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# An efficient mutagenesis screen to generate duplications of polytene section 8 on the X chromosome of *Drosophila melanogaster*.

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## SUMMARY

Duplications of the X chromosome are invaluable tools for conducting detailed genetic analysis of X chromosome genes. Appropriate duplications are essential for defining complementation groups within a given interval. Although 77% of the euchromatic X chromosome is covered by duplications, several regions remain devoid of these tools. Interval 8B is one such region, which prompted us to design a method to create a duplication that was specific to this segment. We initiated a genetic screen to isolate duplications that covered the 8B region. Our strategy utilized an X-Y translocation and an attached X-Y chromosome to generate viable pieces of the X chromosome, connected to a complete Y chromosome. Our results demonstrate an efficient method for generating duplications of the X chromosome and describe five new duplications that span region 8B. Two of these duplications bridge the gap between previously described duplications, while the other three cover only a portion of these segments. These new duplications provide important tools that will permit a systematic genetic analysis of the 8B interval to be conducted.

## 1. INTRODUCTION

Screening for lethal mutations on the X chromosome requires that the region of interest be covered by a duplication that resides elsewhere within the genome. These duplicated segments enable lethal mutations to be carried in hemizygous males that possess only one X chromosome. By carrying mutations in males, complementation tests can then be performed to facilitate the characterization of alleles within a given interval. Unfortunately, regions that lack appropriate duplications cannot be genetically dissected, because complementation analysis is not possible. Thus, the availability of appropriate duplicated segments is essential for conducting detailed genetic analysis of X chromosome genes. Studies have shown that approximately 77% of the euchromatic X chromosome is covered by duplications (Hilliker *et al.*, 1980; Eberl *et al.*, 1992).

One region which is devoid of available duplications is the 8B interval. Our interest in this region initiated with the observation that a cDNA sequence that we had identified, termed *dlim1*, mapped to this segment. The results of which will be presented elsewhere (Lilly *et al.*, 1999, submitted). To our knowledge all pre-existing duplications that spanned this segment had been lost and were unavailable for our use. As a consequence, to carry out a genetic analysis of the region we were compelled to generate a duplication that covered the 8B interval. We designed a screen to create a X chromosome duplication on a complete Y. Y-linked X chromosome duplications are particularly useful because they segregate from the X chromosome in males and facilitate complementation analysis.

The strategy that we used was based on the method of Brosseau *et al.*, (1960), in which they described the generation of Y chromosomes bearing specific sections of the X chromosome. The design

of their screen was dependent upon the fulfillment of two requirements. First, that the translocations used had breakpoints near the region of interest. Secondly, that the irradiated translocations retained small enough fragments to prevent hyperploidy. Using this as a framework, our screen took advantage of a pre-existing X-Y translocation with breakpoints near 8B. By inducing a recombination event between our translocation and an attached X-Y chromosome, we provided an intact Y chromosome. The complete Y chromosome supplied the neccessary elements for proper segregation and fertility. Using the recombined version of the translocation chromosome, we then induced breaks by γ rays and screened for viable males. Viable males with potential 8B duplications were first screened for their ability to complement *oc[1]* in 8A, and subsequently with neighboring alleles.

From our screen we generated five duplications that complemented *oc[1]* and extended into region 8B. Our results describe an efficient method for generating viable pieces of the X chromosome attached to a complete Y chromosome. The strategy we used allowed us to screen very few flies to recover several individual chromosomes with the region of interest. The duplications that were generated cover segments spanning 8B, a region which had previously lacked available duplications. These new duplications provide additional useful tools for the genetic analysis of genes that reside within this interval. These tools will allow for a systematic complementation analysis to be achieved, and facilitate the characterization of genes within region 8B.

## 2. MATERIALS AND METHODS

(i) Fly stocks

All fly strains were maintained on standard cornmeal, molasses, yeast, and agar medium. Detailed description of most stocks can be found in Lindsley and Zimm (1992) and/or in Flybase. Stocks used in this study were, *y[1] btd[1]/FM7c; y[1] lz[89d18-15] f[1]/Dp(1;Y)lz; ct[n] oc[1]/FM1;* and *lz[K];* obtained from Bloomington stock center. Stocks, *FM4, w[1] B[+]/T(1;Y)156, y[1] B[S]; 0/C(1)M4, y[2]/XYL-YS, y[2] su[wa] wa; C(1)RM, y/XYL-YS, y[2] su[wa] wa; 0/C(1)RM, y[1] v[1] bb[**]/C(1;Y)129-16, y[2] y[+] su(w[a]) w[a];* and *w[1] otd[1]/FM7c* were obtained from Mid-American stock center. *C(1)DX, y[1] w[1] f[1]; Dp(1;2)FN107/bw[D]* was provided by Robert Finkelstein. The *Nrg* allele, *In(1)RA35/FM7c*, was obtained from Corey Goodman. New chromosomes generated in this study are diagrammed in Figures 1 and 3.

