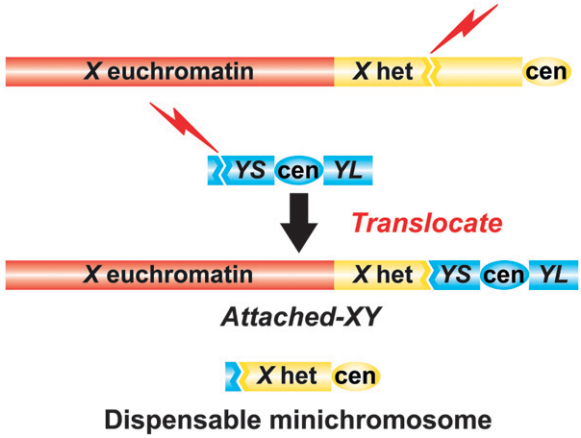

Figure 1.—Generating an *attached-XY* chromosome. Irradiating males can produce a break in *X* centric heterochromatin proximal to all *X*-linked genes and a break near the *Y* tip distal to all genes on the *Y*. Following translocation, the resulting *attached-XY* chromosome carries all *X*- and *Y*-linked genes. The reciprocal minichromosome carries no *X*- or *Y*-linked genes and is dispensable. Irradiation events are indicated by bolts. Breakpoints are shown as interruptions in chromosomal continuity.

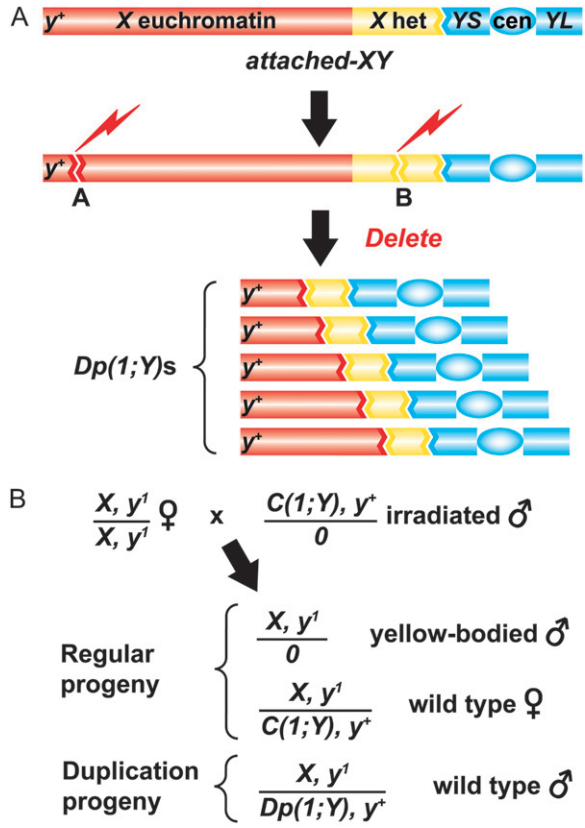

Figure 2.—Generating *Dp(1;Y)* chromosomes from *attached-XY* chromosomes. (A) If *attached-XY* chromosomes are irradiated to introduce a break near the *X* tip (breakpoint A) and a break in *X* centric heterochromatin (breakpoint B), most of the *X* chromosome will be deleted. The resulting *Dp(1;Y)* carries genes from the *X* tip, which will then show a *Y*-linked pattern of inheritance. Because irradiation induces random breaks, different *Dp(1;Y)* chromosomes carry differently sized *X* tip segments, forming a nested set. Though shown at a constant location here, the position of breakpoint B also varies as shown in detail in Figure 3. (B) The *yellow* (*y*) gene allows the identification of new *Dp(1;Y)* chromosomes. When irradiated *attached-XY* (*C(1;Y)*) males are mated to females carrying *y* mutations, *Dp(1;Y)* chromosomes are recovered in male progeny inheriting a wild-type *y* allele and having normal body pigmentation. "*0*" indicates the absence of a normal *Y*.

A *Dp(1;Y)* can be generated from an *attached-XY* by deleting most of the *X* chromosome (Figure 2A). If one breakpoint is positioned near the *X* tip (breakpoint A) and another is positioned in *X* centric heterochromatin (breakpoint B), the resulting *Dp(1;Y)* will carry genes from the end of the *X* and a segment of *X* heterochromatin appended to the end of the *Y*. The *yellow* (*y*) gene, which is located near the *X* tip and necessary for normal pigmentation, is key to identifying *Dp(1;Y)* chromosomes in screens. When males carrying *C(1;Y)* chromosomes are irradiated and mated to females carrying y^1 mutations, most male progeny with normal pigmentation carry a *Dp(1;Y)* (Figure 2B).

If multiple *Dp(1;Y)* chromosomes are isolated from a screen, the *X* tip segments will form a nested set: all the tip segments share the telomeric end, but the ends generated by the breakpoints (breakpoint A) will differ (Figure 2A). In this way, the *X* tip region can be subdivided finely with duplication breakpoints and mutations near the tip of the *X* can be mapped with precision in rescue experiments.

Many of the proximal deletion breakpoints (breakpoint B) will fall in *X* centric heterochromatin as shown in Figure 2A, but they may also fall in the *Y* arm or in basal *X* euchromatin (Figure 3). *Y* breakpoints result in the deletion of *Y*-linked spermatogenesis genes and males carrying these *Dp(1;Y)* chromosomes are sterile. These *Dp(1;Y)* chromosomes are not recovered in stable stocks when irradiated males are crossed to normal females. Breakpoints in basal euchromatin result in *Dp(1;Y)* chromosomes with two sets of duplicated genes: one set from the *X* tip and another from the *X* base. *X* centric heterochromatin and the *Y* arm are much larger targets for irradiation-induced breakpoints than basal *X* euchromatin, so *Dp(1;Y)* chromosomes carrying genes from the base of the *X* are less common than the other two classes. The total number of duplicated genes that a *Dp(1;Y)* can carry from both the tip and base of the *X* is limited by hyperploidy effects. Drosophila is generally quite tolerant of hyperploidy and duplications of up to half a chromosome arm have been recovered (Ashburner *et al.* 2005), but our experience has been that duplications of >10% of *X* euchromatin are rare and flies carrying extremely large duplications have low viability and fertility.

The problem with irradiating a regular *attached-XY* chromosome as described above is that only *X*-linked genes near the tip or base can be recovered in *Dp(1;Y)* chromosomes. What about the genes in the middle of the *X*? Fortunately, the method can be extended by

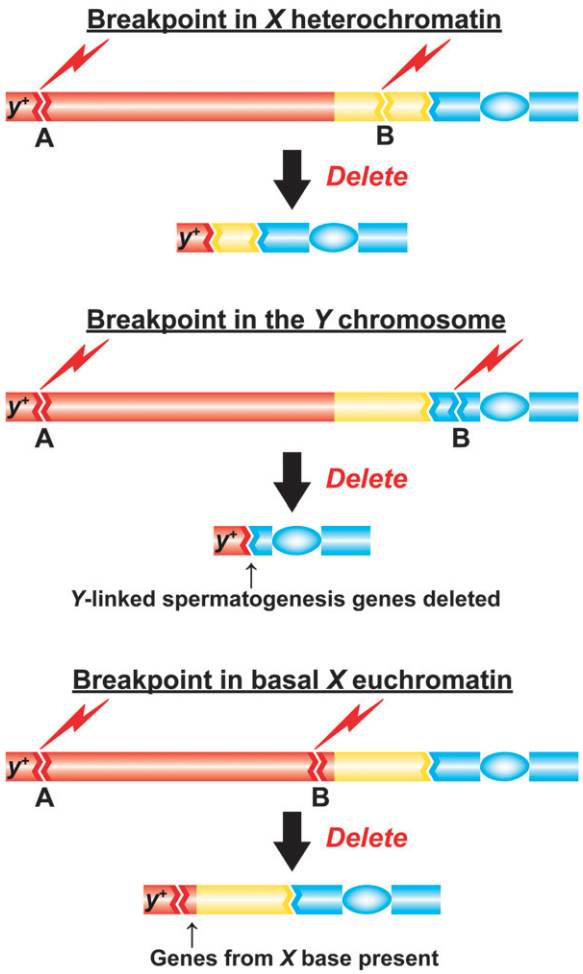

Figure 3.—Position of the proximal deletion breakpoint. The deletion giving rise to a *Dp(1;Y)* from an *attached-XY* can break in *X* centric heterochromatin (top), in the short arm of the *Y* (middle) or in *X* basal euchromatin (bottom). Breaks in *X* centric heterochromatin (top) result in *Dp(1;Y)* chromosomes carrying only genes from the *X* tip. *Y* breaks (middle) result in the deletion of *Y*-linked genes necessary for spermatogenesis. Males carrying these *Dp(1;Y)* chromosomes are sterile. Breaks in *X* basal euchromatin (bottom) result in *Dp(1;Y)* chromosomes carrying genes from the *X* base in addition to genes from the *X* tip. Within a chromosome arm, "proximal" and "basal" refer to positions closer to the centromere of a normal sequence chromosome; "distal" refers to positions closer to the telomere.

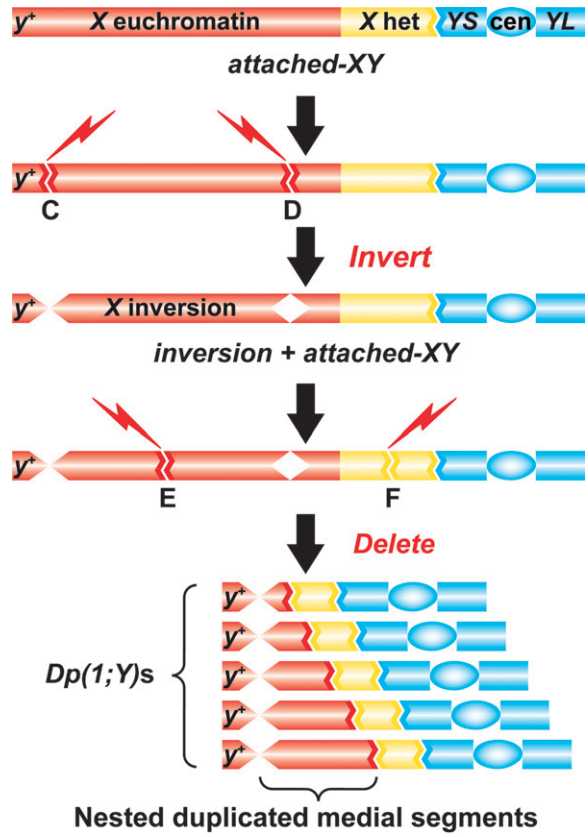

Figure 4.—Generating *Dp(1;Y)* chromosomes from *inversion + attached-XY* chromosomes. To isolate *Dp(1;Y)* chromosomes carrying genes from the middle of the *X*, an inversion is introduced into the *attached-XY* chromosome (breakpoints C and D). The inversion is shown here being induced by irradiation, but, as we will describe, other methods may be used. The inversion places medial *X* genes near the *X* tip so that they will remain in a *Dp(1;Y)* following irradiation to induce a large internal deletion (breakpoints E and F) in the *inversion + attached-XY* chromosome. If multiple *Dp(1;Y)* chromosomes are recovered from irradiating the same *inversion + attached-XY*, they will all share the same region from the *X* telomere to the distal inversion breakpoint (breakpoint C), but will have differently sized segments from the middle of the *X* determined by the position of the distal deletion breakpoint (breakpoint E). Though shown at a constant location here, breakpoint F can fall in *X* centric heterochromatin, in the *Y* or in *X* basal euchromatin in the same way as breakpoint B in Figure 3.

introducing inversions into the *X* portion of the *attached-XY* chromosome (Figure 4). If the inversion has one distal breakpoint (breakpoint C) near the *X* tip and another breakpoint in the middle of the *X* (breakpoint D), irradiating this "*inversion + attached-XY*" chromosome can generate *Dp(1;Y)* chromosomes carrying genes from the middle of the *X* as well as genes from the *X* tip. The size of the segment containing medial *X* genes is determined by the position of the distal deletion breakpoint (breakpoint E).

If multiple *Dp(1;Y)* chromosomes are isolated from an *inversion + attached-XY*, the segments from the middle of the *X* will form a nested set. These nested segments will share a common end corresponding to the inversion breakpoint (breakpoint C), but their other ends will differ by the positions of the distal deletion breakpoints (breakpoint E). All the *Dp(1;Y)* chromosomes will share a common distal segment extending from the *X* telomere to the distal inversion breakpoint (breakpoint C). As in screens with regular *attached-XY* chromosomes, *Dp(1;Y)* chromosomes derived from *inversion + attached-XY* chromosomes will also carry genes from the *X* base if the proximal deletion breakpoint (breakpoint F) falls in basal euchromatin and they will delete *Y*-linked spermatogenesis genes if the breakpoint falls in the *Y* arm (similar to Figure 3).

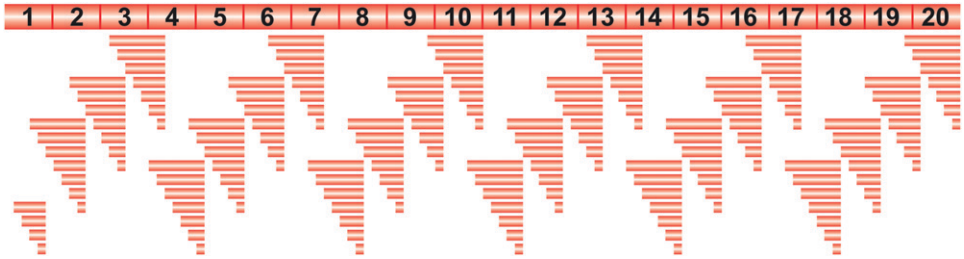

FIGURE 5.—Hypothetical distribution of medial duplicated segments in *Dp(1;Y)* chromosomes derived from different *inversion* + *attached-XY* chromosomes. If the inversions in *inversion* + *attached-XY* chromosomes have different proximal breakpoints, nested sets of duplicated *X* segments in *Dp(1;Y)* chromosomes can be isolated for all *X* regions. The numbers refer to the 20 divisions of the polytene map of the *X* chromosome comprising the 22 Mb of euchromatin. Centric heterochromatin constitutes an additional 10–15 Mb and is not shown.

Inversions with different proximal breakpoints (breakpoint D) move different regions to the *X* tip so that different sets of genes can be recovered in *Dp(1;Y)* chromosomes. Consequently, if a set of *inversion* + *attached-XY* chromosomes existed with proximal inversion breakpoints (breakpoint D) distributed along the *X* chromosome, it would be possible to generate *Dp(1;Y)* chromosomes providing duplication coverage of all *X* regions (Figure 5). The multiple nested sets would also subdivide the entire *X* with duplication breakpoints for use in high-resolution gene mapping.

A preliminary test of the method: To our knowledge, *Dp(1;Y)* chromosomes have been derived from *inversion* + *attached-XY* chromosomes in only three unpublished screens carried out by Abraham Schalet [screens generating $Dp(1;Y)y^+lz^+$, $Dp(1;Y)y^+na^+$, and $Dp(1;Y)y^+g^+$ (LINDSLEY and ZIMM 1992) and a screen for $Dp(1;Y)dx^+1$ through dx^+8 (http://flybase.org/; TWEEDIE *et al.* 2009)]. To assess the method in our hands, we isolated *Dp(1;Y)* chromosomes using the preexisting inversion $In(1)sc^{260\text{-}14}$ (SUTTON 1943). Males with $In(1)sc^{260\text{-}14}$ on an *attached-XY* were irradiated and mated to y^1 females. We recovered 39 y^+ males from ~93,000 progeny. Ten males were fertile and *Dp(1;Y)* stocks were established; the remaining 29 sterile males likely carried duplications lacking one or more *Y*-linked spermatogenesis genes. As shown in Table 1, the *Dp(1;Y)* chromosomes rescued the phenotypes of a variety of mutations in the 10C to 11D region of the *X* chromosome.

The largest duplicated medial segment contained seven polytene subdivisions, suggesting we could cover the entire *X* with *Dp(1;Y)* chromosomes if we had proximal inversion breakpoints spaced roughly every 5 subdivisions on the 120-subdivision *X* map. This would allow the largest duplicated segments from every screen to overlap the common end of the next set of segments (as shown in Figure 5) yet avoid intolerable levels of hyperploidy. Only genes lethal to males in two copies will prevent full coverage. To maximize coverage, inversion breakpoints would need to lie close to the distal sides of the two known *X*-linked diplolethal loci: an unnamed diplolethal in 3F and *Haplo-diplo lethal* (*Hdl*) in 12A (STEWART and MERRIAM 1973; SALZ 1992; J. MERRIAM, personal communication).

Generating *inversion* + *attached-XY* chromosomes: To generate comprehensive duplication coverage of the *X* chromosome with *Dp(1;Y)* chromosomes as shown in Figure 5, it was necessary to generate a large set of inversions on *attached-XY* chromosomes. We wanted the inversions to share the same distal breakpoint (breakpoint C in Figure 4), but to have proximal breakpoints (breakpoint D) distributed along the length of the *X*. To generate the inversions, we used the FLP-FRT site-specific recombination system (GOLIC and GOLIC 1996). As shown in Figure 6, inversions can be recovered upon FLP-induced recombination if the two FRTs are present in opposite orientations on the same chromosome.

To screen for the inversions, we used the FRT-bearing transgenic constructs *P{RS3}* and *P{RS5}*, which were specially designed to reconstitute the *white* (*w*) gene upon recombination (Figure 7) (GOLIC and GOLIC 1996). *P{RS3}* carries FRTs flanking the 5′ exon of *w*. Upon FLP-induced recombination, the 5′ *w* exon is removed. Likewise, *P{RS5}* carries FRTs flanking the 3′ *w* exons so that they are removed upon FLP-mediated recombination. In both cases, removal of *w* exons renders the *w* gene nonfunctional and flies carrying these rearranged transgenes have white eyes in the absence of other functional copies of *w*. When these rearranged transgenes are subsequently combined in the presence of FLP recombinase, recombination between the FRTs will reconstitute a functional *w* gene. In this way, flies carrying chromosomal aberrations can be identified as red-eyed progeny of white-eyed parents.

To isolate *inversion* + *attached-XY* chromosomes, we first placed *P{RS3}* and *P{RS5}* insertions on the *C(1;Y)N12 attached-XY* chromosome (Figure 8; KENNISON 1981). *C(1;Y)N12* is an *X* chromosome broken in centric heterochromatin combined with a *Y* chromosome broken distal to the spermatogenesis genes on its short arm. For convenience in following *C(1;Y)N12* in crosses, it is marked at the tip of the long arm of the *Y* with the dominant B^s mutation affecting eye shape. We first placed proximal *P{RS5}* insertions distributed along the *X* onto *C(1;Y)N12* chromosomes by meiotic recombination following the w^+ eye color marker on *P{RS5}* and B^s. We then placed a common distal *P{RS3}* insertion on these chromosomes by meiotic recombination assaying the amplifi-

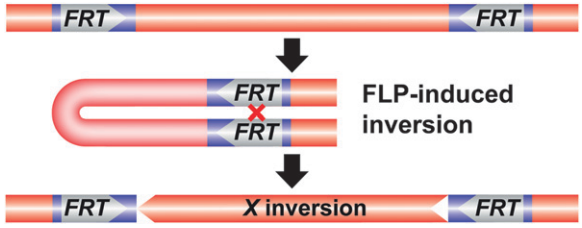

FIGURE 6.—Generating an inversion using the FLP-FRT system. FLP recombinase induces recombination between pairs of FRT sites in an orientation-specific manner. If FRT sites are placed *in cis* in opposite orientations, FLP recombinase will catalyze the formation of chromosomal inversions.

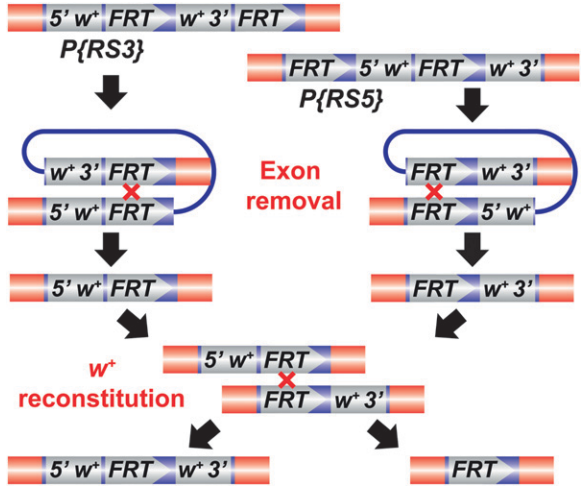

FIGURE 7.—Using *P{RS3}* and *P{RS5}* insertions to detect FLP recombinase-mediated recombination events. *P{RS3}* and *P{RS5}* were designed to allow the detection of recombination between FRT sites by the reconstitution of a functional *w* gene. Insertions of *P{RS3}* and *P{RS3}* carry a functional *w* gene that can be disrupted by FLP-mediated excision of *w* exons flanked by FRT sites. Flies carrying these rearranged constructs in a w^- background have white eyes. FLP-induced recombination between rearranged *P{RS3}* and *P{RS5}* insertions reassembles a functional *w* gene on one of the recombinant chromosomes.

cation of PCR products unique to each construct to identify recombinants.

To generate inversions, we first exposed the *attached-XY* chromosomes carrying *P{RS3}* and *P{RS5}* insertions to heat shock-induced FLP to remove the 5′ *w* exon from *P{RS3}* and the 3′ *w* exons from *P{RS5}*. This was an efficient step: typically, one-third of the progeny were white eyed. We then induced inversions by exposing the chromosomes to FLP again. Inversion-bearing progeny were red eyed from reconstitution of w^+ at the distal inversion breakpoint. The frequency of w^+ flies varied considerably with a range of 1 in 12,600 to 1 in 140 progeny and a median rate of 1 in 1040 progeny. The inversions were verified in polytene chromosome preparations. Figure 9 and Table S2 show the 28 inversions we generated on *C(1;Y)N12*. Six *inversion* + *attached-XY* chromosomes were isolated by a related, but more efficient screening strategy that eliminated PCR screening for the initial recombinant chromosomes carrying both *P{RS3}* and *P{RS5}* (File S1).

The *P{RS3}* and *P{RS5}* insertions were isolated in an isogenic background tested for normal development and behavior (RYDER *et al.* 2004). We substituted all chromosomes used in our crosses into this standard background so that the final *Dp(1;Y)* stocks will be a high-quality genetic resource suitable for experiments involving background-sensitive phenotypes, such as behavioral phenotypes. This genetic uniformity also increases the utility of these strains in screens for dosage-based enhancement and suppression of mutant phenotypes.

***Dp(1;Y)* screens and breakpoint mapping:** Using the *inversion* + *attached-XY* chromosomes to isolate *Dp(1;Y)* chromosomes is straightforward, albeit labor intensive. Males carrying an *inversion* + *attached-XY* chromosome are irradiated and mated to y^1 females. *Dp(1;Y)*-bearing y^+ male progeny are backcrossed to establish stocks. On average, the screens produced one *Dp(1;Y)* chromosome supporting male fertility every ~23,000 progeny. The *Dp(1;Y)* chromosomes isolated and characterized to date provide a minimum of 78% coverage of *X* euchromatin (>17.5 of 22.4 Mb), a minimum of 78% coverage of *X* euchromatic genes (>1742 of 2231 genes), and extensive genomic subdivision (Table 2, Table S3). The largest stretch of contiguous coverage is 5.6 Mb in the 7B–11D region. The *X* tip segment shared by all *Dp(1;Y)* chromosomes accounts for 1.7% (0.3 Mb) of coverage. We have placed 221 *Dp(1;Y)* chromosomes from these screens into public distribution (http://flystocks.bio.indiana.edu/Browse/dp/BDSC-Dps.php).

We located the irradiation-induced breakpoints of the duplicated segments on the genome map by two methods. Our primary mapping strategy localizes the ends of duplicated segments between adjacent transposon insertion sites (Figure 10). We designed PCR primers flanking the insertion sites of transposons located within the region to be duplicated (Table S1). With short extension times, PCR fragments are amplified only when there is no transposon between the primer sites. When females carrying insertions are mated to *Dp(1;Y)*-bearing males and DNA is prepared from their male progeny, PCR fragments are amplified only if the primer sites are present on the *Dp(1;Y)*. In this way, we mapped the ends of duplicated segments to intervals with the target size of 10 protein-coding genes (Table S3). Duplication ends falling in adjacent mapping intervals can lie 0 to ~20 genes apart.

We mapped the breakpoints of a few duplicated segments using comparative genome hybridization (CGH) microarrays. In this technique, genomic DNA samples from wild-type and *Dp(1;Y)*-bearing males are labeled with different fluorochromes and hybridized to the same genomic microarray. Duplicated segments are

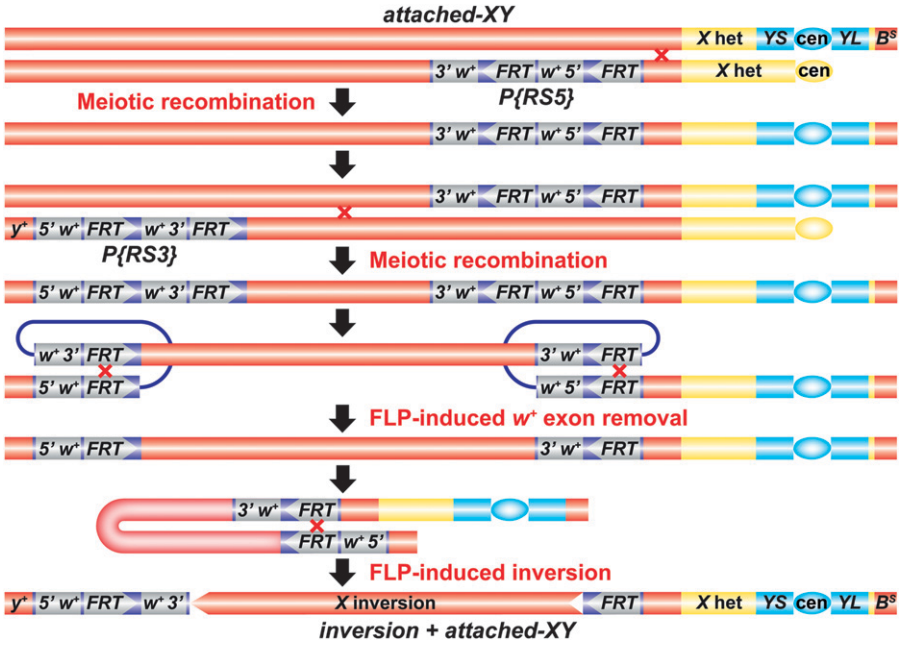

FIGURE 8.—Generating *inversion* + *attached-XY* chromosomes. The construction of *inversion* + *attached-XY* chromosomes proceeded in four steps. First, *P{RS5}* insertions distributed along the *X* were placed on an *attached-XY* chromosome by meiotic recombination. Second, a *P{RS3}* insertion near the *X* tip was placed on each *P{RS5}* + *attached-XY* chromosome by meiotic recombination. Flies carrying these chromosomes had red eyes. Third, *w* exons were removed from the *P{RS3}* and *P{RS5}* insertions by exposure to heat shock-induced FLP recombinase. Flies carrying these chromosomes had white eyes. Finally, inversions were induced by exposing the *attached-XY* chromosomes with rearranged *P{RS3}* and *P{RS5}* insertions to heat shock-induced FLP recombinase. Flies carrying *inversion* + *attached-XY* chromosomes had red eyes due to the reconstitution of a functional *w* gene.

identified as contiguous blocks of genes with twofold-increased relative fluorescence (ERICKSON and SPANA 2006). Because the microarrays contain a probe from most annotated genes, duplication endpoints can usually be mapped with two-gene resolution (see Table S3). Due to its expense, we used this method to analyze only a cytologically preselected subset of *Dp(1;Y)* chromosomes from the *In(1)BSC6* screen.

As an example of genomic coverage and subdivision provided by our *Dp(1;Y)* chromosomes, Figure 11 shows duplicated segments in their uninverted orientation derived from three inversions (*In(1)BSC20*, *In(1)BSC21*, and *In(1)BSC22*). As planned, the nested sets overlap and there is an even distribution of endpoints across the region with a breakpoint in 18 of the 28 PCR mapping intervals targeted by these screens, *i.e.*, between the *In(1)BSC19* and *In(1)BSC22* proximal breakpoints. In this 490-gene region, the largest region between two breakpoints contains at most 42 genes.

Current coverage and subdivision of the entire *X* is depicted in Figure 12. Using the minimal estimates of duplication sizes from completed *Dp(1;Y)* screens, we have calculated that 96% of the intervals between breakpoints contain ≤30 genes, 89% contain ≤20 genes and 62% contain ≤10 genes. The median interval size is 9 genes or ~107 kb. *Dp(1;Y)* chromosomes in specific *X* chromosome regions may be viewed graphically using the GBrowse aberrations viewer on FlyBase (http://flybase.org/cgi-bin/gbrowse/dmelabs/).

It was easier to obtain large duplicated medial segments in some regions than others. For example, the *In(1)BSC3* screen produced a 165-gene (1.68 Mb) duplicated segment even though fewer progeny were screened than in the *In(1)BSC10* screen where a 69-gene (0.84 Mb) segment was the largest recovered (Table 2). All our completed screens were large enough to give duplication endpoints evenly distributed across the desired chromosomal intervals, but screen size correlated poorly to size of the largest duplicated segment ($r = -0.23$). We attribute these regional differences to the effects of hyperploidy for different sets of genes. Other than previously identified diplolethal genes, there are no clear predictors of permissible duplication size.

The positions of proximal deletion breakpoints giving rise to *Dp(1;Y)* chromosomes: As described above, the proximal breakpoint of the deletion that gives rise to a *Dp(1;Y)* from an *inversion* + *attached-XY*

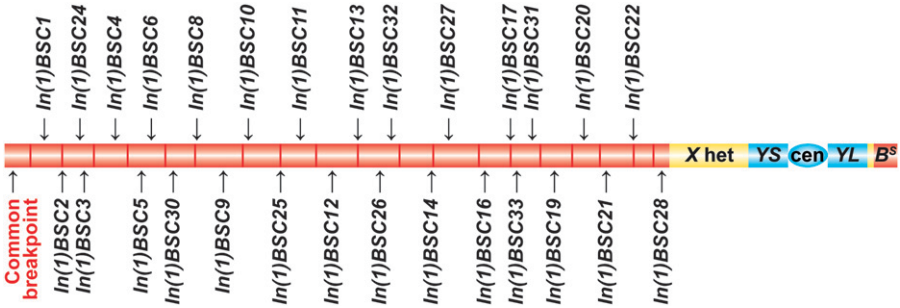

FIGURE 9.—*Inversion* + *attached-XY* chromosomes. Proximal breakpoints for the 28 inversions generated for *Dp(1;Y)* screens are shown. They correspond to the positions of *P{RS5}* insertions. All inversions (except *In(1)BSC30* and *In(1)BSC31*) share the same distal breakpoint corresponding to *P{RS3}CB-5805-3*. The tip segment from the *X* telomere to the common breakpoint is carried by all *Dp(1;Y)* chromosomes.

TABLE 2

***Dp(1;Y)* chromosomes recovered**

Inversion	Progeny screened	Number of *Dp(1;Y)* recovered[a]	Largest *Dp(1;Y)* in stock[b]		
			Breakpoints	Number of genes	Size (Mb)
In(1)BSC2	In progress	6	2C1;2F6	56	0.32
In(1)BSC3	232,000	17	2C1;3E3	165	1.68
In(1)BSC4	341,000	12	3F9;4D7	81	0.98
In(1)BSC6	884,000	21	4D1;5D1	110	1.21
In(1)BSC9	894,000	17	7B1;7D18	92	0.87
In(1)BSC10	330,000	15	7D18;8C3	69	0.84
In(1)BSC25	383,000	17	8A2;8F9	94	0.97
In(1)BSC11	271,000	9	8E4;9E1	113	1.24
In(1)BSC12	In progress	3	9B1;10B14	144	1.37
In(1)BSC13	232,000	11	10B3;11A1	94	0.66
In(1)BSC26	206,000	31	10C5;11D1	143	1.35
In(1)BSC14	384,000	16	12A9;12F4	86	1.20
In(1)BSC27	In progress	1	12E3;13C5	126	1.35
In(1)BSC16	In progress	5	13D3;14A9	89	0.52
In(1)BSC17	In progress	3	14A6;14F5	63	0.65
In(1)BSC19	262,000	16	14F2;16C1	132	1.07
In(1)BSC20	163,000	9	15F9;17C1	106	1.22
In(1)BSC21	441,000	21	17A1;18A7	103	1.02
In(1)BSC22	205,000	16	17C6;19A2	155	1.26

[a] Males carrying some large *Dp(1;Y)* chromosomes were poorly viable and fertile due to hyperploidy. *In(1)BSC10*, *In(1)BSC20*, and *In(1)BSC22* screens each produced one *Dp(1;Y)* too large to be maintained in stock; the *In(1)BSC25* screen produced two.

[b] Minimal extents of medial duplicated region. See Table S3 for breakpoint ranges.

(breakpoint F in Figure 4) can fall in basal *X* euchromatin. Consequently, a *Dp(1;Y)* chromosome can carry genes from the *X* base in addition to genes from the middle and tip of the *X*. To assess how many *Dp(1;Y)* chromosomes carry basal euchromatic genes, we designed PCR primers flanking the insertion sites of transposons in basal *X* euchromatin and assayed for duplication of the insertion sites as described previously. We also examined CGH microarray data when available.

We could not detect breakpoints in the euchromatic gene *stnA* or in the region between it and *X* centric heterochromatin by our PCR mapping approach (Figure 13), because this region is present on all *Dp(1;Y)* chromosomes as a segment of *X* centric heterochromatin and adjacent euchromatin associated with the B^s marker on the *Y* tip (Figure 8; Brosseau and Lindsley 1958). Our microarray analyses showed this region extends distally to the five-gene region between *fog* and *stnA* (Figure 13; *X*:22228492..22384175). Consequently, all *Dp(1;Y)* chromosomes carry at least one copy of five basal euchromatic genes (*stnA*, *stnB*, and three proximal genes). They may also carry euchromatic genes between *fog* and *stnA* and heterochromatic genes. No gene probes in the region showed higher than twofold relative fluorescence in the *Dp(1;Y)* chromosomes analyzed by CGH microarrays.

The proximal deletion breakpoints fell in basal *X* euchromatin at a relatively high frequency. Of the 193 *Dp(1;Y)* chromosomes analyzed for duplication of basal genes by PCR, 36 duplicated *Cda4* (two genes distal to *fog*; Figure 13, Table S4). The duplicated segments extend varying distances distally with the largest segment reaching polytene region 19E (*X*:20631444..20795940). The basal segments carried by these *Dp(1;Y)* chromosomes provide coverage and breakpoint subdivision of 7.2% of the *X* euchromatin.

We also wished to verify that proximal deletion breakpoints often fall in the short arm of the *Y* and that *Dp(1;Y)* chromosomes arising from these events delete *Y*-linked spermatogenesis genes. Across all screens, we saw an approximately sixfold higher recovery of sterile *vs.* fertile y^+ males, suggesting that the proximal de-

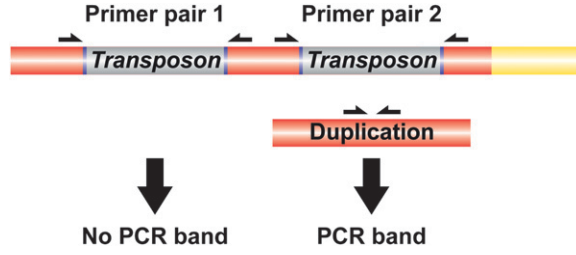

Figure 10.—PCR strategy for mapping the extents of duplicated segments. PCR primers were designed to flank the insertion sites of *X*-linked transposons. When males carry a *Dp(1;Y)* and an *X* with a transposon, a PCR fragment will be amplified from their DNA only if the transposon insertion site is present in the duplicated segment. With a short extension time, no fragment spanning the transposon on the *X* will be amplified.

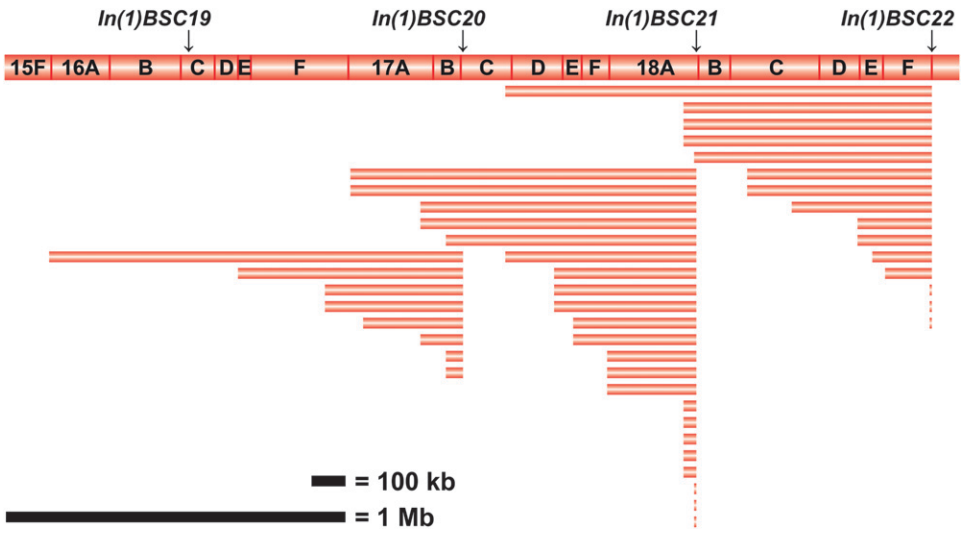

FIGURE 11.—Duplication coverage and genomic subdivision provided by three *Dp(1;Y)* screens. Three nested sets of duplicated segments provide full coverage of the region targeted by the *In(1)BSC20*, *In(1)BSC21*, and *In(1)BSC22* screens. Arrows indicate the positions of the proximal inversion breakpoints. The distal endpoints of the duplicated segments provide fine subdivision of the region for gene mapping. The minimal estimated distal extent of each duplicated segment is depicted. For simplicity, we have not shown the *Dp(1;Y)* chromosomes derived from *In(1)BSC19*. Numbered polytene divisions and lettered subdivisions (six per division, lettered A–F) are shown. Bars, 100 kb and 1 Mb for comparison.

letion breakpoints fall more frequently in the short arm of the *Y* than in *X* centric heterochromatin or the adjacent basal euchromatin. To show that the sterility can be attributed to the deletion of *Y*-linked spermatogenesis genes, we rescued the sterility with a redundant *Y*. In the screen with *In(1)BSC11*, we substituted homozygous *attached-XY* (*C(1;Y)1, y^1*) females for the normal *X, y^1/X, y^1* females usually mated to irradiated *inversion* + *attached-XY* males. Thirteen of the 17 *Dp(1;Y)* chromosomes recovered in fertile y^+ males with a redundant *Y* did not support fertility in the absence of an extra *Y*, demonstrating that the sterility could be rescued by duplicating *Y*-linked spermatogenesis genes. Because male sterile *Dp(1;Y)* chromosomes have limited experimental utility, they were discarded and are not counted in Table 2 or listed in Table S3.

Rescue of mutant phenotypes with *Dp(1;Y)* chromosomes: To verify that duplicated gene copies are functional and to illustrate how *Dp(1;Y)* chromosomes can be used to rescue the phenotypes of *X*-linked mutations, we crossed females bearing a recessive mutation with a lethal or visible phenotype to males carrying a *Dp(1;Y)* containing a wild-type copy of the mutated gene and examined the phenotypes of male progeny. We tested *Dp(1;Y)* chromosomes from 13 of the 16 nested sets and Table 3 shows that, as expected, the duplicated genes completely rescued the mutant phenotypes in nearly every case (85 of 90 crosses). In addition, males carrying *Dp(1;Y)* chromosomes containing the *achaete*, *Notch*, or *Beadex* genes displayed the well-known bristle and wing phenotypes associated with hyperploidy of these genes. These results indicate that duplicated genes are expressed as expected.

The three cases of nonrescue and the two cases of partial rescue are probably explained as suppression of gene expression by heterochromatic position effects. When euchromatic regions are juxtaposed to heterochromatin by chromosomal rearrangements, the compacted chromatin state can spread into the euchromatin and suppress gene expression. The likelihood that a particular gene will be suppressed depends on the distance the gene lies from heterochromatin, the strength of suppression exerted by the heterochromatic sequences and the inherent susceptibility of the gene to suppression.

Rescue of bristle defects caused by a *forked* mutation (f^1) showed the expected pattern for heterochromatic position effect suppression. Of all the *Dp(1;Y)* chromosomes derived from *In(1)BSC19*, the one placing *f* closest to centric heterochromatin (*Dp(1;Y)BSC206*) was the only one unable to rescue. Likewise, the wing defects caused by *upheld* mutations (up^1 and up^{101}) were rescued in 14–50% of males by *Dp(1;Y)BSC185*, a *Dp(1;Y)* derived from *In(1)BSC14* positioning *up* quite close to heterochromatin. [In fact, it is unlikely longer duplicated segments could be recovered using *In(1)BSC14*, because the end of the duplicated segment in *Dp(1;Y)BSC185* defines the proximal boundary of the 1- to 4-gene interval containing the diplolethal locus in region 12A. For further discussion of the *Hdl* region, see VENKEN *et al.* (2010)].

In contrast, the rescue of the wing phenotypes of *miniature* (m^1) and *dusky* (dy^1) mutations shows that position effects can be idiosyncratic. Of the *Dp(1;Y)* chromosomes derived from *In(1)BSC13* and *In(1)BSC26*, only *Dp(1;Y)BSC51* was unable to rescue the phenotypes even though *m* and *dy* are positioned farther from heterochromatin than they are in *Dp(1:Y)BSC54*, *Dp(1;Y)BSC102*, and *Dp(1;Y)BSC103*, which rescued the phenotypes. *Dp(1;Y)BSC52* and *Dp(1;Y)BSC101*, which place *m* and *dy* roughly the same distance from heterochromatin as *Dp(1;Y)BSC51*, also rescued the phenotypes. We attribute the inability of *Dp(1;Y)BSC51* to rescue to the presence of heterochromatic sequences near *m* and *dy* with unusually strong suppressive effects.