(ii) Mutagenesis screen

For the duplication screen, 2100 males of genotype, *XYL-YS, y[+] T(1;Y)156, y[1] B[S]* carrying the newly recombined translocation, diagrammed in Figure 1, were subjected to 3500 rad of gamma irradiation (Gammacell-1000). The irradiated males were allowed to recover for eight hours, and then mated *en masse* to *y[1] w[1]* females for 72 hours, after which the males were discarded. Females were allowed to lay eggs for another 96 hours, 7 days in total. Individual F1 viable male progeny from this cross, that possessed at least one of the Y chromosome markers, *B[S]* and *y[+]*, were crossed to three females of genotype, *ct[n] oc[1]/FM1*. F2 male progeny from this cross carrying the *ct[n] oc[1]* chromosome and a putative duplication on the Y, were screened for the presence of ocelli, indicating the duplication contained X chromosome material that rescued the locus. The F2 males carrying duplications that rescued the *oc* phenotype were established as balanced stocks. Duplications were maintained using the compound chromosome *C(1)DX, y[1] w[1] f[1]* in females, and an embryonic lethal allele, *otd[1]* in males.

(iii) Complementation tests

Complementation tests to map the breakpoints of the duplications were conducted similar to those for the *oc[1]* allele in Figure 2. Males carrying the newly generated duplications *Dp(1;Y)* and *otd[1]* were crossed to females carrying the allele of interest, and an X chromosome balancer. The ability of the duplications to complement lethal alleles was determined by the presence of male progeny that carried markers for the duplication, *y[+] B[S]*, and for the allele of interest. For the viable *lz[K]* allele, all males were screened for the rescue of the rough eye lozenge phenotype. For each cross greater than 100 progeny were scored.

## 3. RESULTS

(i) Generation of a recombined X-Y translocation

The goal of the duplication screen was to generate a piece of the X chromosome that contained region 8B attached to a complete Y chromosome. An entire Y chromosome was an essential requirement to provide all the fertility genes and the Y centromere for proper segregation. We utilized the availability of a previously described X-Y translocation stock that had breakpoints near region 8B. This provided an advantage to creating a viable piece of the X chromosome with breakpoints near the region 8B. We chose X-Y translocation *T(1;Y)156* (Lindsley and Zimm, 1992) that has the long arm of the Y chromosome attached to the X chromosome portion 7D-20 to the centromere, while the short arm of the Y chromosome is attached to X chromosome portion 1A-7C.

The starting translocation is shown in Figure 1A. Since our interest was in the 8B region we sought to place the portion of the X chromosome 7D-20 containing 8B on to a complete Y chromosome. To do this we recombined the sequences of the translocation *T(1;Y)156* with that of an attached X-Y chromosome, *XYL-YS 129-16, y[2] y[+] su(w[a]) w[a]* (Figure 1B). By doing so, we generated a translocation that contains the original short arm of the Y chromosome attached to X chromosome 1-7C, and a newly recombined chromosome. The recombined chromosome contained the original long arm of the Y chromosome attached to X chromosome 7D-20, but was now joined to a complete Y chromosome from the attached X-Y chromosome (Figure 1E). The newly recombined translocation was designated,

Table 1. Results of the duplication screen

| | | |
|---|---|---|
| Number of irradiated males | 2100 | |
| Number of males recovered | 1608 | |
| $y^1w^1$ | 1113 | |
| $y^1w^1B^S$ | 329 | 495 |
| $y^+w^1$ | 56 | |
| $y^+w^1B^S$ | 110 | |
| Number of males crossed to $oc^1$ | 495 | |
| Number of fertile males | 153 | |
| Number that rescued $oc^1$ | 5 | |

Recovered males were catagorized based on *y* and *B* markers. Only males with a $y^+$ and/or $B^S$ marker were tested to complement $oc^1$.