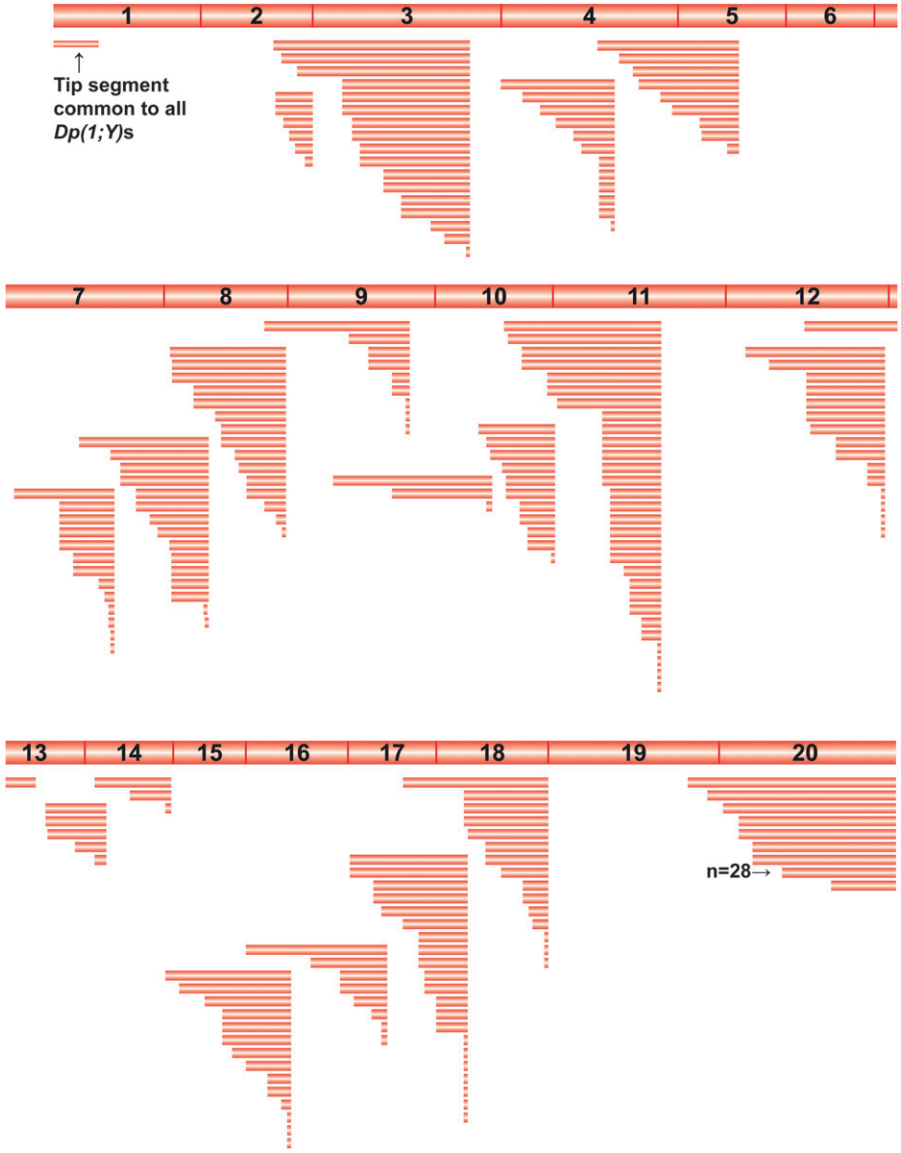

FIGURE 12.—Current duplication coverage and subdivision of the *X* chromosome. The nested sets of duplicated segments from the 14 completed screens and 5 screens in progress provide 78% *X* coverage and extensive subdivision. The *X* tip region common to all *Dp(1;Y)* chromosomes is shown. The most basal set of duplicated segments is detailed in Figure 12. It contains basal segments carried by *Dp(1;Y)* chromosomes from all screens and, as shown, includes 28 segments with breakpoints falling in the same mapping interval. The minimal estimated distal extent of each duplicated segment is depicted. Numbered polytene divisions are indicated.

The nonrescue of *f*, *m*, or *dy* phenotypes is probably not explained by the disruption of these genes during irradiation. The probability of mutating a particular gene with 4 kR exposure is ~1 in 5000 (ASHBURNER *et al.* 2005). We used a slightly higher dose (4.5 kR), but the likelihood of a duplicated segment carrying a mutated gene is still low. Likewise, nonrescue is not explained by mitotic loss of *Dp(1;Y)* chromosomes during development, because we have seen no y^+ or B^s mosaicism. To demonstrate gene expression is suppressed by heterochromatic position effects, it is sometimes possible to restore it with well-established position effect suppressors such as low temperature. Though the *f* and *dy* phenotypes were not rescued by *Dp(1;Y)BSC51* and *Dp(1;Y)BSC206* in flies reared at 18°, the nature of the chromosomal rearrangements in the *Dp(1;Y)* chromosomes suggests heterochromatic suppression is still the most likely explanation for the lack of rescue.

Using *Dp(1;Y)* chromosomes to rescue mutant phenotypes in females: The *Y*-linked inheritance pattern of *Dp(1;Y)* chromosomes makes it easy to track duplicated genes in experimental crosses, but it may not be apparent how *Dp(1;Y)* chromosomes can be used to rescue mutant phenotypes in females where a *Y* chromosome is not usually present. As we will show, recovering *Dp(1;Y)* chromosomes in *XXY* females and using them to rescue the phenotypes of recessive *X*-linked mutations is straightforward. Such experiments are useful in mapping *X*-linked mutations with female-specific phenotypes, such as defects in oogenesis.

XXY females arise from primary nondisjunction in both males and females. In females, nondisjunction results in *XX* and *0* gametes. *XX* eggs fertilized by *Y* sperm generate *XXY* females. In males, nondisjunction results in *XY* and *0* gametes. *XY* sperm fertilizing *X* eggs also generate *XXY* females. Consequently, any cross of

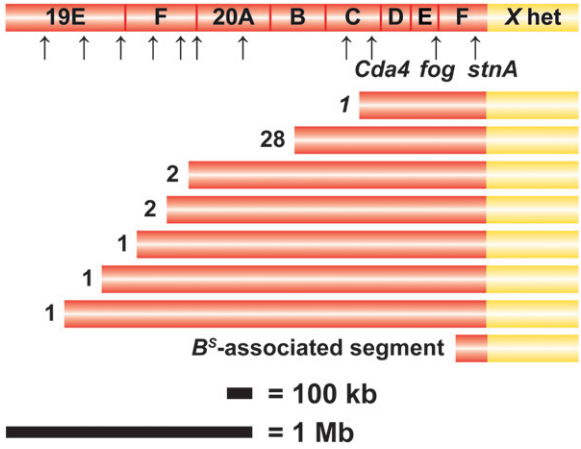

FIGURE 13.—Duplications in *Dp(1;Y)* chromosomes with breakpoints in basal *X* euchromatin. When the proximal breakpoints of deletions giving rise to *Dp(1;Y)* chromosomes fall in basal *X* euchromatin, *Dp(1;Y)* chromosomes can carry genes from the *X* base in addition to genes from the middle and tip of the *X*. PCR mapping cannot detect duplicated segments carrying the most basal euchromatic genes, because all *Dp(1;Y)* chromosomes carry a chromosomal segment associated with the *Y* tip B^S marker that extends from the euchromatic *fog* to *stnA* interval into centric heterochromatin (shown at the bottom). Thirty-six duplicated segments extend from *X* heterochromatin varying distances distal to *Cda4*. The arrows indicate the sites of PCR primer pairs used in mapping. The minimal estimated extent of each duplicated segment is depicted. The number of duplicated segments ending in each mapping interval is indicated.

XX females to males with a *Dp(1;Y)* chromosome can result in *XXY* females carrying a *Dp(1;Y)*. *XXY* females themselves produce mostly *XY* and *X* eggs, though they can produce *XX* and *Y* eggs by secondary nondisjunction (XIANG and HAWLEY 2006). The dominant B^S marker is quite useful in identifying females inheriting a *Dp(1;Y)BSC* chromosome, but crosses can be adapted to use y^+, w^+, or any marker present in a duplicated segment.

Rescue of a recessive female sterile (*fs*) phenotype can be shown by crossing *fs/balancer* females to *Dp(1;Y)* males to recover *fs/balancer/Dp(1;Y)* female progeny arising from nondisjunction in the mother and then crossing these *XXY* females to *fs/Y* males to recover fertile *fs/fs/Dp(1;Y)* females. We have successfully rescued the recessive sterility of an *ovarian tumor* mutation (otu^4) with *Dp(1;Y)BSC35* and *Dp(1;Y)BSC36* by this approach (see File S1 for details of *XXY* crosses), but it relies on the relatively low rate of nondisjunction in normal females. Spontaneous nondisjunction occurs at a rate of ~1 in 5000 female meioses (ASHBURNER *et al.* 2005), so large crosses must be set up to recover the initial *XXY* female.

The less labor-intensive approach is to recover *XXY* females following nondisjunction in males. Most of our *Dp(1;Y)* chromosomes are maintained in stock with *winscy*, a homozygous viable *X* balancer, and *XXY* females produced by nondisjunctional *winscy/Dp(1;Y)* sperm are readily obtained. Rescue can be demonstrated in two ways. First, *fs/balancer* females can be crossed to *winscy/Dp(1;Y)* males to produce *fs/winscy/Dp(1;Y)* females. These females can be crossed to *fs/Y* males to produce fertile *fs/fs/Dp(1;Y)* females. We have rescued otu^4 sterility with *Dp(1;Y)BSC35* by this approach as well. Alternatively, *winscy/winscy/Dp(1;Y)* females can be recovered directly from the stock and crossed to *fs/Y* males to produce *fs/winscy/Dp(1;Y)* females, which can then be crossed to *fs/Y* males to produce fertile *fs/fs/Dp(1;Y)* females. We have used this method to rescue the recessive lethality and sterility phenotypes of $N^{1N\text{-}ts1}$ females with *Dp(1;Y)BSC77* and *Dp(1;Y)BSC79* and the wing and sterility phenotypes of fu^1 with *Dp(1;Y)BSC15*. We prefer the latter alternative, because all it requires is expansion of the *Dp(1;Y)* stock until a *XXY* female is recovered.

Relying on male nondisjunction to recover *XXY* females is efficient, because nondisjunction in *Dp(1;Y)BSC* males is elevated. Typically, spontaneous nondisjunction in males occurs at a rate of ~1 in 2000 meioses (ASHBURNER *et al.* 2005), but we measured nondisjunction in *Dp(1;Y)BSC182* males at ~1 in 200 meioses. This rate is probably typical for all *Dp(1;Y)BSC* chromosomes, because *XXY* females and *X0* males are commonly seen in the stocks. The reason for elevated nondisjunction is not apparent—it is not a property of all *Dp(1;Y)* chromosomes (ZIMMERING and WU 1964). Nevertheless, it simplifies the use of these *Dp(1;Y)* chromosomes for rescuing phenotypes in females, because experiments can be initiated with *XXY* females directly from the stocks.

Regardless of the specific crosses, the first step in using a *Dp(1;Y)* to rescue a female-specific phenotype is the recovery of a *XXY* female carrying a *Dp(1;Y)*. She can be used directly in a rescue experiment, or she can be used to establish a stock with a high proportion of *XXY* females. Because 30–50% of the female progeny of *XXY* females are themselves *XXY*, selecting for high numbers of *XXY* females in subsequent generations is easy.

In conclusion, *Y* linkage is not a significant barrier to the use of *Dp(1;Y)* chromosomes to rescue the phenotypes of *X*-linked mutations in females. Such experiments require only an appreciation of *Y* inheritance in *XXY* females—a straightforward variation of normal sex chromosome behavior. As this is the most complicated use of *Dp(1;Y)* chromosomes most investigators are likely to encounter, we feel the flexibility and ease of use provided by *Y*-linked duplications in most other experiments outweigh their potential disadvantages in this one situation.

DISCUSSION

We have presented results of an ongoing project to provide coverage of the *D. melanogaster X* chromosome

TABLE 3

Phenotypic rescue by nested sets of *Dp(1;Y)* chromosomes

Mutation	Progenitor inversion	Rescuing *Dp(1;Y)*	Nonrescuing *Dp(1;Y)*
l(1)G1044^{G1044}	*In(1)BSC2*	*BSC217,219*	
N$^{1N\text{-}ts1}$	*In(1)BSC3*	*BSC77,79*	
rb^{1}	*In(1)BSC4*	*BSC158-161*	
cv^{1}	*In(1)BSC6*	*BSC91-99*	
sn^{3}	*In(1)BSC9*	*BSC172-178*	
oc^{1}	*In(1)BSC10*	*BSC33-39*	
lz^{77a7}	*In(1)BSC25*	*BSC144-151*	
flw^{1}	*In(1)BSC11*	*BSC58,59*	
m^{1}	*In(1)BSC13*	*BSC47-50,52,54*	*BSC51*
dy^{1}	*In(1)BSC13*	*BSC47-50,54*	*BSC51*
m^{1}	*In(1)BSC26*	*BSC100-103*	
dy^{1}	*In(1)BSC26*	*BSC100-103*[a]	
g^{1}	*In(1)BSC14*	*BSC189*	
up^{1}, *up*^{101}	*In(1)BSC14*	*BSC185*[b]	
Top1^{112}	*In(1)BSC27*	*BSC231*	
drd^{1}	*In(1)BSC27*	*BSC231*	
para^{ST76}	*In(1)BSC17*	*BSC228*	
if^{1}	*In(1)BSC19*	*BSC201*	
f^{1}	*In(1)BSC19*	*BSC200-205*	*BSC206*[a]
os^{o}	*In(1)BSC20*	*BSC67-70,157*	
os^{o}	*In(1)BSC21*	*BSC11,12*	
fu^{1}	*In(1)BSC21*	*BSC15*	
l(1)G0156^{G0156}	*In(1)BSC22*	*BSC129-135*	

[a] *dy*^{1}*/Dp(1;Y)BSC102*, *dy*^{1}*/Dp(1;Y)BSC103*, and *f*^{1}*/Dp(1;Y)BSC206* males were tested at 18°, 24°, and 29°. Other tests performed at 24° only.

[b] Rescue seen in some *up*^{1}*/Dp(1;Y)BSC185* and *up*^{101}*/Dp(1;Y)BSC185* males, but not others.

with nested sets of *Y*-linked duplications in a genetically uniform background. The project was initiated to address the poor selection of material resources for the genetic analysis of *X*-linked genes. Construction of all the necessary progenitor *inversion + attached-XY* chromosomes for comprehensive *Dp(1;Y)* screens is complete and we have isolated duplications providing at least 78% coverage. These *Dp(1;Y)* chromosomes also provide extensive breakpoint subdivision of the *X* with the median interval between breakpoints containing nine genes. This is a far better selection of duplications than existed previously. With the possible exception of two small regions containing diplolethal genes, our continuing efforts should provide complete duplication coverage within the coming year. We anticipate that the full set of *Dp(1;Y)* chromosomes will comprise ~300 stocks.

The Bloomington Stock Center duplication project is currently one of two large-scale efforts generating *X* chromosome duplications with molecularly defined breakpoints in Drosophila. The accompanying article by Venken *et al.* (2010) describes a collection of small, 70–120 kb *X* segments inserted into a third chromosome target site using the ΦC31 transgenesis system. The size of duplicated segments is the most significant consequence of the different approaches. The largest segment recovered by the transgenesis method to date is ~146 kb (Venken *et al.* 2006), while the size of segments isolated by our method is limited only by aneuploidy effects. The largest segment we have isolated is 1.68 Mb (165 genes). It remains to be seen whether transgenesis methods can be developed to transform larger duplicated segments, but *in vivo* chromosome manipulation approaches currently provide the only means of recovering duplicated segments of more than ~10 genes.

The two sets of duplications are complementary resources. We anticipate that mapping mutations and identifying dosage-dependent modifiers will involve three successive steps. First, a gene will be localized with coarse resolution to a large *X* interval using a tiling set of the largest duplications from our *Dp(1;Y)* screens. This will be an efficient step, because maximal coverage of the *X* can be provided with approximately two dozen duplications. Second, the gene will be mapped at medium resolution using duplications within a nested set of *Dp(1;Y)* chromosomes. Finally, the gene will be mapped with fine resolution using the transgenic duplications. While the resolution provided by *Dp(1;Y)* breakpoints is equivalent to the resolution provided by the transgenic duplications in some *X* regions, the average resolution of 3–5 genes provided by the transgenic duplications exceeds the median resolution of nine genes provided by our *Dp(1;Y)* chromosomes. Mapping to successively smaller intervals using duplications from both projects should prove to be an effective and efficient process.

The creation of *X* duplications has been accompanied by a large-scale project at the Bloomington Stock Center to improve the selection of chromosomal deletions. We have generated >830 deletions with sequence-mapped endpoints using the FLP-FRT system described by Thibault *et al.* (2004) and Parks *et al.* (2004). These and similar deletions isolated by Exelixis (Parks *et al.* 2004) and the DrosDel Project (Ryder *et al.* 2007) combine to provide the best genomic deletion coverage and breakpoint subdivision in any multicellular eukaryote. Phenotypic rescue with duplications from the two duplication projects and complementation with molecularly defined deletions from the three deletion projects will enable *X*-linked genes to be localized with near single gene resolution. The *Dp(1;Y)* chromosomes will enhance the utility of the deletion collection, because, unlike the transgenic duplications, they are large enough to rescue the lethality of most *X* deletions.

For all methods of gene rescue, the chromosomal context of duplicated genes is important. Regulatory elements near transgene insertion sites often suppress the expression of transformed genes. While individual genes are not removed from their normal chromosomal sites in the *Dp(1;Y)* chromosomes and their expression will probably not be affected by novel regulatory elements, the chromosomal rearrangements make heterochromatic position-effect suppression a potential concern. Because we saw evidence of heterochromatic suppression in only a small number of rescue experiments, it should affect the use of the *Dp(1;Y)* chromosomes in relatively few instances. The diversity in size among the duplicated segments and the substantial overlap of adjacent sets of nested duplicated segments should make it possible to identify *Dp(1;Y)* chromosomes rescuing most *X*-linked mutations—even if heterochromatic position effects occasionally necessitate the use of other genetic tools, such as the transgenic duplications, for fine mapping. In fact, the redundancy of coverage and experimental flexibility provided by the *Dp(1;Y)* chromosomes and the transgenic duplications are beneficial outcomes of two independent projects.

In summary, we have presented an important new research resource that will alleviate longstanding difficulties associated with the analysis of *X*-linked gene function. We anticipate these *Dp(1;Y)* chromosomes will be useful for many rescue, mapping, and modifier experiments. Because the isolation of these duplications required complicated progenitor chromosomes and multiple large screens, they would never have resulted from hypothesis-driven research. Their creation required a focused project and targeted resource-development funding. The same is true for many of the material resources that have propelled model organism research in recent years (for examples, see Hayashi *et al.* 2002; Bellen *et al.* 2004; Dietzl *et al.* 2007; Ryder *et al.* 2007; Ni *et al.* 2009; Guan *et al.* 2010). Because research resources to a large extent determine the kinds of experiments that are possible, resource-development projects such as ours are significant in the breadth of their impact. We are confident we have expanded "what is possible" with this new resource and hope it will be used heavily by the research community.

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GENETICS

Supporting Information
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A New Resource for Characterizing *X*-Linked Genes in *Drosophila melanogaster*: Systematic Coverage and Subdivision of the *X* Chromosome With Nested, *Y*-Linked Duplications

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FILE S1

Supporting Methods

Isolation and characterization of *C(1;Y)N12*: *T(1;Y)N12* is a reciprocal translocation between *X* centric heterochromatin of a $y^1\ w^1\ f^1$ chromosome and the tip of *YS* of $Dp(1;Y)B^sYy^+$, a *Y* chromosome marked with B^s at the tip of *YL* and y^+ at the tip of *YS* (KENNISON 1981). By isolating the B^s-marked chromosome of the translocation chromosome pair, we obtained a chromosome, which we call *C(1;Y)N12*, with all the *Y* genes needed for male fertility and all *X* genes distal to the *bobbed* heterochromatic gene cluster (though *bb* on the *X* is likely deleted, the redundant *bb* on the *Y* is present). Mitotic chromosome preparations stained with chromomycin A3 and DAPI showing that the translocation breakpoints fell at the distal end of band h29 in *X* heterochromatin and distal to *Y* chromosome band h24. Males bearing *C(1;Y)N12* in the absence of a free *Y* are viable and fertile.

Genetic background: To assure that the *Dp(1;Y)* chromosomes retained the genetic background of the *P{RS3}* and *P{RS5}* insertions (RYDER *et al.* 2004), all chromosomes used in the following crosses were first substituted into the standard background. Details of these substitution crosses will be provided upon request.

Crosses to generate *inversion + attached-XY* chromosomes

Step 1. Placing the proximal *FRT*-bearing transposon insertions onto the *attached-XY* chromosome by meiotic recombination:

G0: $w^{1118}\ P\{w^{+mW.Scer\backslash FRT.hs}=RS5\}$ ♀ x $C(1;Y)N12,\ y^1\ w^1\ f^1,\ B^s/Dp(1;Y)y^+$ ♂

G1: $w^{1118}\ P\{w^{+mW.Scer\backslash FRT.hs}=RS5\}/C(1;Y)N12,\ y^1\ w^1\ f^1,\ B^s$ ♀ x $w^{1118}\ P\{w^{+mW.Scer\backslash FRT.hs}=RS5\}/Dp(1;Y)y^+$ sib ♂

G2: $C(1)RA,\ In(1)sc^{7l1},\ In(1)sc^8,\ l(1)1Ac^1/Dp(1;Y)y^+$ ♀ x $C(1;Y)N12,\ w^{1118}\ P\{w^{+mW.Scer\backslash FRT.hs}=RS5\}\ (f^1),\ B^s/Dp(1;Y)y^+$ ♂

These crosses were completed for each of the *P{RS5}* insertions. The f^1 marker was present on some recombinant chromosomes. As shown in the final cross, *attached-XY* chromosomes may be maintained in stock by mating males carrying *attached-XY* chromosomes to females carrying *attached-X* chromosomes (also known as *compound-X* or *C(1)* chromosomes). *Attached-X* chromosomes consist of two *X* chromosomes sharing the same centromere. Stocks with *attached-X* females and *attached-XY* males may have a free *Y* as shown, or they may lack a free *Y*. As discussed below, we do not recommend maintaining *attached-XY* chromosomes with free *Y* chromosomes in long term cultures.

Step 2. Placing the distal *FRT*-bearing transposon insertion on the *attached-XY* chromosome by meiotic recombination:

G0: $P\{w^{+mW.Scer\backslash FRT.hs}=RS3\}CB\text{-}5805\text{-}3\ w^{1118}$ ♀ x $C(1;Y)N12,\ w^{1118}\ P\{w^{+mW.Scer\backslash FRT.hs}=RS5\}\ (f^1),\ B^s/Dp(1;Y)y^+$ ♂

G1: $P\{w^{+mW.Scer\backslash FRT.hs}=RS3\}CB\text{-}5805\text{-}3\ w^{1118}/C(1;Y)N12,\ w^{1118}\ P\{w^{+mW.Scer\backslash FRT.hs}=RS5\}\ (f^1),\ B^s$ ♀ x $FM7j,\ y^{93j}\ w^1\ B^+/Dp(1;Y)y^+$ ♂

G2: $C(1;Y)N12,\ P\{w^{+mW.Scer\backslash FRT.hs}=RS3\}CB\text{-}5805\text{-}3\ w^{1118}\ P\{w^{+mW.Scer\backslash FRT.hs}=RS5\}\ (f^1),\ B^s/FM7j,\ y^{93j}\ w^1\ B^+$ ♀ x $FM7j,\ y^{93j}\ w^1\ B^+/Dp(1;Y)y^+$ ♂

These crosses were completed for each of the *P{RS5}* insertions. Recombinant chromosomes were recovered in females, because *C(1;Y)N12* males had low viability and fertility. We usually could not determine the number of *miniwhite* markers present based on eye color, so recombinant chromosomes were identified using the following PCR primers specific to *P{RS3}* and *P{RS5}*.

P{RS3} Set A

Forward primer: CAAAAACGCACCGGACTGTAAC

Reverse primer: CATTGTTCAGATGCTCGGCAG

P{RS3} Set B

Forward primer: CGCACATACAGCTCACTGTTCAC

Reverse primer: GATAGCCGAAGCTTACCGAAGT

P{RS5} Set C

Forward primer: CAAAAACGCACCGGACTGTAAC

Reverse primer: GATAGCCGAAGCTTACCGAAGT

P{RS5} Set D

Forward primer: AAGCATGCTGCGACGTGAAC

Reverse primer: GATAGCCGAAGCTTACCGAAGT

Step 3. Disrupting *miniwhite* markers in the *P{RS3}* and *P{RS5}* insertions by FLP-mediated recombination:

G0: *FM7j, y^{93j} w^{1} B^{+}* ♀ x *w^{1118}/Y; noc^{Sco}/SM6b, P{$ry^{+t7.2}$=70FLP}7* ♂

G1: *C(1;Y)N12, P{$w^{+mW.Scer\FRT.hs}$=RS3}CB-5805-3 w^{1118} P{$w^{+mW.Scer\FRT.hs}$=RS5} (f^{1}), B^{S}/FM7j, y^{93j} w^{1} B^{+}* ♀ x *FM7j, y^{93j} w^{1} B^{+}/Y; +/SM6b, P{$ry^{+t7.2}$=70FLP}7* ♂

G2: *C(1;Y)N12, P{$w^{+mW.Scer\FRT.hs}$=RS3}CB-5805-3 w^{1118} P{$w^{+mW.Scer\FRT.hs}$=RS5} (f^{1}), B^{S}/FM7j, y^{93j} w^{1} B^{+}; +/SM6b, P{$ry^{+t7.2}$=70FLP}7* ♀ (heat shocked as larvae at 37° for one hour three days after cultures established) x *FM7j, y^{93j} w^{1} B^{+}/Dp(1;Y)y^{+}* ♂

G3: *C(1;Y)N12, P{w^{RS3r}=RS3r}CB-5805-3 w^{1118} P{$w^{RS5r.hs}$=RS5r} (f^{1}), B^{S}/FM7j, y^{93j} w^{1} B^{+}* (white-eyed) ♀ x *FM7j, y^{93j} w^{1} B^{+}/Dp(1;Y)y^{+}* ♂

P{RS3r} and *P{RS5r}* refer to the rearranged versions of *P{RS3}* and *P{RS5}* lacking *w* exons. These crosses were completed for each of the *P{RS3}* *P{RS5}* + *attached-XY* chromosomes.

Step 4. Inducing inversions by FLP-mediated recombination:

G0: *winscy* ♀ x *C(1;Y)N12, P{w^{RS3r}=RS3r}CB-5805-3 w^{1118} P{$w^{RS5r.hs}$=RS5r} (f^{1}), B^{S}/Dp(1;Y)y^{+}* ♂

G0: *winscy* ♀ x w^{1118}*/Y;* noc^{Sco}*/SM6b,* $P\{ry^{+t7.2}=70FLP\}7$ ♂

G1: *winscy/C(1;Y)N12,* $P\{w^{RS3r}=RS3r\}CB\text{-}5805\text{-}3$ w^{1118} $P\{w^{RS5r.hs}=RS5r\}$ (f^1), B^S ♀ x *winscy/Y; +/SM6b,* $P\{ry^{+t7.2}=70FLP\}7$ ♂

G2: *C(1;Y)N12,* $P\{w^{RS3r}=RS3r\}CB\text{-}5805\text{-}3$ w^{1118} $P\{w^{RS5r.hs}=RS5r\}$ (f^1), B^S*/winscy; +/SM6b,* $P\{ry^{+t7.2}=70FLP\}7$ ♀ (heat shocked as larvae at 37° for one hour five days after cultures established) x *winscy/Dp(1;Y)*y^+ ♂

G3: *C(1;Y)N12, In(1)BSC,* $P\{w^{+mW.Scer\backslash FRT.hs3}=3'.RS5+3.3'\}BSC$ w^{1118} (f^1), B^S*/winscy* (red-eyed) ♀ x *winscy/Dp(1;Y)*y^+ ♂

P{3'.RS5+3.3'} refers to the recombinant construct carrying the reconstituted *w* gene. These crosses were completed for each of the *P{RS3r} P{RS5r}* + *attached-XY* chromosomes.

The *inversion* + *attached-XY* chromosomes were maintained as either balanced stocks or *attached-X* stocks until their use in the *Dp(1;Y)* screens described below. We initially established these stocks with a free *Y* chromosome in addition to the *Y* chromosome present on the *attached-XY*. We did not appreciate the speed at which *Y* chromosomes accumulate spontaneous mutations in male fertility genes when selective pressure is relieved by the presence of a redundant *Y*. One-third of our *inversion* + *attached-XY* chromosomes were no longer male fertile in the absence of a free *Y* after less than two years in stock with a free *Y*. The accumulation of mutations by *Y* chromosomes that have not been kept under selection has been noted previously (HAZELRIGG *et al.* 1982; KENNISON 1981; J. Kennison, personal communication). Though we have not measured the rate of mutation in detail, spontaneous disruption of the six *Y*-linked male fertility genes seems higher than spontaneous mutation rates for other genes (estimated at <0.005 lethals per chromosome per generation or $<10^{-5}$ mutations per gene per generation (ASHBURNER *et al.* 2005; WOODRUFF 1983). The male sterility necessitated the replacement of the *Y* and basal *X* portions of sterile *inversion* + *attached-XY* chromosomes by meiotic recombination with a fertile *attached-XY*. All *inversion* + *attached-XY* stocks were rebuilt to eliminate free *Y* chromosomes as shown in Step 5 below. Based on these experiences, we strongly advise against maintaining *Dp(1;Y)*s in stock long term with other *Y* chromosomes.

Step 5. Establishing *attached-X* stocks of the *inversion* + *attached-XY* chromosomes lacking a free *Y* chromosome:

G0: *C(1)M3,* $y^2/0$ ♀ x *C(1;Y)N12, In(1)BSC,* $P\{w^{+mW.Scer\backslash FRT.hs3}=3'.RS5+3.3'\}BSC$ w^{1118} (f^1), B^S*/Dp(1;Y)*y^+ ♂

G1: *C(1)M3,* $y^2/0$ ♀ x *C(1;Y)N12, In(1)BSC,* $P\{w^{+mW.Scer\backslash FRT.hs3}=3'.RS5+3.3'\}BSC$ w^{1118} (f^1), $B^S/0$ ♂

Alternative crosses to generate *inversion* + *attached-XY* chromosomes

Background: The method in the previous section for generating *inversion* + *attached-XY* chromosomes was labor intensive and had steps that were difficult and inefficient. Particularly problematic was the need to screen for meiotic recombinants by PCR. The method was used to isolate most of the *inversion* + *attached-XY* chromosomes, but *In(1)BSC1*, *In(1)BSC2, In(1)BSC30*, *In(1)BSC31*, *In(1)BSC32* and *In(1)BSC33* were generated by a more efficient method.

The key to understanding this alternative strategy is the fact that heat shock-induced expression of FLP recombinase occurs in all cells. Consequently, it can catalyze recombination between *FRT*s and produce inversions in somatic cells as well as germ line cells. When FLP-

induced recombination between *P{RS3r}* and *P{RS5r}* insertions produces inversions and reconstitutes the *w* gene during eye development, clonal patches of red eye facets result. We realized we could use the ability to form inversion-bearing, red eye clones as a indication that rearranged *P{RS3}* and *P{RS5}* constructs had been placed *in cis* by meiotic recombination. We simply changed the order of the steps described in the last section to eliminate the need for PCR assays to detect recombinant chromosomes.

Step 1. Disrupting *miniwhite* markers in the *P{RS3}* and *P{RS5}* insertions by FLP-mediated recombination: We first exposed the individual *P{RS3}* and *P{RS5}* chromosomes to FLP recombinase to remove the 5' and 3' *w* exons, respectively.

G0: w^{1118} $P\{w^{+mW.Scer\backslash FRT.hs}=RS\}$ ♀ x w^{1118}/Y; $noc^{Sco}/SM6b$, $P\{ry^{+t7.2}=70FLP\}7$ ♂

G1: $C(1)RA$, $In(1)sc^{71}$, $In(1)sc^{8}$, $l(1)1Ac^{1}/Dp(1;Y)y^{+}$ ♀ x w^{1118} $P\{w^{+mW.Scer\backslash FRT.hs}=RS\}/Y$; +/$SM6b$, $P\{ry^{+t7.2}=70FLP\}7$ ♂ (heat shocked as larvae at 37° for one hour three days after cultures established)

G2: $P\{w^{+mW.Scer\backslash FRT.hs}=RS3\}l(1)CB\text{-}6411\text{-}3^{1}$, $w^{1118}/FM7h$, y^{93j} w^{1} B^{1} ♀ x $Dp(1;Y)y^{+}/w^{1118}$ $P\{w^{RS5r.hs}=RSr\}$ ♂ (white-eyed male)

G3: $FM7h$, y^{93j} w^{1} B^{1}/w^{1118} $P\{w^{RS5r.hs}=RSr\}$ ♀ x $FM7h$, y^{93j} w^{1} B^{1}/Y ♂

Step 2. Recovering recombinant chromosomes by meiotic recombination and inducing inversions by FLP-mediated recombination: *X* chromosomes carrying the rearranged constructs were placed *in trans* in females where meiotic crossing over could place them *in cis*. These recombinant chromosomes were recovered in males carrying a heat shock-inducible FLP recombinase transgene. Only those males inheriting a chromosome with both a rearranged *P{RS3}* and a rearranged *P{RS5}* transgene on the same *X* chromosome were able to generate inversions in somatic cells upon FLP recombinase expression to produce red eye clones.

G0: w^{1118} $P\{w^{RS5r.hs}=RS5r\}$ ♀ x $w^{1118}/Dp(1;Y)y^{+}$; $TM2/TM6C$, Sb^{1} ♂

G1: $P\{w^{RS3r}=RS3r\}$ w^{1118} ♀ x w^{1118} $P\{w^{RS5r.hs}=RS5r\}/Dp(1;Y)y^{+}$; +/$TM6C$, Sb^{1} ♂

G2: $P\{w^{RS3r}=RS3r\}$ w^{1118}/w^{1118} $P\{w^{RS5r.hs}=RS5r\}$ ♀ x w^{1118}/Y; $noc^{Sco}/SM6b$, $P\{ry^{+t7.2}=70FLP\}7$ ♂

G3: $P\{w^{+mW.Scer\backslash FRT.hs}=RS3\}l(1)CB\text{-}6411\text{-}3^{1}$, $w^{1118}/FM7h$, y^{93j} w^{1} B^{1} ♀ x $P\{w^{RS3r}=RS3r\}$ w^{1118} $P\{w^{RS5r.hs}=RS5r\}/Y$; +/$SM6b$, $P\{ry^{+t7.2}=70FLP\}7$ ♂ (heat shocked as larvae at 37° for one hour for three days beginning three days after cultures established; males carrying recombinant chromosomes recognized from w^{+} clonal eye sectoring)

G4: $FM7h$, y^{93j} w^{1} $B^{1}/In(1)BSC$, $P\{w^{+mW.Scer\backslash FRT.hs3}=3'.RS5+3.3'\}BSC$ w^{1118} ♀ x $FM7h$, y^{93j} w^{1} B^{1}/Y ♂ (red-eyed females carry inversions)

While we did not initially know if we would be able to recover inversion-bearing progeny directly from males showing red eye clones, we found that FLP-induced germ line recombination was high enough that red eyed progeny could be recovered from germ line clones in every case. This obviated the need to recover recombinant chromosomes in stock and undertake a later screen for germ line recombination events. Depending on the *P{RS3}*-*P{RS5}* pair, anywhere from 5 to 100% of males with red eye clones produced red-eyed, inversion-bearing progeny, though 30% was typical.

Step 3. Placing inversions onto the *attached-XY* by meiotic recombination: Once we isolated inversions, we placed them onto *attached-XY* chromosomes by meiotic recombination.

G0: *In(1)BSC, P{w$^{+mW.Scer\FRT.hs3}$=3′.RS5+3.3′}BSC w^{1118}/FM7h, y^{93j} w^{1} B^{1}* ♀ x *C(1;Y)N12, In(1)BSC25, P{w$^{+mW.Scer\FRT.hs3}$=3′.RS5+3.3′}BSC w^{1118} f^{1}, B^{S}/0* ♂

G1: *In(1)BSC, P{w$^{+mW.Scer\FRT.hs3}$=3′.RS5+3.3′}BSC w^{1118}/C(1;Y)N12, In(1)BSC25, P{w$^{+mW.Scer\FRT.hs3}$=3′.RS5+3.3′}BSC w^{1118} f^{1}, B^{S}* ♀ x *C(1;Y)1, y^{1}/0* ♂

G2: *C(1)M3, y^{2}/0* ♀ x *C(1;Y)N12, In(1)BSC, P{w$^{+mW.Scer\FRT.hs3}$=3′.RS5+3.3′}BSC w^{1118}, B^{S}/0* ♂

We used a preexisting *inversion + attached-XY* stock (*C(1;Y)N12, In(1)BSC25*) as the source of the *attached-XY* to combine with the new inversions.

The crosses above are shown with a distal *P{RS3}* and proximal *P{RS5}* insertion, but *In(1)BSC30* was generated with distal *P{RS5}* and proximal *P{RS3}* insertions.

Screens to isolate new *Dp(1;Y)* chromosomes:

G0: *winscy/winscy* ♀ x *winscy/Dp(2;Y)G, P{w^{+mC}=hs-hid}Y* ♂ (to kill larval males, stock cultures were heat shocked at 37° for one hour five days after being set up)

G1: *winscy/winscy* ♀ x *C(1;Y)N12, In(1)BSC, P{w$^{+mW.Scer\FRT.hs3}$=3′.RS5+3.3′}BSC w^{1118}, B^{S}/0* ♂ (adult males irradiated at 4,500 R)

G2: *winscy/winscy* ♀ x *winscy/Dp(1;Y)BSC, y^{+} P{w$^{+mW.Scer\FRT.hs3}$=3′.RS5+3.3′}BSC, B^{S}* ♂ (*Dp(1;Y)*-bearing males recognized by wild type body color from *y^{+}* allele at distal *X* tip)

All putative *Dp(1;Y)* chromosomes are assessed for *Y*-linked segregation patterns. A subset has been examined in polytene chromosome preparations and has looked as expected.

Rescuing female-specific phenotypes in *XXY* females

Three sets of crosses were undertaken to recover *Dp(1;Y)*-bearing *XXY* females homozygous for female sterile mutations. In the first crosses, *y^{1} cv^{1} otu^{4} v^{1} f^{1}/FM0, y^{31d} w^{1} v^{Of} f^{1} B^{1}* females were mated to *winscy, y^{1} w^{1}/Dp(1;Y)BSC, y^{+} P{w$^{+mW.Scer\FRT.hs3}$}BSC w^{1118}, B^{S}* males. *XXY* progeny resulting from nondisjunction in the mothers (*y^{1} cv^{1} otu^{4} v^{1} f^{1}/FM0, y^{31d} w^{1} v^{Of} f^{1} B^{1}/Dp(1;Y)BSC, y^{+} P{w$^{+mW.Scer\FRT.hs3}$}BSC w^{1118}, B^{S}* females) were crossed to *y^{1} cv^{1} otu^{4} v^{1} f^{1}/Y* males to recover *y^{1} cv^{1} otu^{4} v^{1} f^{1}/y^{1} cv^{1} otu^{4} v^{1} f^{1}/Dp(1;Y)BSC, y^{+} P{w$^{+mW.Scer\FRT.hs3}$}BSC w^{1118}, B^{S}* females. *XXY* progeny resulting from nondisjunction in the fathers (*y^{1} cv^{1} otu^{4} v^{1} f^{1}/winscy, y^{1} w^{1}/Dp(1;Y)BSC, y^{+} P{w$^{+mW.Scer\FRT.hs3}$}BSC w^{1118}, B^{S}* females) were crossed to *y^{1} cv^{1} otu^{4} v^{1} f^{1}/Y* males to recover *y^{1} cv^{1} otu^{4} v^{1} f^{1}/y^{1} cv^{1} otu^{4} v^{1} f^{1}/Dp(1;Y)BSC, y^{+} P{w$^{+mW.Scer\FRT.hs3}$} w^{1118}, B^{S}* females. In the second set of crosses, *winscy, y^{1} w^{1}/winscy, y^{1} w^{1}/Dp(1;Y)BSC77, y^{+} P{w$^{+mW.Scer\FRT.hs3}$}BSC77 w^{1118}, B^{S}* females recovered directly from the stock were crossed to *y^{1} N$^{1LN-ts1}$ g^{2} f^{1}/Y* males to produce *y^{1} N$^{1LN-ts1}$ g^{2} f^{1}/winscy, y^{1} w^{1}/Dp(1;Y)BSC77, y^{+} P{w$^{+mW.Scer\FRT.hs3}$} BSC77 w^{1118}, B^{S}* females. These females were crossed to *y^{1} N$^{1LN-ts1}$ g^{2} f^{1}/Y* males to produce *y^{1} N$^{1LN-ts1}$ g^{2} f^{1}/y^{1} N$^{1LN-ts1}$ g^{2} f^{1}/Dp(1;Y)BSC77, y^{+}*

P{w+mW.Scer\FRT.hs3}BSC77 w1118, B^S females. Similar crosses were undertaken with *Dp(1;Y)BSC79, y+ P{w+mW.Scer\FRT.hs3}BSC79 w1118*, B^S. In the third set of crosses, *winscy, y1 w1/winscy, y1 w1/Dp(1;Y)BSC15, y+ P{w+mW.Scer\FRT.hs3}BSC15 w1118*, B^S females recovered directly from the stock were crossed to f^1 *fu1/Y* males to produce f^1 *fu1/winscy, y1 w1/Dp(1;Y)BSC15, y+ P{w+mW.Scer\FRT.hs3} BSC15 w1118*, B^S females. These females were crossed to f^1 *fu1/Y* males to produce f^1 *fu1/f1 fu1/Dp(1;Y)BSC15, y+ P{w+mW.Scer\FRT.hs3}BSC15 w1118*, B^S females.