Table 2. Complementation tests

| | | Alleles | | | | |
|---|---|---|---|---|---|---|
| | | *nrg* | *oc* | *dlim-1* | *lz* | *btd* |
| Duplications | *Dp(1;Y)578* | − | + | + | − | − |
| | *Dp(1;Y)619* | − | + | + | − | − |
| | *Dp(1;Y)850* | − | + | + | − | − |
| | *Dp(1;Y)867* | − | + | + | + | − |
| | *Dp(1;Y)921* | − | + | + | + | − |

Complementation tests with the new duplications. (-) duplication fails to complement allele. (+) duplication complements the allele.

*XYL-YS, y[+] T(1;Y)156, y[1] B[S]*. Each recombined translocation was established as an individual stock using a compound chromosome *C(1)M4, y[2]*. Because the two pieces of the translocation segregated independently, the stock consisted of females with the compound chromosome *C(1)M4* and *Y[S]* with X chromosome 1-7C. The males of this stock contained both portions of the X-Y translocation (Figure 1E). All other combinations of these three chromosomes resulted in non-viable progeny which permitted the stock to be balanced. The females of this stock, containing the compound chromosome and a part of the translocation required additional care to maintain. Presumably the combination of the translocation segment 1-7C, and the compound chromosome had an adverse affect on their viability. With the generation of the recombined translocation we had the primary tool that we needed for the production of duplications that covered our region of interest, 8B.

(ii) Mutagenesis screen for X chromosome duplications

Our mutagenesis screen was designed to induce double strand breaks in the newly recombined chromosome, that would generate viable pieces of the translocation in males that possessed an additional X chromosome. By using a translocation that had breakpoints near region 8B, we essentially predetermined the distal breakpoint. Therefore, our screen was simplified by having only to generate a proximal breakpoint that was small enough to be compatible with viability. Since the two pieces of the translocation segregated independently from one another, it allowed us to screen by markers for viable males that carried the portion of the translocation that contained region 8B. Males that retained too much of the translocation would not survive as a consequence of hyperploidy (Patterson *et al.*, 1937). Thus, only males that had lost a considerable part of the X chromosome material would be viable. This provided us with a powerful and efficient screen by eliminating all males that retained unuseable translocations.

The design of the screen is diagrammed in Figure 2. Males carrying the translocation were subjected to γ irradiation, allowed to recover and crossed to *y[1] w[1]* females. By discarding the irradiated males after 72 hours we insured that the resulting progeny would be derived from irradiated sperm. As expected, the majority of the progeny recovered were males. Table 1 summarizes the results of the mutagenesis screen. 2100 irradiated males produced 1608 viable males, possessing four different phenotypes. Only 15 females were recovered, and were probably a result of low frequency non-disjunction from the parents. The four phenotypic groups of the males recovered (Table 1), reflected the probable outcomes resulting from chromosome breaks due to the γ rays. As shown in Figure 2 the dominant markers, *y[+]* and *B[S]* were transmitted from the Y portion of the recombinant translocation. Males that lacked both of these markers made up 69% of the viable progeny. The absense of these markers in viable males suggested that these flies had recieved translocations that had lost significant portions of the chromosome, or lacked it altogether. The three other phenotypes, making up 31% of the progeny, contained one or both of the dominant markers, *y[+]* and *B[S]*. However as a consequence of hyperploidy, these males must have lost most the X chromosome to be viable. Surprisingly, no males were recovered that retained the *w[+]* marker of the smaller translocation. The reason for this is unclear; however, its proximity to the tip of the X may have reduced the number events which would allow for its transmission.

The males that displayed one or more of the translocation markers with which the 8B region segregated with, *y[+]* and *B[S]*, shown in Figure 2C were crossed to *oc[1]* females. 495 males were crossed to *oc[1]* females, of which only 153 were fertile and produced progeny. From the 153 crosses, five individual lines rescued the *oc[1]* phenotype and were presumed to contain a duplication of the 8A1 region of the X chromosome. Four of these retained the *y[+]* and *B[S]* markers, and were designated, *Dp(1;Y)578, Dp(1;Y)619, Dp(1;Y)867,* and *Dp(1;Y)921, w*hile *Dp(1;Y)850* retained only the *y[+]*