Male nondisjunction was assayed in the cross f^1 females x *winscy, y1 w1/Dp(1;Y)BSC182, y+ P{w+mW.Scer\FRT.hs3}BSC182 w1118*, B^S males. The frequency of male nondisjunction was calculated as the fraction of exceptional progeny arising from *XY* or nullo-*X* male gametes: (*XXY* + *X0*)/(*XX* + *XY* + *XXY* +*X0*). Exceptional *XXY* and *X0* progeny arising from nondisjunction in f^1 females were included only in the total progeny count. We showed that nondisjunction was not elevated in homozygous *winscy* females by measuring nondisjunction in *winscy, y1 w1/winscy, y1 w1* females crossed to *C(1;Y)2, y1 B1/0* males.

LITERATURE CITED IN SUPPORTING METHODS

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TABLE S1

Insertions and primers for mapping distal ends of duplicated segments by PCR

Insertion	Forward primer		Reverse primer	
	Sequence	Coordinates	Sequence	Coordinates
P{SUPor-P}KG01655	GACCTGTGAGCCATCGTCGC	*X*:1055741..1055760	GCTTAGTGGAGTTCGGATTAGGCC	*X*:1056568..1056591
P{EPgy2}EY11509	GAATGCCAAGAGAGCAGCATGGC	*X*:1103871..1103893	CCTTGAAGTGACTGGGGTAATCGG	*X*:1104395..1104418
P{EPgy2}CG11412^{EY10202}	GCCGATAGTCACAGTTGGC	*X*:1236080..1236098	CCAAGCGGCCGAGCCTCCTGC	*X*:1236761..1236781
P{EPgy2}CG3719^{EY14694}	CGATGATTGCAAATTCTGCCTCGGC	*X*:1272985..1273009	CCGGCAGCCGCAGGTCC	*X*:1273577..1273593
P{EP}CG14777^{G1158}	GAGGGCGTGAGATGCGGACG	*X*:1360823..1360842	CTGAAACTGCACGATGAGTGG	*X*:1361546..1361566
Mi{ET1}Nmdar2^{MB09441}	CTGCCATTCATAAGGATGAGCAGC	*X*:1381640..1381663	GCTTAGGACGACTTTGTGGTGC	*X*:1382522..1382543
P{EPgy2}EY03391	CATCAACTCGCGCGTATCTATGG	*X*:1563016..1563038	GGGCGGTTCTGCAGGCTCG	*X*:1563763..1563781
P{GT1}Adar^{BG02235}	CCGCATCGAGGAACCAAATCG	*X*:1667892..1667912	GCAATGCAGCAGTGAAGCCTTTCCC	*X*:1668680..1668704
P{SUPor-P}deltaCOP^{KG07426}	GCCAGCAGTCATCAGCTTGGG	*X*:1755327..1755347	GTTGGTAAAGGCGATGCTTGG	*X*:1756095..1756115
P{EPgy2}CG14806^{EY15916}	CCGATTGCGTGTCACGAACAGGG	*X*:1774273..1774295	GCAGGCGGATGAAGTCTTCC	*X*:1775058..1775077
P{EPgy2}CG3573^{EY15890}	CATGAGAGCGTAGTCAAGCATCCGC	*X*:1816653..1816677	CAGCGCGATCTTACTAGGCTCGGCC	*X*:1817206..1817182
P{SUPor-P}CG3600^{KG00928}	CTTTGGCCGCCAGGACCGTCC	*X*:1841903..1841923	GTGGCTTCGTATTGCAACTCG	*X*:1842697..1842717
P{SUPor-P}KG06944	CAGAAGAACACCAACTAAACTAAACGC	*X*:1903879..1903905	GTCTCCTCTTTTCGTCATTGCAGCG	*X*:1904294..1904270
P{EPgy2}CG14054^{EY07071}	CCGCTGTCCTCCTGTCCGCTCGC	*X*:1959038..1959060	GTGGACTGCCTGGCGCTTGTGTCC	*X*:1959591..1959568
Mi{ET1}MB01363	CTATGCTCTATCATACATGTGGCGTCC	*X*:2010175..2010201	CCGACTCACCACTTCTCACAGCACG	*X*:2010776..2010752
P{EPgy2}EY03702	CCAAAGCGGTCGCCAGGAGAATCGC	*X*:2069227..2069251	GATCTAATCAGCAGAACCAGAGTGG	*X*:2069764..2069740
Mi{ET1}msta^{MB00924}	CTACAGTGCTTGCCAAGACGATCCC	*X*:2099704..2099728	GATGGCCAAGTACCAGGACTTCGC	*X*:2100362..2100339
PBac{RB}CG3191^{e02435}	GCTGTCCGCGTCTAGAGGATCCC	*X*:2145248..2145270	GCTGCGTCCGATAGCCGATAGC	*X*:2145950..2145929
P{GT1}BG01975	CTGTTATCAGTGCAAGACAGAAACGC	*X*:2211467..2211492	GTTCTTCCTAAGGGCGTAAATGCCGC	*X*:2212081..2212056
P{SUPor-P}KG06050	GTCGGATTGACTGCATCTTTGTTGG	*X*:2219491..2219515	GAGAACGTGATAACTTTCTGCCGC	*X*:2220057..2220034
P{SUPor-P}KG06050	GTCGGATTGACTGCATCTTTGTTGG	*X*:2219491..2219515	CCGCAGCTCTCTGAACCGCTCC	*X*:2219892..2219913
Mi{ET1}MB04449	CCAAAAGTCGGAGCATGGAAACATAGC	*X*:2302387..2302413	CAACGGCGATGCAGGTTGGGAACTGGG	*X*:2302925..2302899
P{EPgy2}egh^{EY03917}	GAAGGGGTGAGAGAGTGGAAGAAGG	*X*:2483034..2483058	GTTTGGGTTTTATATCTGTCTCCGCC	*X*:2483487..2483462
Mi{ET1}CG2652^{MB03796}	GCTCGGTAGCCAGGCCATCTCTCCG	*X*:2575674..2575698	CGTACATCTTCCGGCCGCGCGTGG	*X*:2576478..2576455
P{EPgy2}CG2694^{EY21207}	GGGAATAGCTAACATCCCATACGC	*X*:2608030..2608053	CGTCCCCGCAGCCTGGCCAGAGACG	*X*:2608618..2608594
P{EPgy2}SyX4^{EY00005}	GGTTTGAGTGATGCCAAACCACTGTGC	*X*:2637387..2637413	GCCCAGGCTGTAGCCAGAGTGTTTGC	*X*:2638207..2638182
Mi{ET1}MB09143	CATTCTCGTCTGATTTGGTTTCCCTCG	*X*:2779902..2779928	GGCATTACTTTTGGTCAGCGTCTCGG	*X*:2780463..2780438

P{EPgy2}CG17959^{EY03513}	GCCTGTGGATCGAGACTCCAGTTGC	*X*:2841198..2841222	GCTGGGGAGTCAAGTTCGCACTCC	*X*:2841552..2841529
P{SUPor-P}kirre^{KG05552}	CATTGCGATGTGGGCCATGCTGTTGG	*X*:2992911..2992936	CCGAATTACTCGACATCGGAACACG	*X*:2841198..2841222
PBac{RB}Fcp3C^{e04212}	GTTGTGCTGCTACTTGCAACACC	*X*:3068122..3068144	CAAATGTTGGCCGCCCAATATTGATTGG	*X*:3069002..3068975
P{SUPor-P}KG06782	GCCGCTGCTTCGCCTGGCTTCCTGG	*X*:3253742..3253766	GACGAGTTTTCGGCGTTTAGATCATTGG	*X*:3254392..3254365
PBac{WH}CG12206^{f07347}	GCACCCGGATGGTCTCGTCCTGC	*X*:3360464..3360486	CGCCTTGGGAAATTCGATCGTGTTC	*X*:3361427..3361403
Mi{ET1}CG32791^{MB01249}	CTGCGGCATTATCGTCGCATGGG	*X*:3422188..3422210	CGGCACGGTCTTTATTGTTTGCGATTC	*X*:3422825..3422799
P{SUPor-P}tlk^{KG06931}	CTATGGCCACATTCAAGAAACGCC	*X*:3634322..3634345	CTGCAGTCTACAGAAAGTAACAGGC	*X*:3635061..3635037
P{SUPor-P}ec^{KG09175}	CCAGAACGCAGCACTATATCGAC	*X*:3736678..3736700	GGCCAACGTCTTCAGTGCGACG	*X*:3737602..3737581
P{Mae-UAS.6.11}Vap-33-1^{GG01069}	CGCTTTCTATTGCCATCTCGCTCGATTGGC	*X*:3843899..3843928	GCGAGGGGGTTGCAAAAGAATGGGG	*X*:3844623..3844599
P{EPgy2}CG6379^{EY08403}	GTTCTCATCGTCCGAAGGTTCGTCC	*X*:3994648..3994672	CTAGACCAAGTGTGGTATGCTCG	*X*:3995304..3995282
PBac{RB}GlcAT-I^{e04384}	CCACACTGGAGACGAAGAATATCG	*X*:4025177..4025200	GTGGCTTAATCCAGTTGGGGTCC	*X*:4025778..4025756
P{SUPor-P}KG00475	GGCTAAGCCAGTTTACAGGATCG	*X*:4186834..4186856	GTTATCCTCGTTAAGTGCGTTAACCAC	*X*:4187481..4187455
P{XP}bi^{d05964}	GAGTTCCATCTCGATTTAAGGTGGC	*X*:4317564..4317588	GCATCCTACTCTCATTCGCTCGCC	*X*:4318287..4318264
Mi{ET1}MB01991	GTTCTAAGGCACAGGTCAGGGC	*X*:4478617..4478638	GGTGATGACAGCAGACACCAGGC	*X*:4479348..4479326
P{EP}HLH4C^{G351}	CTCAGTCAGAGCAGCTGGTGTCC	*X*:4539074..4539096	CCAACTTAACCGAAGTGTTCGATGG	*X*:4539664..4539640
P{SUPor-P}KG02802	CGACGCGAACTCGTTTCTCGCCG	*X*:4573372..4573394	CGCTTGTAAGGTGTTCACCACTTGCG	*X*:4574098..4574073
P{SUPor-P}KG06705	CGAATTAATGACGGTTGGGGTTCGC	*X*:4698591..4698615	CATCCAAAGGACCACCAAACTGC	*X*:4699234..4699212
P{EPgy2}CG6903^{EY08878}	GTCCAATCCAGACGCTCGTCGCG	*X*:4813070..4813092	GGCATAGTGACCCTGATCGAGGTCG	*X*:4813615..4813591
P{SUPor-P}CG3011^{KG08318}	CTCTGCTTGGAGTCCGAATATCG	*X*:5810306..5810328	GATAGCGCAGCGATCGGGTCGG	*X*:5810719..5810740
P{GT1}BG01736	GGCAGAGCGGATCGGCTTCC	*X*:5882264..5882283	CGAGTTACGATGGGATCGATCG	*X*:5883053..5883074
P{GT1}BG02331	GGATCGCCAAAGTCCGTCGCCG	*X*:5971289..5971310	CATAGTCGCTTTTTCACCGCTTGC	*X*:5971849..5971872
P{GT1}CG3774^{BG02156}	GGAGAGCCCATAGGCGAGCG	*X*:6114141..6114160	CAGTCGTACGTCGTATGTGC	*X*:6114957..6114976
P{EPgy2}EY06102	CAGGATTCTCACCTAATTGGGC	*X*:6170249..6170270	CAGGTACATTGGAATTATTGCTTAGGTACC	*X*:6170819..6170848
P{EPgy2}EY16428	CGACACTGGTGCCTGGACGG	*X*:6188987..6189006	GCAGGCGTCACAATAGTCGTGG	*X*:6189850..6189871
PBac{WH}swa^{f01372}	GCTCGTCCGTCGGAAAACTCTCG	*X*:6259593..6259615	CATTGCCGACTAGAATCCAGTTGC	*X*:6260082..6260105
P{XP}CG3918^{d02940}	CGGGCGGATTGAACTGGCGC	*X*:6418107..6418126	CTATGCGATTGGTGCGCTTACC	*X*:6418827..6418848
P{EPgy2}EY07268	GAAATGGACACTTTCGACCTGAAGGC	*X*:6434427..6434452	GATGTTGGGTTTGTGCGGCAAGCC	*X*:6434848..6434871
P{EPgy2}l(1)G0148^{EY07177}	GTATATCAAGTGCCAGCTTTGGTGC	*X*:6548351..6548375	GCAGTAACTGAGATGACAACTCC	*X*:6548939..6548961
PBac{WH}CG14442^{f06399}	GTTGGATCTCCACCATCGATCG	*X*:6583433..6583454	CTGGTGACCATCAGTCAGCACC	*X*:6584031..6584052
PBac{WH}CG14439^{f06522}	CATCTCCTAACTAGCCCTATAAGC	*X*:6642256..6642279	GAAAGATTCATTAGACTCATTCTCATTGGG	*X*:6642746..6642775
PBac{WH}AtX-1^{f01201}	GCAGGTGCAGCCGGGTCATCC	*X*:6717733..6717753	CCATTAGAATGCTTGACAGGAGG	*X*:6718403..6718425

P{EPgy2}EY03050	GGTCCGCCTATCCTTTGTCCC	*X*:6777690..6777710	GATAGTAGCAGCGTTGCCAGGC	*X*:6778164..6778185
PBac{WH}ogre^{f07788}	CGTGTACAAGGGGTTTTCACG	*X*:6875585..6875605	CAAGTTATTCCGTACCTTTTCGTAGGCC	*X*:6876203..6876230
P{XP}CG14427^{d06860}	GTCACCCACATTCCGGAGGC	*X*:6935287..6935306	GGCCCCTGACCTTTGACCCGC	*X*:6935833..6935853
P{SUPor-P}CG9650^{KG00935}	CGATTCGTCGTCACCTACGTGATCC	*X*:7089520..7089544	GCTTCTTAGGCGAACATGTCCG	*X*:7090100..7090121
Mi{ET1}Dok^{MB03742}	GTCGGCATGGGATTGCCCGCC	*X*:7219606..7219626	GCCTCAAAGGTGAACTTGCC	*X*:7220307..7220326
Mi{ET1}MB07442	CGTCAATCCGACTTTCCCAAGG	*X*:7310589..7310610	GCCAAGCAGCTCTGACCC	*X*:7311355..7311372
PBac{WH}CG15478^{f07358}	GATCCACCCACTCAAGGCTGCCCAGC	*X*:7610424..7610449	CGAATACGGACAACATACACGGGAC	*X*:7611227..7611203
Mi{ET1}CG1402^{MB01998}	CGATTGAGACAATAACCCGAAAGCC	*X*:7726814..7726838	CTGCGATTCACTTCCAGGACTTCAC	*X*:7727326..7727302
PBac{WH}CG10932^{f04498}	CTCCTGGACATCGGTCTTCGC	*X*:7782491..7782511	GCTTGTAACCGTTAAGTTCAATTTACGC	*X*:7783020..7783047
P{EPgy2}CG1444^{EY08252}	GCCGTTGACAATTGACATTCAATCGC	*X*:7802982..7803007	GACCTTCCGGAAGACCTGGAAGCC	*X*:7803488..7803465
PBac{WH}CG15332^{f05798}	CCCTCGGTATGCCTGCAATGCC	*X*:7849656..7849677	GGGCGACGAACGGTGGCTGC	*X*:7850292..7850311
P{EPgy2}fs(1)h^{EY10625}	CGCGAGTTTCTTCTGAGTCGCC	*X*:7954973..7954994	CCGAGCCTAGGAACAGTGTTGC	*X*:7955600..7955621
P{EPgy2}Gclc^{EY05904}	CATGGGCGAAATTTACGCG	*X*:8009881..8009899	GCAAGTTGTGGTGAAATCACAAACTGC	*X*:8010552..8010578
P{SUPor-P}CG2116^{KG00028}	CGATGTATGAAAGCGGAAGGAAGCAAGC	*X*:8023765..8023792	CCAATTCCCTGACCACAAATTGCG	*X*:8024252..8024229
PBac{PB}CG10959^{c02347}	GTGCACTTCGACAAGCAGGG	*X*:8043108..8043127	GACTTGCCGCAGTTCTCGTGC	*X*:8043859..8043879
PBac{PB}CG10959^{c02347}	CCGCTCACCAAGACGGTCACGGG	*X*:8043132..8043154	CCGTGTGCTCCTTGCGACGATGC	*X*:8043761..8043739
P{EPgy2}EY20665	GCAGAGAAGTCGGGGAAATCCATGG	*X*:8071764..8071788	GGTCCTGGGCCGTAGTGTCC	*X*:8072637..8072656
P{XP}sdt^{d05058}	GGGTAAAACAGATGCTGTATCGCG	*X*:8086758..8086781	CAGTCCGCCGGTGGGAACGAGACG	*X*:8087345..8087322
P{XP}CG1632^{d05362}	GTGATGAGGTTGGTCTCGCCCTGG	*X*:8165237..8165260	CTCTCTCCAATTGCCATTCAATG	*X*:8165644..8165622
P{SUPor-P}CG32711^{KG09043}	CAGCTAAGGACTTTGTTCGCATATCC	*X*:8302771..8302796	GAGAGCACAAGTCTGAGCACACAC	*X*:8303364..8303341
Mi{ET1}CG15347^{MB04140}	GCAGTGCCAACCCGTTGACACCTCG	*X*:8352348..8352372	GGTATCTTTCGCAGATGGCTACAC	*X*:8353216…8353193
P{XP}Nrg^{d11128}	GAATGGCAATGCAATATTGTACGAAGCAC	*X*:8412006..8412034	GCCAAAGCGTGAGAGACGGAGCG	*X*:8412305..8412283
P{EPgy2}EY19827	GGACCACTATTCGTAAACCAAAATGTGC	*X*:8483158..8483134	CGCTGTGGGCTGCGAGCCGTGTAGC	*X*:8483158..8483134
PBac{RB}CG11284^{e04402}	CCAAAGTCCAAGTTATAATGTGCTTGCC	*X*:8582193..8582220	CAACTTGTCTTTCTCGGCGTCGAAG	*X*:8582905..8582881
P{SUPor-P}CG2004^{KG10420}	CGGAATGTCTAAGTGATCACACCC	*X*:8608364..8608364	GCGCATCTCTAACCCTGCCTTCA	*X*:8608747..8608725
PBac{RB}Moe^{e03902}	CGACTTCATGCTAATGGGATAAGTGTGC	*X*:8791314..8791341	GAGGGGTAGAGTGATTGGAGTGTGAGTC	*X*:8791733..8791706
Mi{ET1}rdgA^{MB06886}	CGCGTAAGTGAAAGAGAACCGATGGTATGG	*X*:8919421..8919450	GGCTGAAGGTAAATTGGGTGGTATATC	*X*:8920102..8920076
P{EP}CG7267^{EP1030}	CAATGTGTCTATCAATATTCTTATGACACAGC	*X*:8972175..8972206	CTTTCGGACTGTCTATAGTGATTCG	*X*:8972920..8972896
P{EPgy2}fend^{EY02774}	GTGGACAAGGAATGAGAATGAGG	*X*:9023264..9023286	GTGCCCATGGTTCCCACACTTGTCG	*X*:9023975..9023951
P{EPgy2}EY09570	CCACTATCTCGTCGGTCTGCTCCGC	*X*:9055896..9055920	GGCCTTACCGTGTACCGCTACAGG	*X*:9056299..9056276
Mi{ET1}CG12119^{MB05824}	CAGGAATTTCGAAAAGGCTGGCAGC	*X*:9102623..9102647	GATTGAAGTTCCGCAGTTTAGCC	*X*:9103310..9103288
P{EPgy2}EY14474	GCAGGACAATCGACTCCATGGCTGG	*X*:9137219..9137243	GCTGGGATGCCAGGTATTTGCAGC	*X*:9137810..9137787

PBac{RB}CG16892^{e03860}	CCTAAAACAGTGTCCGCCGTTCTCC	*X*:9167564..9167588	GTCTACACCGGCTACGGCTAAGC	*X*:9168080..9168058
P{EPgy2}EY00880	CATTGGTGTTAGTATCGGTGTTAGC	*X*:9249724..9249748	GCTACGATGTCACTTCGATGGC	*X*:9250310..9250289
P{SUPor-P}KG00777	CTATCGCACTGTCACTACAGTTGG	*X*:9394100..9394123	GAACCACCAACACGCACTGTGGCAGC	*X*:9394691..9394666
P{GT1}BG00175	GCTAACCGATCCCAAACTCGC	*X*:9488176..9488196	GTATCCGTAATAGTAGCCACATCTATAGC	*X*:9488647..9488619
P{SUPor-P}KG07347	CCAGATAACGAAGCAGCGAGTCC	*X*:9579966..9579988	GTTTGCATGACCACCGGTTCGTCGG	*X*:9580497..9580473
P{SUPor-P}KG07347	GTAAGAAACATCATTGGTTGGAGACC	*X*:9580068..9580093	GCGCCAGCCAGTCATATGGCCGTC	*X*:9580803..9580780
P{EPgy2}EY02055	CGGACGTTTACCGTTGGATGAGGACG	*X*:9801096..9801121	CCGCTGACTCGAACCGCTAAAGTAG	*X*:9801732..9801708
Mi{ET1}CG32694^{MB05037}	CTTCCTTTGGCATTCCGCACGTGGC	*X*:9889597..9889621	CCGACAAGACTATTGGATAATGGCCG	*X*:9890260..9890235
PBac{WH}CG2974^{f02346}	CACCGGCGGCTTGAAGCTGAATCC	*X*:9979553..9979576	GGCTAGCTTACAGCACCTCCCGC	*X*:9980412..9980390
PBac{WH}CG15308^{f00264}	GCAGGGATGTTAAGAATCGGAAATGGTC	*X*:10112413..10112440	CCAGTCCTCCAAGCACAACTTACTC	*X*:10113158..10113134
P{SUPor-P}KG10244	GGAGCGTCTCGTTGAACCAATCGAG	*X*:10225396..10225420	GGCGCGTATAGAACGATGTTGAAGG	*X*:10226273..10226249
P{EPgy2}EY12447	CTAGCCGTATGATCATCAAGTCGTCG	*X*:10279580..10279605	CGGTGGGCCGCTGATGTTCACTG	*X*:10280250..10280228
PBac{WH}Neb-cGP^{f02352}	GCACTTTCAACACTGTTCGCCCAGC	*X*:10354712..10354736	GGATCCAAGACATCGATTGTGGCGG	*X*:10355246..10355222
P{SUPor-P}CG1628^{KG08894}	CGAGGGGCTGGGACACATACACC	*X*:10495487..10495509	CGCCAGTAGCATAACACTAACACGC	*X*:10496184..10496160
P{SUPor-P}CG32676^{KG04888}	GCAAGTCTTGTGTAACCTAATCTCCG	*X*:10632983..10633008	GTTGAGTTTTGCCCTATTGACGC	*X*:10633589..10633567
P{EP}G733	GGCGGGCCAACTATCGCACAGGTCG	*X*:10741132..10741156	CGTTAGCAACTGGACGAGGGAACCACC	*X*:10741828..10741802
P{XP}Ork1^{d09258}	GGAGGTGCGGCACTGATTGATTTACC	*X*:10784066..10784091	CACACATTATCGCCAAGCCACCCAACC	*X*:10784544..10784518
P{EPgy2}EY00595	GAAATCGATAAGACTCGATAATCCCG	*X*:10824618..10824643	GTTTCTTTTGGCGTCACTTCGTCACCGC	*X*:10825279..10825252
PBac{WH}sev^{f02355}	GGTGTGTTATTGACACCATTATTGTCCC	*X*:10975227..10975254	GAGCTGGTGATGATTTAGCTCCAGG	*X*:10975778..10975754
P{EPgy2}EY09320	CCGCTACAATGTGTTAGCACTTTCC	*X*:11030920..11030944	GTCTTATTAAACAGCTAATTTTGTTAGGGC	*X*:11031560..11031531
Mi{ET1}MB06515	CACCACCGTCATTCCGGCTAGG	*X*:11062961..11062982	CCACCGTATGATTATCTTGAGCACTTCG	*X*:11063521..11063494
PBac{RB}CG1657^{e02476}	GCAATCAGCGCCCACATAACGGACC	*X*:11219857..11219881	GTTGCCTGTGACGAAAATAGTAGG	*X*:11220419..11220396
PBac{RB}CG11752^{e04370}	CGCACTTTCCGCGTTACCCAACACG	*X*:11244026..11244050	GCTGGCGAAATTCCACGCGATCG	*X*:11244810..11244788
P{XP}CG15196^{d06689}	CGACTAAACGAACGCCATCGTGAAATTCGG	*X*:11303997..11304026	GGCGTCGCTTATGCCTCTTCGTCC	*X*:11304560..11304537
P{SUPor-P}CG11727^{KG00813}	GTAATTAATGGTTTACAGAAGAGTGGACG	*X*:11347096..11347124	CGCTGTCTGTGTGGGTTTATGCTCGCG	*X*:11347686..11347660
P{EPgy2}CG1572^{EY23597}	CGCTCTCGTCTATATGTTCACCTAGG	*X*:11451050..11451075	CCTCTGTATGGGTCTTACAAGGC	*X*:11451545..11451523
PBac{WH}nod^{f04008}	GAGCGAGATGACAATAGAGAGGCG	*X*:11474221..11474244	CGCGGACCGCAATCCGAACTGCG	*X*:11474999..11474977
PBac{RB}FucT6^{e02394}	CCCACAGCCCAGAGTTGCAGAAAAGG	*X*:11600469..11600494	GGATCTACAGCTGCGTGTTGGTGAC	*X*:11601204..11601180
PBac{WH}dy^{f04509}	CATGCACACACTTGGACTCACACG	*X*:11670262..11670285	AACACTTTCATATAGCAGG	*X*:11671080..11671062
Mi{ET1}MB01008	CGAACACGTTCCTTGATCGACTACG	*X*:11733351..11733375	GGCGTGGCCAAATCATGTTGGGAAAGG	*X*:11733796..11733770
P{SUPor-P}CG10353^{KG03540}	GATTCCGAGAGATCCGGTAAGAAGC	*X*:11775943..11775967	GCCCCGCTGCTGTAATGATGCACAC	*X*:11776559..11776535
PBac{WH}f03985	CTTACCATCCCTACTTTTTGACGGGC	*X*:11814681..11814706	GAGAGAGTGCAGATTGTTAGCTCC	*X*:11815341..11815318

P{SUPor-P}KG05404	CCATCCACATTCCGCAGCAAACCG	*X*:11900875..11900898	CGGATCGTGAGTGCAACTGTACG	*X*:11901255..11901233
P{XP}d08667	GCCTTCTACAAGACCGCACTTTTCCC	*X*:11900970..11900995	CAAAGCACCGGTGCGTTAAGATTAGTC	*X*:11901942..11901916
Mi{ET1}Cyp318a1^{MB02480}	GGGCACCTTGAGTGTCTGAATCCG	*X*:11926365..11926388	CGATATAGAGCAGGACTCGG	*X*:11927161..11927142
PBac{WH}CG2750^{f01388}	GGCACAACCACGTCGTCAAGCGTATG	*X*:12001848..12001873	CATCGATGCTTCATGATACGAGGGC	*X*:12002412..12002388
PBac{WH}f01428	GTATGGCTAGGTGAGCTATGTTTGCAC	*X*:12289367..12289393	CCTGCGAATTCCCATCATCCTG	*X*:12290184..12290163
P{XP}CG42258^{d01896}	GTGTGCTATTTGGCTGACCACAG	*X*:12364084..12364106	GCTCACTCTACCGCTCCTCGCTC	*X*:12364498..12364476
P{XP}d06616	ACTTAGGCACGCGCGCCGCAGAGTG	*X*:12477049..12477073	GGTTGAAGTCTTCGCTACAGTCTC	*X*:12477408..12477385
P{EP}tomosyn^{EP1359}	CACATGATCCTTACTCGCGAACACC	*X*:12534787..12534811	GGTCGGCGTGAATAGTATAGCATAC	*X*:12535194..12535170
PBac{WH}Smx^{f02932}	CGCCCACTCATCTATCATTCGATAG	*X*:12627909..12627933	CCTTGCCAAATCCTCGGTCCTGCC	*X*:12628571..12628548
P{XP}d05563	CGCCACTATAGCAACATTGACGTTCC	*X*:12679058..12679083	CGCTTTTATTGGGACAAAGAGCTG	*X*:12679580..12679557
P{EPgy2}EY20029	GGTTCGATAACAGTGCCCGGTATGC	*X*:12790564..12790588	CTCAAATGTACTTGTGCGCGCCACCTG	*X*:12791066..12791040
P{EP}Tango13^{EP1218}	CTTAACACTATCATTGGCGCCACC	*X*:13457567..13457590	GTTGTTGCTGCCGCTAACTATTGTTGC	*X*:13458193..13458219
P{EPgy2}CG2691^{EY08204}	CTGCCGGCCACATCGTTAACCG	*X*:13518037..13518058	CGATAACAGGTTGGTGTGTTAGC	*X*:13518713..13518735
P{EPgy2}NFAT^{EY07123}	CACGGCCTGAGGTGTGCGCGTGC	*X*:13534311..13534333	CGCTTGGGCGCGAACAACTATTTGGC	*X*:13535124..13535149
Mi{ET1}mus101^{MB08064}	GTTGTTGAAAATTAATTCTAAGTCAAAGCGC	*X*:13620991..13621021	GTTGCCTAGAGCATGAGATTCC	*X*:13621561..13621582
P{EPgy2}EY00885	GAGAGTGACGCTTTCTCGCGCGC	*X*:13656333..13656355	CATTATTATCATTGCGGGCAGCTGG	*X*:13657098..13657122
PBac{RB}jub^{e03614}	GTGTGACCGCCGCGGGTTACG	*X*:13724330..13724350	CAACGACATCACGCGCACC	*X*:13725065..13725083
Mi{ET1}MB00659	GATGCGTTCGCCACATGAGGTG	*X*:14040471..14040492	GTTTGGCCTTCGTCATATACTCG	*X*:14040950..14040972
P{EPgy2}EY01770	CACCGCTGACATCATGAACGGGC	*X*:14086953..14086975	GTCTGCGAGGTTAGGTTGAATCC	*X*:14087493..14087515
PBac{RB}CG42271^{e02366}	CTCGTTTAGCAACTCCATAGATGG	*X*:14127636..14127659	CACACTCTTGCATTGCTCAGCGC	*X*:14128306..14128284
PBac{RB}na^{e04385}	GCCATGCTGACGCTGTTCGAGG	*X*:14166500..14166521	GCTGGTGAGATTCAGGCATGCGG	*X*:14167142..14167164
Mi{ET1}dpr8^{MB03631}	GATGAAAGACTAAGAGCCGGCGCG	*X*:14292263..14292286	CACGCATTCTGTGGTCTACTCCG	*X*:14293028..14293050
Mi{ET1}MB07827	CACTTTGCTCTGAACCAGAGTCG	*X*:14573279..14573301	GGTATTCCTGCTGTTTATCAGGCTCG	*X*:14573990..14574015
P{GT1}rut^{BG00139}	GGTGCTGATGACCCTTTGGCG	*X*:14703387..14703407	CGATTTGGATAGTGATGTCATGGG	*X*:14704073..14704096
P{EPgy2}CG14407^{EY04278}	GTCCTTGACACCTGCACGCAAGC	*X*:14731776..14731798	GAGGCCCAGGCCGCGACATCC	*X*:14732506..14732526
P{GT1}Flo-2^{BG00596}	CTGCACTTTCATTTAGGCCTCG	*X*:14790697..14790718	CATCAGCCATCAACCGCAACCGC	*X*:14791407..14791429
P{EPgy2}CG9009^{EY02124}	CATTACATATCGGCACTTTCCTGCG	*X*:14844078..14844102	GATTGATCGCATCTCGAAGAGC	*X*:14844703..14844724
P{EPgy2}EY07971	GACTCAAGGTGCGGCAGAAGG	*X*:14969858..14969878	GACCCTTGACTAGGCATAAATCTTGG	*X*:14970447..14970472
P{EPg}HP10680	GGCCACAATTAGCAGTAGTAGATAGC	*X*:15019331..15019356	CTCGAGCGCCTGACTACTGGC	*X*:15020111..15020131
Mi{ET1}MB01800	GGAGATCTGTAGCTTCCAGCTAGC	*X*:15154894..15154917	GAGTTCCTGTCCCGGATACCG	*X*:15155792..15155812
Mi{ET1}HDAC6^{MB06564}	GACGTGTCCGCACTTGAGGC	*X*:15232911..15232930	CTCCGCTCCGCTGTCTAGTGC	*X*:15233659..15233679
P{EP}Ahcy13^{EP1007}	CTTTGGATACGGCTGTTCAATGACC	*X*:15343811..15343835	TTCCGCCAGACTGATATCGGC	*X*:15344699..15344719

P{GT1}CG6340^{BG01111}	CGACTGCATGCGCTCCAACCCG	*X*:15378270..15378291	GCTAGATGCTTAGGATGCTGG	*X*:15379041..15379061
P{XP}CG6340^{d02850}	GAAAATTCTGTTTGTTCGGTCCGCTTGCG	*X*:15378358..15378386	CGTGCGCCTTGATCTCTCTTTCGC	*X*:15378922..15378899
PBac{WH}CG42300^{f06338}	GGCCCAGCGCATTCTGTCCGCCATG	*X*:15464027..15464051	CTAGCCATAAGCTATAGAAATGTGC	*X*:15464595..15464571
PBac{WH}CG8097^{f06511}	CGGTACCTGACACTCAGTGTGGC	*X*:15476924..15476946	CGGTCAGATCATCGTTGTTGTAAC	*X*:15477648..15477625
Mi{ET1}MB01710	CAAGATCGGCTGGCATATAACTGGC	*X*:15561084..15561108	GCACTGAGCTAGAGGGTGCGGGAG	*X*:15561590..15561567
P{EPgy2}EY01689	GAATCGGAAATCCAAGTCGTACACC	*X*:15607555..15607579	CGGCCGTTAGCTCAGAGGAGCCC	*X*:15608199..15608177
PBac{WH}Graf^{f02954}	GACGATGCATTCTTCGAACTCCAGAG	*X*:15652004..15652029	GACTGCCCACTTTTTATCGTGGCAC	*X*:15652843..15652819
P{EPgy2}Paf-AHalpha^{EY05630}	GGGATTCACATTGTCCAGCGCACCG	*X*:15678824..15678848	CAGTAACATATGTGACCGTGTAACG	*X*:15679690..15679666
P{XP}sd^{d04263}	GTCATACAAAGACACACCTCGTAAATCC	*X*:15708354..15708381	CCCACACTGCATTTACAAGCTTCTGGCC	*X*:15708953..15708926
P{EP}Gbeta13F^{EP1071}	CGGTCGAAGATACGTCTTAAGGGT	*X*:15753717..15753740	GTAGGCGCAAAACTATCGAGACTGC	*X*:15754450..15754426
PBac{WH}f07337	CGTCGGAATGTTGATGTCTGGACC	*X*:15815512..15815535	GAATGGAACTAGCGATACTCGAACC	*X*:15816137..15816161
P{EPgy2}CG9170^{EY21976}	GGGATTTGGTTAACTCAGCGGAC	*X*:15876487..15876509	GCATATTAAGCTTGTTAATCAGG	*X*:15877234..15877212
PBac{WH}CG12698^{f01404}	CCACACACCCAACTTATCGGACGAA	*X*:15899836..15899860	GGTTACGGGAAAATCCTCCTCCTTC	*X*:15900799..15900775
P{SUPor-P}Tob^{KG06291}	GGGAATTTCCTTGCAGCGCTTAGTGG	*X*:15983253..15983278	GGCATTTTGTCGCGGTGTTCGTCGC	*X*:15984029..15984053
P{SUPor-P}CG42353^{KG01000}	GCACTAACGTTCGTTCACACACACAACC	*X*:15998497..15998524	GAAATCTTGTGGTGAGCGGACG	*X*:15998942..15998963
Mi{ET1}CG3632^{MB03514}	CTATATATTGCGCACCTTGTGGAACTGC	*X*:16191223..16191250	GATCTCGCACCTGTACGGATTCG	*X*:16192006..16192028
P{EP}Cyp1^{EP1073}	CGCATTGTCATGCAATTAGTCATGG	*X*:16213821..16213845	GCATGAACGCGACCATTTATCCG	*X*:16214521..16214543
PBac{WH}nonA^{f00870}	GGGTTCCTCACGGCTAATGC	*X*:16260878..16260897	GCTCATCCGGAATACTCACCAGATTCTCG	*X*:16261565..16261593
P{GT1}BG00710	CGGTATAACTGCGAAGTAACTGC	*X*:16316402..16316424	CGTTCGCAATCGTTCCACACCATCC	*X*:16317166..16317190
P{EPgy2}EY08038	GTGCTGCTGTTTCGTTACCCG	*X*:16427074..16427094	GATGCCCGCTGTTATCCTGGCG	*X*:16427494..16427473
P{EPgy2}mbt^{EY08341}	GAAGTCTATGTTGAGAGAGAAACGG	*X*:16504426..16504450	GGATGGACAAATCGAGATGCGC	*X*:16504885..16504906
PBac{RB}r^{e02423}	GGATAACTTGATGGCGATACTAATGC	*X*:16549682..16549707	CTTTAGTACCTGTCACTCGAAAACCG	*X*:16550363..16550388
P{EPgy2}AXs^{EY00887}	CCTGACAGTGTCTTAGCTTGGCC	*X*:16577115..16577137	CGTCACGCGCCACGCAGCTGGG	*X*:16577773..16577794
PBac{WH}CG18358^{f05802}	CCAATCAATCGCGCACACACCCACG	*X*:16609614..16609638	CAACGAATGCGTACAGCTTTAACC	*X*:16610367..16610390
P{SUPor-P}mRpL22^{KG10050}	CGCGACGCGCTGTTGAGAACG	*X*:16677554..16677574	CAAGTGGATTAGAGGATTGCAGC	*X*:16678418..16678440
P{SUPor-P}CG4768^{KG09304}	GAACATAGTGATTCGTGACTGGTTCG	*X*:16685900..16685925	CCCTTCCAAGTTACGGGTTCC	*X*:16686531..16686551
P{SUPor-P}KG04053	CAAACTGCTTAACTTCACGAATATGC	*X*:16729993…16730018	CAGACAGAAGGTGGAAAGACAGGC	*X*:16730511..16730534
P{EP}EP1337	CGCGCCCCGTTATATTACATTATGC	*X*:16837341..16837365	CTCTGCTAAATTGCTAAGCTGATTTCCC	*X*:16837767..16837740
PBac{WH}CG8945^{f08057}	GGGAGTACTCACCCGACGAGC	*X*:16980041..16980061	GGCCAAATACACGTAGGAAGAAGCG	*X*:16980768..16980744
Mi{ET1}CG4991^{MB03239}	CCGACCGGAGAAGTTTAGCACC	*X*:16997281..16997302	GATTGAGCTTGGGAACGACCAGC	*X*:16997887..16997909
P{EPgy2}baz^{EY09846}	GCTCTTCCTATGAATTTCGTCGAGCTAATCC	*X*:17070780..17070810	GTTAGTTAGCTAGGCATTTATTCCGC	*X*:17071658..17071633
Mi{ET1}CG5172^{MB05660}	GATGCTGACTTGTGTTCCATGGC	*X*:17116553..17116575	GACACCACTATCCACTGCTCAACC	*X*:17117104..17117081