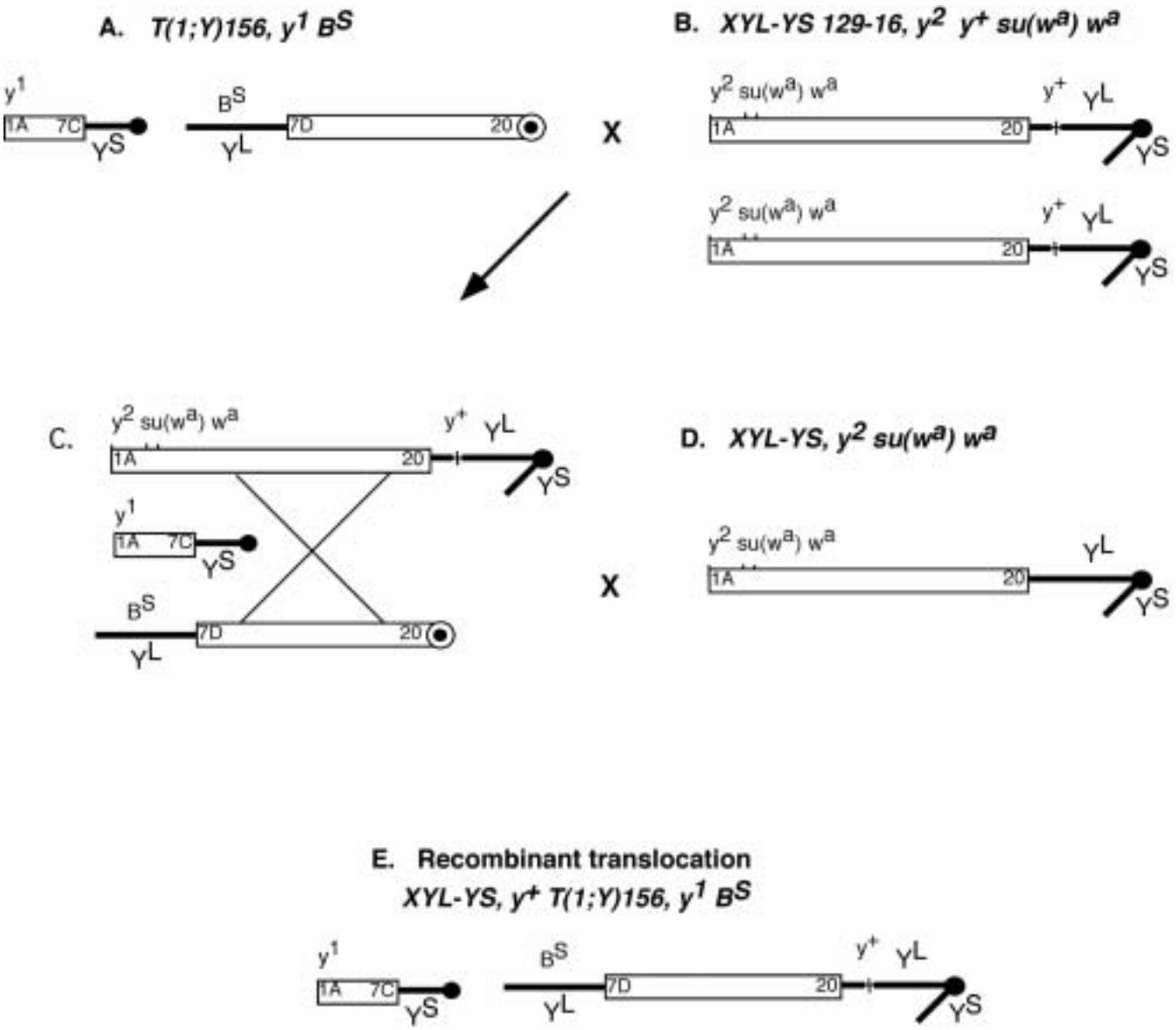


Figure 1. Generation of a recombined translocation chromosome. Open boxes represent X chromosome material and thick black lines represent Y chromosomes. (A) Males carrying translocation *T(1;Y)156, y[1] B[S]* were crossed to females (B), *XYL-YS 129-16, y[2] y[+] su(w[a]) w[a],* having two attached X-Y chromosomes, to generate females, with an attached X-Y and the translocation (C). These females, from which the recombination event was designed to occur, were crossed to males (D), *XYL-YS, y[2] su(w[a]) w[a],* an attached X-Y chromosome. Recombination events were identified by the presence of *y[+]* and *B[S]* markers segregating together in males (E).

marker and lacked *B[S]*. Males with duplications that rescued *oc[1]* were maintained as stocks over a lethal *oc* allele, *otd[1]* in males and a compound X chromosome, *C(1)DX* in females. To verify the regions that these duplications covered, the five duplications were mapped by complementation tests using surrounding alleles outlined in Table 2.