P{XP}Fim^{d02114}	GCTCCACGTTGAGAATATCGGCC	*X*:17185337..17185359	CGCGTTTTCTAAAGGTGTTCGTCTGCC	*X*:17185864..17185838
PBac{WH}CG8557^{f03948}	CGCTGATTATGAGGATGGCACGC	*X*:17370704..17370726	GCACCTTGTCTAATTTATGCCTCG	*X*:17371436..17371413
PBac{RB}X11L^{e03317}	CTTGTACTATCCGTTCGAATGTTGC	*X*:17493278..17493302	GCACTACTCCTTCAGCAACTCG	*X*:17493693..17493672
P{SUPor-P}CG32556^{KG01967}	GCTACGTGGTCACATAGATACACC	*X*:17575254..17575277	GTGCACACTCTCCCGAAGGCG	*X*:17575754..17575734
P{SUPor-P}KG00022	CTCACACGCGGACATTTGGAGCCG	*X*:17592431..17592454	GCGGCTTGTCCTGCTCCGAAGTTAGG	*X*:17592920..17592895
P{GT1}e(y)1^{BG00948}	CTTGGCTAGAACGTGGCGCTCCAACGG	*X*:17736655..17736629	CACTAGACGTCTGCATCGATAGTATCGC	*X*:17736155..17736182
P{SUPor-P}mnb^{KG04573}	CCGTTTTTCCAGCGGCCACACACGGC	*X*:17781089..17781114	GCCGCTATTAGCACTGGCTTGCAGG	*X*:17781523..17781499
P{EP}EP970	CGAGCATACTGCGAGCTGC	*X*:17805012..17805030	CAAATGTCACTTGCACGCCAAACGC	*X*:17805791..17805767
PBac{RB}CG7192^{e04401}	GTCAACGCAGCATCCACACACATCC	*X*:17995075..17995099	CACTATACCATAGATTCCTCAAATTGCC	*X*:17995501..17995474
Mi{ET1}MB05922	CACACTCCGCAATAACGAGTCGACCG	*X*:18067979..18068004	CACAATAATTGTTATCATCGACGTTGCC	*X*:18068543..18068516
Mi{ET1}CG33639^{MB04209}	GAATGGCCATCGGCGAGACGCAGACC	*X*:18107466..18107491	CGTTTGCCATCCAGGATTTGCTCGACC	*X*:18108028..18108002
PBac{WH}CG6179^{f08025}	GATGGCTGCACGCAGCTCTGCAGGC	*X*:18273551..18273575	CTTCTCGTTGTAGGTGTACACGGC	*X*:18274277..18274254
PBac{WH}CG32547^{f06408}	GCTGAACTTGGTCGGCACTGAAAGTCG	*X*:18352471..18352497	CCATGATCTCCCACATCCTGATCGC	*X*:18352991..18352967
P{EPgy2}Wnt5^{EY03178}	GCGATTCCATTCAAGACGATTCAGTTCG	*X*:18399005..18399032	CCGTAGGTACGTGTGAAGCTGCTTCC	*X*:18399796..18399771
P{SUPor-P}CG6461^{KG03971}	CTCTTGAGTTGCACTTTCCTAGGCC	*X*:18400752..18400776	GGCCGAACCTGTGCGCAACCATGTTG	*X*:18401285..18401260
PBac{RB}wgn^{e00637}	GCAATAAACATCGATGATATGGAGGAGG	*X*:18526115..18526142	GCTTGTTGGCTTTGATCCCGTAGTG	*X*:18526876..18526852
P{SUPor-P}KG01373	GCATTGGCATAGTAGTCGAGCAGTGC	*X*:18667730..18667755	GCGGCTGATTTGATGATTAGCGCGG	*X*:18668339..18668315
PBac{WH}CG7101^{f01197}	GGCGAAGAAGCCGGCCAAGAAGCG	*X*:18724761..18724784	GTAGCAGCAGTCCAGGGCACTGCGTTCG	*X*:18725506..18725479
P{SUPor-P}CG32541^{KG02698}	GTGCTTCGAACACGGTCCACACGGGC	*X*:18823963..18823988	CGCACCGAAGAACGGGTGGTCAAAAACCG	*X*:18824772..18824744
P{SUPor-P}RhoGAP18B^{KG00160}	CCTGACTATCGCTGTCCCTCTTCGCC	*X*:19047385..19047410	CCAGCGCGCGATCCTTCATCCTCCG	*X*:19048071..19048047
PBac{WH}Mec2^{f06342}	CAATCGTTGAGCACGGATTACCAGCC	*X*:19083868..19083893	GGCTGTTTTCTTTGTCCGCCATCGTTTAG	*X*:19084330..19084302
P{GT1}BG01439	GGACACATCATTGGGCACAGACCC	*X*:19114334..19114357	GGAACATAGATCAACCTACTACTCGC	*X*:19115069..19115044
P{EPgy2}rictor^{EY08986}	CAAGAGGAGGTGCACACATGCATGC	*X*:19153875..19153899	CGGTCGCCACAGCATAGAGAGAGCC	*X*:19154382..19154358
P{EPgy2}gfA^{EY10801}	GGCTTAAGTGGTTCTGCATTAGC	*X*:19235474..19235496	CTTCAACTGACAGGTGTGCCCGG	*X*:19236086..19236064
PBac{RB}CG32533^{e00904}	GCTCCTATCGTCACTTGAATGGG	*X*:19368749..19368771	GAAGTAGTTGGCCACGCTAGTTGGC	*X*:19369403..19369379
P{XP}l(1)G0156^{d06039}	GCTGATAAGGCCGCAAATCAGGG	*X*:19415066..19415088	CAGCAGTTGATTACGTGCTGGCC	*X*:19415616..19415594
P{EPgy2}CG14204^{EY05761}	GCCCGTGCCACTTCGCGTTCGC	*X*:19478642..19478663	GTGCTGCAGGCGCCATCTATCG	*X*:19479376..19479355
P{SUPor-P}MKP-4^{KG03420}	CTCCGCAAGCTGAAAGTACTCCGG	*X*:19516790..19516813	CGAATCCAGCGTGAGGATGTGGG	*X*:19517523..19517501
P{SUPor-P}KG05538	CCAAATACGACGTGATTCTAGGTAGG	*X*:19560935..19560960	GCCCAAAGCTGCGATACTCACCTTCC	*X*:19561674..19561649
P{EPgy2}meso18E^{EY07842}	CGTCTAGAGCGTGGCGATTGTGCG	*X*:19606268..19606291	GGAATCTCAGGACGGCGGACTACGG	*X*:19607025..19607001
P{SUPor-P}CG12703^{KG08105}	GTGATAACTTGTTCGATAGCTCTTCG	*X*:19644723..19644748	CTTCCTGTACTACAACTGGTGGTGG	*X*:19645089..19645065
P{SUPor-P}CG32529^{KG03876}	CACACGGACGGAATGGACCTCACCG	*X*:19769718..19769742	GCGATTTACACTAGTGTTGAAATTGGC	*X*:19770395..19770369

P{SUPor-P}KG10095	GCATTGCCGACCTTTGAGCTGTGC	*X*:20631444..20631467	GTTAAACCTGAATTGAGCATTGCTAGC	*X*:20632215..20632241
P{SUPor-P}KG06210	GTGGATATGGATGTGTGGCAGG	*X*:20795940..20795961	CTCCAATCCCACTTCCCTTTGTTGC	*X*:20796429..20796453
P{SUPor-P}bves^{KG09159}	GATCACTGACAGCTCAATTAGCACTG	*X*:20942269..20942294	CATTCGCACTCGAGAGCCATTGAACC	*X*:20943012..20943037
P{GT1}BG02205	CTTGCATGTCCAACAACTTTACAGCC	*X*:21075861..21075886	GTGATTGGTTGTTGTGTAAATGGGCC	*X*:21076293..21076318
P{SUPor-P}CG33713^{KG06423}	CTCGTTCTCGATCTTCTCCAGCGCGG	*X*:21189930..21189955	GCAGCTTATGCTTACTAGACATCAGCG	*X*:21190646..21190672
P{SUPor-P}slgA^{KG07965}	CCTTTGCAGCTGCAACTGTCCGAGG	*X*:21253567..21253591	CGGCTGGAAGTAAGTCTGCTCCG	*X*:21254094..21254116
P{EPgy2}EY09781	CGCACAGGTCTTGAAGGCATTGCC	*X*:21443126..21443149	GTTTCTAGCAGCCCCCTTCGCACCG	*X*:21443688..21443712
Mi{ET1}DIP1^{MB00541}	CATTGGACAAATACTCACTCTCATCAAG	*X*:21495758..21495785	CCTTTTCTGTCAAAGTGAAGAGGC	*X*:21496197..21496220
P{GT1}flam^{BG02658}	GAATATGGGACAGCTCGACTCG	*X*:21502251..21502272	CTTGCGTCCATACCGAAACG	*X*:21502999..21503018
P{Mae-UAS.6.11}CG14619^{GG01842}	CATCGCTCGCGCGCACAATCTCGGC	*X*:21858117..21858141	GTGCCGCCAGGCTGTCTACGCTCC	*X*:21858613..21858636
P{GT1}BG01274	CTAGTGTATTTGCCTTCACCATAATCG	*X*:21961730..21961756	GAGCGAGGAATATCTGATATGCGG	*X*:21962214..21962237
PBac{WH}f02323	GATGGCGGTGTCCTTGTCTCTGGC	*X*:22366703..22366726	CCTGTGACATTAATGCAGGCGACGG	*X*:22367213..22367237

TABLE S2

Inversions constructed for *Dp(1;Y)* screens

Inversion	Distal insertion	Proximal insertion	Genomic coordinates	Cytology
In(1)BSC1	*P{RS3}CB-5805-3*	*P{RS5}CG3600$^{5\text{-}HA\text{-}1598}$*	*X*:387562;1837325	1B5;2B17
In(1)BSC2	*P{RS3}CB-5805-3*	*P{RS5}5-SZ-3121*	*X*:387562;2219975	1B5;2F6
In(1)BSC24	*P{RS3}CB-5805-3*	*P{RS5}5-HA-1961*	*X*:387562;3266986	1B5;3D2
In(1)BSC3	*P{RS3}CB-5805-3*	*P{RS5}Mnt$^{5\text{-}SZ\text{-}3142}$*	*X*:387562;3583172	1B5;3E3
In(1)BSC4	*P{RS3}CB-5805-3*	*P{RS5}CG4068$^{5\text{-}SZ\text{-}3655}$*	*X*:387562;4825473	1B5;4D7
In(1)BSC5	*P{RS3}CB-5805-3*	*P{RS5}CG3125$^{5\text{-}SZ\text{-}4068}$*	*X*:387562;5641035	1B5;5B6
In(1)BSC6	*P{RS3}CB-5805-3*	*P{RS5}5-SZ-3429*	*X*:387562;5882812	1B5;5D1
In(1)BSC30[a]	*P{RS5}arg$^{5\text{-}SZ\text{-}4074}$*	*P{RS3}CB-0332-3*	*X*:417311;6589040	1B8;6C7
In(1)BSC8	*P{RS3}CB-5805-3*	*P{RS5}CG9650$^{5\text{-}HA\text{-}1616}$*	*X*:387562;7090126	1B5;7A3
In(1)BSC9	*P{RS3}CB-5805-3*	*P{RS5}sdt$^{5\text{-}SZ\text{-}3206}$*	*X*:387562;8087225	1B5;7D18
In(1)BSC10	*P{RS3}CB-5805-3*	*P{RS5}CG10962$^{5\text{-}SZ\text{-}4103}$*	*X*:387562;8924088	1B5;8C3
In(1)BSC25	*P{RS3}CB-5805-3*	*P{RS5}5-HA-1967*	*X*:387562;9580686	1B5;8F9
In(1)BSC11	*P{RS3}CB-5805-3*	*P{RS5}ras$^{5\text{-}SZ\text{-}4112}$*	*X*:387562;10638967	1B5;9E1
In(1)BSC12	*P{RS3}CB-5805-3*	*P{RS5}CG11727$^{5\text{-}SZ\text{-}3419}$*	*X*:387562;11347991	1B5;10B14
In(1)BSC13	*P{RS3}CB-5805-3*	*P{RS5}5-SZ-4084*	*X*:387562;11901120	1B5;11A1
In(1)BSC26	*P{RS3}CB-5805-3*	*P{RS5}CG4407$^{5\text{-}HA\text{-}1857}$*	*X*:387562;12797208	1B5;11D1
In(1)BSC32	*P{RS3}CB-5805-3*	*P{RS5}HDAC4$^{5\text{-}HA\text{-}2919}$*	*X*:387562;13178324	1B5;11E8
In(1)BSC14	*P{RS3}CB-5805-3*	*P{RS5}5-SZ-4073*	*X*:387562;14720102	1B5;12F4
In(1)BSC27	*P{RS3}CB-5805-3*	*P{RS5}Gmap$^{5\text{-}HA\text{-}1831}$*	*X*:387562;15392986	1B5;13C5
In(1)BSC16	*P{RS3}CB-5805-3*	*P{RS5}5-SZ-3670*	*X*:387562;15985699	1B5;14A9
In(1)BSC17	*P{RS3}CB-5805-3*	*P{RS5}r$^{5\text{-}HA\text{-}1737}$*	*X*:387562;16549850	1B5;14F5
In(1)BSC33	*P{RS3}CB-5805-3*	*P{RS5}CG4768$^{5\text{-}HA\text{-}1883}$*	*X*:387562;16686364	1B5;15A8
In(1)BSC31[a]	*P{RS3}UM-8274-3*	*P{RS5}5-HA-1765*	*X*:580813;17001665	1C2;15E3
In(1)BSC19	*P{RS3}CB-5805-3*	*P{RS5}CG32556$^{5\text{-}HA\text{-}1561}$*	*X*:387562;17576847	1B5;16C1
In(1)BSC20	*P{RS3}CB-5805-3*	*P{RS5}CG6461$^{5\text{-}HA\text{-}1134}$*	*X*:387562;18400974	1B5;17C1
In(1)BSC21	*P{RS3}CB-5805-3*	*P{RS5}CG14194$^{5\text{-}SZ\text{-}3651}$*	*X*:387562;19087625	1B5;18A7
In(1)BSC22	*P{RS3}CB-5805-3*	*P{RS5}amn$^{5\text{-}SZ\text{-}3656}$*	*X*:387562;19781188	1B5;19A2
In(1)BSC28	*P{RS3}CB-5805-3*	*P{RS5}5-HA-1907*	*X*:387562;21961315	1B5;20C3

[a]*In(1)BSC30* and *In(1)BSC31* were constructed with nonstandard insertion combinations, because standard insertions did not exist in the regions of the desired proximal breakpoints.

TABLE S3

Extents of duplicated medial segments in *Dp(1;Y)*s

Duplication	Genomic coordinates	Size of duplicated segment	Cytological breakpoints
A. *In(1)BSC2* screen[a]			
Dp(1;Y)BSC214	*X*:1841903..1903879;2219975	316 - 378 kb	2B17-2C1;2F6
Dp(1;Y)BSC215	*X*:1841903..1903879;2219975	316 - 378 kb	2B17-2C1;2F6
Dp(1;Y)BSC216	*X*:1903879..1959038;2219975	261 - 316 kb	2C1-2C8;2F6
Dp(1;Y)BSC217	*X*:1959038..2010175;2219975	210 - 261 kb	2C8-2D2;2F6
Dp(1;Y)BSC218	*X*:2010175..2069227;2219975	151 - 210 kb	2D2-2E1;2F6
Dp(1;Y)BSC219	*X*:2099704..2145248;2219975	75 - 120 kb	2E2-2F2;2F6
B. *In(1)BSC3* screen			
Dp(1;Y)BSC74	*X*:1816653..1903879;3583172	1679 - 1767 kb	2B16-C1;3E4
Dp(1;Y)BSC75	*X*:1903879..1959038;3583172	1624 - 1679 kb	2C1-8;3E4
Dp(1;Y)BSC76	*X*:2069227..2099704;3583172	1483 - 1514 kb	2E1-2;3E4
Dp(1;Y)BSC77	*X*:2302387..2483034;3583172	1100 - 1281 kb	3A2-6;3E4
Dp(1;Y)BSC78	*X*:2302387..2483034;3583172	1100 - 1281 kb	3A2-6;3E4
Dp(1;Y)BSC79	*X*:2302387..2483034;3583172	1100 - 1281 kb	3A2-6;3E4
Dp(1;Y)BSC80	*X*:2483034..2575674;3583172	1007 - 1100 kb	3A6-B1;3E4
Dp(1;Y)BSC81	*X*:2483034..2575674;3583172	1007 - 1100 kb	3A6-B1;3E4
Dp(1;Y)BSC82	*X*:2608030..2637387;3583172	946 - 975 kb	3B3-4;3E4
Dp(1;Y)BSC83	*X*:2608030..2637387;3583172	946 - 975 kb	3B3-4;3E4
Dp(1;Y)BSC84	*X*:2779902..2841198;3583172	742 - 803 kb	3C2-3;3E4
Dp(1;Y)BSC85	*X*:2779902..2841198;3583172	742 - 803 kb	3C2-3;3E4
Dp(1;Y)BSC86	*X*:2841198..2992911;3583172	590 - 742 kb	3C3-6;3E4
Dp(1;Y)BSC87	*X*:2841198..2992911;3583172	590 - 742 kb	3C3-6;3E4
Dp(1;Y)BSC88	*X*:2992911..3253742;3583172	329 - 590 kb	3C6-D2;3E4
Dp(1;Y)BSC89	*X*:3253742..3360464;3583172	223 - 329 kb	3D2-4;3E4
Dp(1;Y)BSC90	*X*:3422188..3583172;3583172	0 - 161 kb	3D5-E4;3E4
C. *In(1)BSC4* screen			
Dp(1;Y)BSC158	*X*:3736678..3843899;4825473..4825859	982 - 1089 kb	3F3-9;4D7
Dp(1;Y)BSC159	*X*:3994648..4025177;4825473..4825859	800 - 831 kb	4A5-B1;4D7
Dp(1;Y)BSC160	*X*:4025177..4186834;4825473..4825859	639 - 800 kb	4B1-5;4D7
Dp(1;Y)BSC161	*X*:4186834..4317564;4825473..4825859	508 - 639 kb	4B5-C3;4D7
Dp(1;Y)BSC162	*X*:4317564..4478617;4825473..4825859	347 - 508 kb	4C3-8;4D7
Dp(1;Y)BSC163	*X*:4478617..4539074;4825473..4825859	286 - 347 kb	4C8-10;4D7
Dp(1;Y)BSC164	*X*:4573372..4698591;4825473..4825859	127 - 252 kb	4C12-D2;4D7
Dp(1;Y)BSC165	*X*:4573372..4698591;4825473..4825859	127 - 252 kb	4C12-D2;4D7
Dp(1;Y)BSC166	*X*:4573372..4698591;4825473..4825859	127 - 252 kb	4C12-D2;4D7
Dp(1;Y)BSC167	*X*:4573372..4698591;4825473..4825859	127 - 252 kb	4C12-D2;4D7
Dp(1;Y)BSC168	*X*:4573372..4698591;4825473..4825859	127 - 252 kb	4C12-D2;4D7
Dp(1;Y)BSC169	*X*:4698591..4813070;4825473..4825859	12 - 127 kb	4D2-6;4D7

D. *In(1)BSC6* screen[b]

Dp(1;Y)BSC91	*X*:4665273..4670742;5882812	1212 - 1218 kb	4D1;5D1
Dp(1;Y)BSC92	*X*:4821917..4849103;5882812	1034 - 1061 kb	4D6-7;5D1
Dp(1;Y)BSC93	*X*:4961358..4969249;5882812	914 - 921 kb	4E2;5D1
Dp(1;Y)BSC94	*X*:4969249..5026586;5882812	856 - 914 kb	4E2-F1;5D1
Dp(1;Y)BSC95	*X*:5198244..5201097;5882812	682 - 685 kb	4F4;5D1
Dp(1;Y)BSC96	*X*:5299568..5314625;5882812	568 - 583 kb	4F9-10;5D1
Dp(1;Y)BSC97	*X*:5523433..5537021;5882812	346 - 359 kb	5A8-9;5D1
Dp(1;Y)BSC98	*X*:5559109..5565679;5882812	317 - 324 kb	5A10-11;5D1
Dp(1;Y)BSC99	*X*:5772623..5775863;5882812	107 - 110 kb	5C6;5D1

E. *In(1)BSC9* screen

Dp(1;Y)BSC172	*X*:7089520..7219606;8087225	868 - 998 kb	7A3-7B1;7D18
Dp(1;Y)BSC173	*X*:7310589..7610424;8087225	477 - 777 kb	7B2-7B6;7D18
Dp(1;Y)BSC174	*X*:7310589..7610424;8087225	477 - 777 kb	7B2-7B6;7D18
Dp(1;Y)BSC175	*X*:7310589..7610424;8087225	477 - 777 kb	7B2-7B6;7D18
Dp(1;Y)BSC176	*X*:7310589..7610424;8087225	477 - 777 kb	7B2-7B6;7D18
Dp(1;Y)BSC177	*X*:7610424..7726814;8087225	360 - 477 kb	7B6-7C1;7D18
Dp(1;Y)BSC178	*X*:7610424..7726814;8087225	360 - 477 kb	7B6-7C1;7D18
Dp(1;Y)BSC179	*X*:7849656..7954973;8087225	132 - 238 kb	7D1-7D5;7D18
Dp(1;Y)BSC180	*X*:7954973..8009881;8087225	77 - 132 kb	7D5-7D6;7D18
Dp(1;Y)BSC181	*X*:8009881..8043108;8087225	44 - 77 kb	7D6-7D16;7D18
Dp(1;Y)BSC182	*X*:8009881..8043108;8087225	44 - 77 kb	7D6-7D16;7D18
Dp(1;Y)BSC183	*X*:8043108..8071764;8087225	15 - 44 kb	7D16-7D17;7D18
Dp(1;Y)BSC184	*X*:8043108..8071764;8087225	15 - 44 kb	7D16-7D17;7D18

F. *In(1)BSC10* screen

Dp(1;Y)BSC32 [c]	*X*:7726814..7802982;8924088	1121 - 1197 kb	7C1-2;8C3
Dp(1;Y)BSC33	*X*:8023765..8086758;8924088	837 - 900 kb	7D12-18;8C3
Dp(1;Y)BSC34	*X*:8086758..8165237;8924088	759 - 837 kb	7D18-E1;8C3
Dp(1;Y)BSC35	*X*:8086758..8165237;8924088	759 - 837 kb	7D18-E1;8C3
Dp(1;Y)BSC36	*X*:8165237..8302771;8924088	621 - 759 kb	7E1-6;8C3
Dp(1;Y)BSC37	*X*:8165237..8302771;8924088	621 - 759 kb	7E1-6;8C3
Dp(1;Y)BSC38	*X*:8352348..8412006;8924088	512 - 572 kb	7E11-F2;8C3
Dp(1;Y)BSC39	*X*:8412006..8483158;8924088	441 - 512 kb	7F2-7;8C3
Dp(1;Y)BSC40	*X*:8483158..8582193;8924088	342 - 441 kb	7F7-8A2;8C3
Dp(1;Y)BSC41	*X*:8582193..8608364;8924088	316 - 342 kb	8A2;8C3
Dp(1;Y)BSC42	*X*:8582193..8608364;8924088	316 - 342 kb	8A2;8C3
Dp(1;Y)BSC43	*X*:8582193..8608364;8924088	316 - 342 kb	8A2;8C3
Dp(1;Y)BSC44	*X*:8582193..8608364;8924088	316 - 342 kb	8A2;8C3
Dp(1;Y)BSC45	*X*:8791314..8919421;8924088	5 - 133 kb	8B6-C3;8C3
Dp(1;Y)BSC46	*X*:8791314..8919421;8924088	5 - 133 kb	8B6-C3;8C3

G. *In(1)BSC25* screen

Dp(1;Y)BSC170	*X*:8483158..8582193;9580686	998 - 1098 kb	7F7-8A2;8F9
Dp(1;Y)BSC171	*X*:8582193..8608364;9580686	972 - 998 kb	8A2-8A2;8F9
Dp(1;Y)BSC144	*X*:8582193..8608364;9580686	972 - 998 kb	8A2;8F9
Dp(1;Y)BSC145	*X*:8608364..8791314;9580686	789 - 972 kb	8A2-B6;8F9
Dp(1;Y)BSC146	*X*:8608364..8791314;9580686	789 - 972 kb	8A2-B6;8F9
Dp(1;Y)BSC147	*X*:8919421..8972175;9580686	609 - 661 kb	8C3-4;8F9
Dp(1;Y)BSC148	*X*:8972175..9023264;9580686	557 - 609 kb	8C4-12;8F9
Dp(1;Y)BSC149	*X*:8972175..9023264;9580686	557 - 609 kb	8C4-12;8F9
Dp(1;Y)BSC150	*X*:9102623..9137219;9580686	443 - 478 kb	8D1-2;8F9
Dp(1;Y)BSC151	*X*:9137219..9167564;9580686	413 - 443 kb	8D2-4;8F9
Dp(1;Y)BSC152	*X*:9167564..9249724;9580686	331 - 413 kb	8D4-9;8F9
Dp(1;Y)BSC153	*X*:9167564..9249724;9580686	331 - 413 kb	8D4-9;8F9
Dp(1;Y)BSC154	*X*:9249724..9394100;9580686	187 - 331 kb	8D9-E4;8F9
Dp(1;Y)BSC155	*X*:9394100..9488176;9580686	93 - 187 kb	8E4-12;8F9
Dp(1;Y)BSC156	*X*:9488176..9579966;9580686	1 - 93 kb	8E12-F9;8F9

H. *In(1)BSC11* screen

Dp(1;Y)BSC58	*X*:9249724..9394100;10638967	1245 - 1389 kb	8D9-E4;9E2
Dp(1;Y)BSC59	*X*:9979553..10112413;10638967	527 - 659 kb	9B1-4;9E2
Dp(1;Y)BSC60	*X*:10225396..10279580;10638967	359 - 414 kb	9B7-14;9E2
Dp(1;Y)BSC61	*X*:10225396..10279580;10638967	359 - 414 kb	9B7-14;9E2
Dp(1;Y)BSC62	*X*:10354712..10495487;10638967	143 - 284 kb	9C4-D4;9E2
Dp(1;Y)BSC63	*X*:10354712..10495487;10638967	143 - 284 kb	9C4-D4;9E2
Dp(1;Y)BSC64	*X*:10495487..10632983;10638967	6 - 143 kb	9D4-E1;9E2
Dp(1;Y)BSC65	*X*:10495487..10632983;10638967	6 - 143 kb	9D4-E1;9E2
Dp(1;Y)BSC66	*X*:10495487..10632983;10638967	6 - 143 kb	9D4-E1;9E2

I. *In(1)BSC12* screen[a]

Dp(1;Y)BSC220	*X*:9889597..9979553;11347991	1368 - 1458 kb	9A4-9B1;10B14
Dp(1;Y)BSC221	*X*:10354712..10495487;11347991	853 - 993 kb	9C4-9D4;10B14
Dp(1;Y)BSC222	*X*:11244026..11303997;11347991	44 - 104 kb	10B3-10B10;10B14

J. *In(1)BSC13* screen

Dp(1;Y)BSC47	*X*:11219857..11244026;11901120	657 - 681 kb	10B3;11A1
Dp(1;Y)BSC48	*X*:11244026..11303997;11901120	597 - 657 kb	10B3-10;11A1
Dp(1;Y)BSC49	*X*:11303997..11347096;11901120	554 - 597 kb	10B10-13;11A1
Dp(1;Y)BSC50	*X*:11347096..11451050;11901120	450 - 554 kb	10B13-C5;11A1
Dp(1;Y)BSC51	*X*:11451050..11474221;11901120	427 - 450 kb	10C5-7;11A1
Dp(1;Y)BSC52	*X*:11451050..11474221;11901120	427 - 450 kb	10C5-7;11A1
Dp(1;Y)BSC53	*X*:11474221..11600469;11901120	301 - 427 kb	10C7-D5;11A1
Dp(1;Y)BSC54	*X*:11474221..11600469;11901120	301 - 427 kb	10C7-D5;11A1
Dp(1;Y)BSC55	*X*:11600469..11670262;11901120	231 - 301 kb	10D5-E2;11A1
Dp(1;Y)BSC56	*X*:11600469..11670262;11901120	231 - 301 kb	10D5-E2;11A1

Dp(1;Y)BSC57	*X*:11900875..11901120;11901120	0 - 0 kb	11A1;11A1

K. *In(1)BSC26* screen

Dp(1;Y)BSC100	*X*:11347096..11451050;12797208	1346 - 1450 kb	10B14-C5;11D1
Dp(1;Y)BSC101	*X*:11451050..11474221;12797208	1323 - 1346 kb	10C5-7;11D1
Dp(1;Y)BSC102	*X*:11474221..11600469;12797208	1197 - 1323 kb	10C7-D5;11D1
Dp(1;Y)BSC103	*X*:11474221..11600469;12797208	1197 - 1323 kb	10C7-D5;11D1
Dp(1;Y)BSC104	*X*:11775943..11814681;12797208	983 - 1021 kb	10F3-7;11D1
Dp(1;Y)BSC105	*X*:11775943..11814681;12797208	983 - 1021 kb	10F3-7;11D1
Dp(1;Y)BSC106	*X*:11814681..11900970;12797208	896 - 983 kb	10F7-11A1;11D1
Dp(1;Y)BSC107	*X*:12001848..12289367;12797208	508 - 795 kb	11A4-9;11D1
Dp(1;Y)BSC108	*X*:12001848..12289367;12797208	508 - 795 kb	11A4-9;11D1
Dp(1;Y)BSC109	*X*:12001848..12289367;12797208	508 - 795 kb	11A4-9;11D1
Dp(1;Y)BSC110	*X*:12001848..12289367;12797208	508 - 795 kb	11A4-9;11D1
Dp(1;Y)BSC111	*X*:12001848..12289367;12797208	508 - 795 kb	11A4-9;11D1
Dp(1;Y)BSC112	*X*:12001848..12289367;12797208	508 - 795 kb	11A4-9;11D1
Dp(1;Y)BSC113	*X*:12289367..12364084;12797208	433 - 508 kb	11A9-11;11D1
Dp(1;Y)BSC114	*X*:12289367..12364084;12797208	433 - 508 kb	11A9-11;11D1
Dp(1;Y)BSC115	*X*:12289367..12364084;12797208	433 - 508 kb	11A9-11;11D1
Dp(1;Y)BSC116	*X*:12289367..12364084;12797208	433 - 508 kb	11A9-11;11D1
Dp(1;Y)BSC117	*X*:12289367..12364084;12797208	433 - 508 kb	11A9-11;11D1
Dp(1;Y)BSC118	*X*:12289367..12364084;12797208	433 - 508 kb	11A9-11;11D1
Dp(1;Y)BSC119	*X*:12364084..12477049;12797208	320 - 433 kb	11A11-B1;11D1
Dp(1;Y)BSC120	*X*:12477049..12534787;12797208	262 - 320 kb	11B1-7;11D1
Dp(1;Y)BSC121	*X*:12477049..12534787;12797208	262 - 320 kb	11B1-7;11D1
Dp(1;Y)BSC122	*X*:12477049..12534787;12797208	262 - 320 kb	11B1-7;11D1
Dp(1;Y)BSC123	*X*:12534787..12627909;12797208	169 - 262 kb	11B7-14;11D1
Dp(1;Y)BSC124	*X*:12534787..12627909;12797208	169 - 262 kb	11B7-14;11D1
Dp(1;Y)BSC125	*X*:12679058..12790564;12797208	7 - 118 kb	11C2-D1;11D1
Dp(1;Y)BSC126	*X*:12679058..12790564;12797208	7 - 118 kb	11C2-D1;11D1
Dp(1;Y)BSC127	*X*:12679058..12790564;12797208	7 - 118 kb	11C2-D1;11D1
Dp(1;Y)BSC128	*X*:12679058..12790564;12797208	7 - 118 kb	11C2-D1;11D1

L. *In(1)BSC14* screen

Dp(1;Y)BSC185	*X*:13457567..13518037;14720102	1202 - 1263 kb	12A4-12A9;12F4
Dp(1;Y)BSC186	*X*:13656333..13724330;14720102	996 - 1064 kb	12C1-12C6;12F4
Dp(1;Y)BSC187	*X*:13724330..14040471;14720102	680 - 996 kb	12C6-12E2;12F4
Dp(1;Y)BSC188	*X*:13724330..14040471;14720102	680 - 996 kb	12C6-12E2;12F4
Dp(1;Y)BSC189	*X*:13724330..14040471;14720102	680 - 996 kb	12C6-12E2;12F4
Dp(1;Y)BSC190	*X*:13724330..14040471;14720102	680 - 996 kb	12C6-12E2;12F4
Dp(1;Y)BSC191	*X*:14040471..14086953;14720102	633 - 680 kb	12E2-12E3;12F4
Dp(1;Y)BSC192	*X*:14166500..14292263;14720102	428 - 554 kb	12E7-12E9;12F4
Dp(1;Y)BSC193	*X*:14166500..14292263;14720102	428 - 554 kb	12E7-12E9;12F4
Dp(1;Y)BSC194	*X*:14292263..14573279;14720102	147 - 428 kb	12E9-12F2;12F4

Dp(1;Y)BSC195	*X*:14292263..14573279;14720102	147 - 428 kb	12E9-12F2;12F4
Dp(1;Y)BSC196	*X*:14573279..14703387;14720102	17 - 147 kb	12F2-12F4;12F4
Dp(1;Y)BSC197	*X*:14573279..14703387;14720102	17 - 147 kb	12F2-12F4;12F4
Dp(1;Y)BSC198	*X*:14573279..14703387;14720102	17 - 147 kb	12F2-12F4;12F4
Dp(1;Y)BSC199	*X*:14573279..14703387;14720102	17 - 147 kb	12F2-12F4;12F4

M. *In(1)BSC27* screen[a]

Dp(1;Y)BSC230	*X*:13724330..14040471;15392986	1353 - 1669 kb	12C6-12E3;13C5

N. *In(1)BSC16* screen[a]

Dp(1;Y)BSC223	*X*:15378358..15464027;15985699	522 - 607 kb	13C5-13D3;14A9
Dp(1;Y)BSC224	*X*:15378358..15464027;15985699	522 - 607 kb	13C5-13D3;14A9
Dp(1;Y)BSC225	*X*:15464027..15476924;15985699	509 - 522 kb	13D3-13D4;14A9
Dp(1;Y)BSC226	*X*:15678824..15708354;15985699	277 - 307 kb	13E18-13F1;14A9
Dp(1;Y)BSC227	*X*:15815512..15876487;15985699	109 - 170 kb	14A1-14A5;14A9

O. *In(1)BSC17* screen[a]

Dp(1;Y)BSC228	*X*:15876487..15899836;16549850	650 - 673 kb	14A5-14A6;14F5
Dp(1;Y)BSC229	*X*:15998497..16191223;16549850	359 - 551 kb	14A9-14B9;14F5
Dp(1;Y)BSC230	*X*:16427074..16504426;16549850	45 - 123 kb	14E1-14F2;14F5

P. *In(1)BSC19* screen

Dp(1;Y)BSC200	*X*:16427074..16504426;17576847	1072 - 1150 kb	14E1-14F2;16C1
Dp(1;Y)BSC201	*X*:16577115..16609614;17576847	967 - 1000 kb	15A1-15A3;16C1
Dp(1;Y)BSC202	*X*:16729993..16837341;17576847	740 - 847 kb	15A11-15C4;16C1
Dp(1;Y)BSC203	*X*:16837341..16980041;17576847	597 - 740 kb	15C4-15E1;16C1
Dp(1;Y)BSC204	*X*:16837341..16980041;17576847	597 - 740 kb	15C4-15E1;16C1
Dp(1;Y)BSC205	*X*:16837341..16980041;17576847	597 - 740 kb	15C4-15E1;16C1
Dp(1;Y)BSC206	*X*:16997281..17070780;17576847	506 - 580 kb	15E3-15F1;16C1
Dp(1;Y)BSC207	*X*:17116553..17185337;17576847	392 - 460 kb	15F4-15F9;16C1
Dp(1;Y)BSC208	*X*:17185337..17370704;17576847	206 - 392 kb	15F9-16B1;16C1
Dp(1;Y)BSC209	*X*:17185337..17370704;17576847	206 - 392 kb	15F9-16B1;16C1
Dp(1;Y)BSC210	*X*:17370704..17493278;17576847	84 - 206 kb	16B1-16B7;16C1
Dp(1;Y)BSC211	*X*:17493278..17575254;17576847	2 - 84 kb	16B7-16C1;16C1
Dp(1;Y)BSC212	*X*:17493278..17575254;17576847	2 - 84 kb	16B7-16C1;16C1
Dp(1;Y)BSC213	*X*:17493278..17575254;17576847	2 - 84 kb	16B7-16C1;16C1

Q. *In(1)BSC20* screen

Dp(1;Y)BSC67	*X*:17116553..17185337;18400974	1216 - 1284 kb	15F4-9;17C1
Dp(1;Y)BSC68	*X*:17592431..17736655;18400974	664 - 809 kb	16C1-E1;17C1
Dp(1;Y)BSC69	*X*:17805012..17995075;18400974	406 - 596 kb	16F2-6;17C1
Dp(1;Y)BSC157	*X*:17805012..17995075;18400974	406 - 596 kb	16F2-6;17C1
Dp(1;Y)BSC70	*X*:18067979..18107466;18400974	294 - 333 kb	17A1-2;17C1
Dp(1;Y)BSC71	*X*:18107466..18273551;18400974	127 - 294 kb	17A2-8;17C1

Dp(1;Y)BSC72	*X*:18273551..18352471;18400974	49 - 127 kb	17A8-B3;17C1
Dp(1;Y)BSC73	*X*:18273551..18352471;18400974	49 - 127 kb	17A8-B3;17C1

R. *In(1)BSC21* screen

Dp(1;Y)BSC11	*X*:17995075..18067979;19087625	1020 - 1093 kb	16F6-17A1;18A7
Dp(1;Y)BSC12	*X*:17995075..18067979;19087625	1020 - 1093 kb	16F6-17A1;18A7
Dp(1;Y)BSC13	*X*:18107466..18273551;19087625	814 - 980 kb	17A2-8;18A7
Dp(1;Y)BSC14	*X*:18107466..18273551;19087625	814 - 980 kb	17A2-8;18A7
Dp(1;Y)BSC15	*X*:18273551..18352471;19087625	735 - 814 kb	17A8-B3;18A7
Dp(1;Y)BSC16	*X*:18400752..18526115;19087625	562 - 687 kb	17C1-6;18A7
Dp(1;Y)BSC17	*X*:18526115..18667730;19087625	420 - 562 kb	17C6-D4;18A7
Dp(1;Y)BSC18	*X*:18526115..18667730;19087625	420 - 562 kb	17C6-D4;18A7
Dp(1;Y)BSC19	*X*:18526115..18667730;19087625	420 - 562 kb	17C6-D4;18A7
Dp(1;Y)BSC20	*X*:18667730..18724761;19087625	363 - 420 kb	17D4-E1;18A7
Dp(1;Y)BSC21	*X*:18667730..18724761;19087625	363 - 420 kb	17D4-E1;18A7
Dp(1;Y)BSC22	*X*:18724761..18823963;19087625	264 - 363 kb	17E1-F3;18A7
Dp(1;Y)BSC23	*X*:18724761..18823963;19087625	264 - 363 kb	17E1-F3;18A7
Dp(1;Y)BSC24	*X*:18724761..18823963;19087625	264 - 363 kb	17E1-F3;18A7
Dp(1;Y)BSC25	*X*:18823963..19047385;19087625	40 - 264 kb	17F3-18A3;18A7
Dp(1;Y)BSC26	*X*:18823963..19047385;19087625	40 - 264 kb	17F3-18A3;18A7
Dp(1;Y)BSC27	*X*:18823963..19047385;19087625	40 - 264 kb	17F3-18A3;18A7
Dp(1;Y)BSC28	*X*:18823963..19047385;19087625	40 - 264 kb	17F3-18A3;18A7
Dp(1;Y)BSC29	*X*:18823963..19047385;19087625	40 - 264 kb	17F3-18A3;18A7
Dp(1;Y)BSC30	*X*:19047385..19083868;19087625	4 - 40 kb	18A3-6;18A7
Dp(1;Y)BSC31	*X*:19047385..19083868;19087625	4 - 40 kb	18A3-6;18A7

S. *In(1)BSC22* screen

Dp(1;Y)BSC129	*X*:18400752..18526115;19781188	1255 - 1380 kb	17C1-6;19A2
Dp(1;Y)BSC130	*X*:18823963..19047385;19781188	734 - 957 kb	17F3-18A3;19A2
Dp(1;Y)BSC131	*X*:18823963..19047385;19781188	734 - 957 kb	17F3-18A3;19A2
Dp(1;Y)BSC132	*X*:18823963..19047385;19781188	734 - 957 kb	17F3-18A3;19A2
Dp(1;Y)BSC133	*X*:19047385..19083868;19781188	697 - 734 kb	18A3-6;19A2
Dp(1;Y)BSC134	*X*:19153875..19235474;19781188	546 - 627 kb	18B6-C2;19A2
Dp(1;Y)BSC135	*X*:19153875..19235474;19781188	546 - 627 kb	18B6-C2;19A2
Dp(1;Y)BSC136	*X*:19235474..19368749;19781188	412 - 546 kb	18C2-7;19A2
Dp(1;Y)BSC137	*X*:19516790..19560935;19781188	220 - 264 kb	18D7-13;19A2
Dp(1;Y)BSC138	*X*:19516790..19560935;19781188	220 - 264 kb	18D7-13;19A2
Dp(1;Y)BSC139	*X*:19560935..19606268;19781188	175 - 220 kb	18D13-E3;19A2
Dp(1;Y)BSC140	*X*:19606268..19644723;19781188	136 - 175 kb	18E3-F1;19A2
Dp(1;Y)BSC141	*X*:19769718..19781188;19781188	0 - 11 kb	18F4-19A2;19A2
Dp(1;Y)BSC142	*X*:19769718..19781188;19781188	0 - 11 kb	18F4-19A2;19A2
Dp(1;Y)BSC143	*X*:19769718..19781188;19781188	0 - 11 kb	18F4-19A2;19A2

[a]Screen still in progress.

[b]*Dp(1;Y)*s derived from *In(1)BSC6* were characterized by CGH microarrays

[c]*Dp(1;Y)BSC32* had low fertility due to hyperploidy effects and was not maintained in culture. Other *Dp(1;Y)* chromosomes that could not be maintained were not named.

TABLE S4

***Dp(1;Y)*s with duplicated basal *X* genes**

Duplication	Genomic Coordinates	Cytological breakpoints
Dp(1;Y)BSC21	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC22	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC29	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC30	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC31	*X*:20631444..20795940;het	19E2-19E5;*X* het
Dp(1;Y)BSC39	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC43	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC47	*X*:21189930..21253567;het	19F4-20A1;*X* het
Dp(1;Y)BSC52	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC53	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC54	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC60	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC64	*X*:21075861..21189930;het	19F2-19F4;*X* het
Dp(1;Y)BSC71	*X*:21075861..21189930;het	19F2-19F4;*X* het
Dp(1;Y)BSC72	*X*:20795940..20942269;het	19E5-19E7;*X* het
Dp(1;Y)BSC85	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC90	*X*:20942269..21075861;het	19E7-19F2;*X* het
Dp(1;Y)BSC100	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC105	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC109	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC118	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC120	*X*:21189930..21253567;het	19F4-20A1;*X* het
Dp(1;Y)BSC136	*X*:21858117..21961730;het	20C1-20C3;*X* het
Dp(1;Y)BSC140	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC143	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC149	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC152	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC156	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC169	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC194	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC195	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC203	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC205	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC208	*X*:21443126..21858117;het	20A3-20C1;*X* het
Dp(1;Y)BSC220	*X*:21443126..21858117;het	20A3-20C1;*X* het