(iii) Complementation tests of duplications

In generating duplicated segments that complemented the *oc[1]* allele, we had predicted that the neighboring region, 8B would be included in one or more of these new duplications. To determine if the 8B region was covered, and to define the breakpoints of these duplications, we carried out complementation tests using alleles that surrounded the *oc* locus at region 8A. Because of the lack of duplications that span 8B-C, few alleles exist that have been cytologically mapped to this region. For purposes of clarity we used only well characterized alleles whose cytological locations had been verified by molecular means for our complementation analysis. Table 2 outlines the results of the complementation tests. The most distal and most proximal alleles that failed to complement these duplications are shown from left to right, respectively. Figure 3 illustrates the mapping data of the five new duplications. Shown are two previously described duplications that flank the 8B region. *Dp(1;2)FN107*, covers the X chromosome region 7A8-8A5 (Craymer and Roy, 1980), and *Dp(1;Y)lz* spans region 8D(7-9)-9A(4-5) (Santamaria and Randsholt, 1995).

The new duplications span the gap that was previously not covered by any available duplications. All five duplications have a distal breakpoint that lies between *Nrg* at 7F1, and *oc* located at 8A1. This is not surprising, considering the original translocation had a breakpoint that was reported to be near 7D (Lindsley and Zimm, 1992). The duplications overlap *Dp(1;2)FN107* and fill the gap that had existed within this interval. The proximal breakpoints of these five duplications vary to a much greater degree. Two duplications *Dp(1;Y)867* and *Dp(1;Y)921* complement *lz*, and thus overlap with the previously described duplication *Dp(1;Y)lz*. Neither of these two duplications complement the *btd* allele, indicating that their breakpoints lie between the interval 8D(8-9) and 9A1. The three other duplications, *Dp(1;Y)578*, *Dp(1;Y)619*, and *Dp(1;Y)850*, failed to complement the *lz* allele. The most proximal allele tested, for which these duplications complement is a newly described gene, *dlim1*, (Lilly *et al*., 1999, submitted) which has been mapped to region 8B(1-2) by cytology and deficiency analysis. Thus, the proximal breakpoint of these duplications lies somewhere between 8B(1-2) and the *lz* allele at 8D(8-9). Using a host of other alleles scattered along the X chromosome, these duplications failed to complement any of them. This suggests that the duplications are made up of a contiguous stretch of the X chromosome within the 8A-B region and lack any other X chromosome material. In addition, each new duplication was analyzed by cytology using the *dlim1* cDNA, as a marker. Because the Y chromosome does not polytenize it was difficult to map the duplicated segments by cytology. The analysis of the squashes supported our complementation data in that we were able to see pieces of the X chromosome that hybridized with our probe (not shown).

## 4. DISCUSSION

To facilitate the analysis of a newly discovered gene found in the 8B region of the X chromosome, we undertook a genetic screen to create duplications that covered interval 8B. This region has lacked sufficient characterization because of the absence of duplications that span it. Duplications of the X chromosome enable one to test by complementation the allelelism of mutations within a given region. Thus, without appropriate duplicated segments, comprehensive genetic screens can not be performed. To generate new duplications we utilized an efficient mutagenesis scheme, that was based on that described by Brousseau *et al.*, (1961). The screen design took advantage of a pre-existing X-Y translocations with breakpoints near region 8B, and the lethality associated with hyperploidy of the *Drosophila* X chromosome. Through a recombination event we attached an intact Y chromosome to the translocation which was a neccessary element for generating usuable duplications.

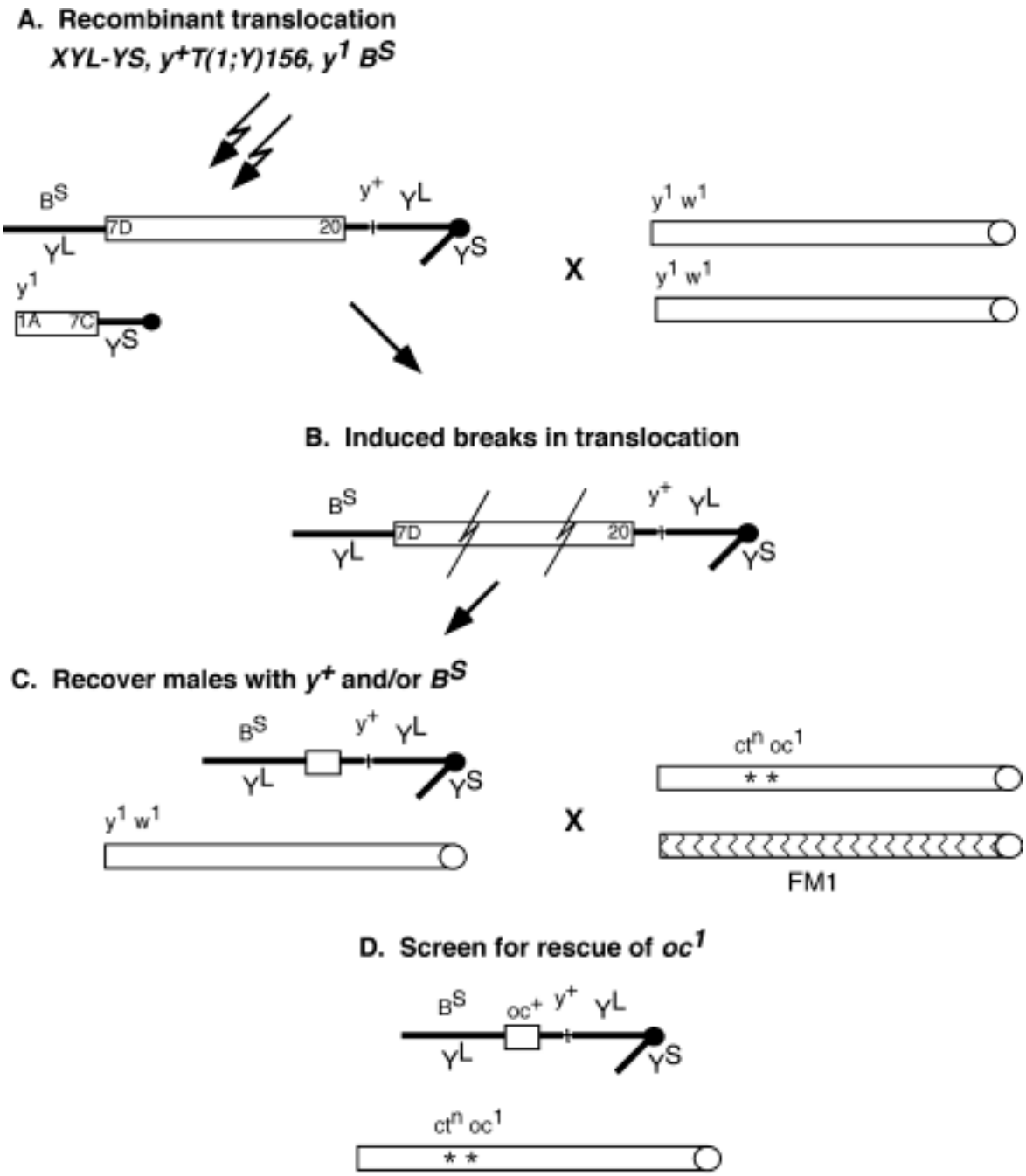


Figure 2. Mutagenesis screen for X chromosome duplications. Open boxes represent X chromosomes and thick black lines represent Y chromosomes. (A) Males carrying the recombined translocation chromosome were subjected to γ irradiation and crossed to *y[1] w[1]* females. (B) Chromosomal breaks produced viable male progeny that retained one or both markers from the recombined translocation chromosome. These males (C), shown with both the *y[+]* and *B[S]* markers, were crossed to *ct[n] oc[1]/FM1* females. The progeny from this cross were screened for rescue of the *oc* phenotype (D). The diagrammed duplicated segment (C), represents just one example of the potential viable chromosomes. Many other possibilities exist due to the random breaks induced by the γ rays. Only males with a *y[+]* and/or *B[S]* markers were crossed to *ct[n] oc[1]* females.

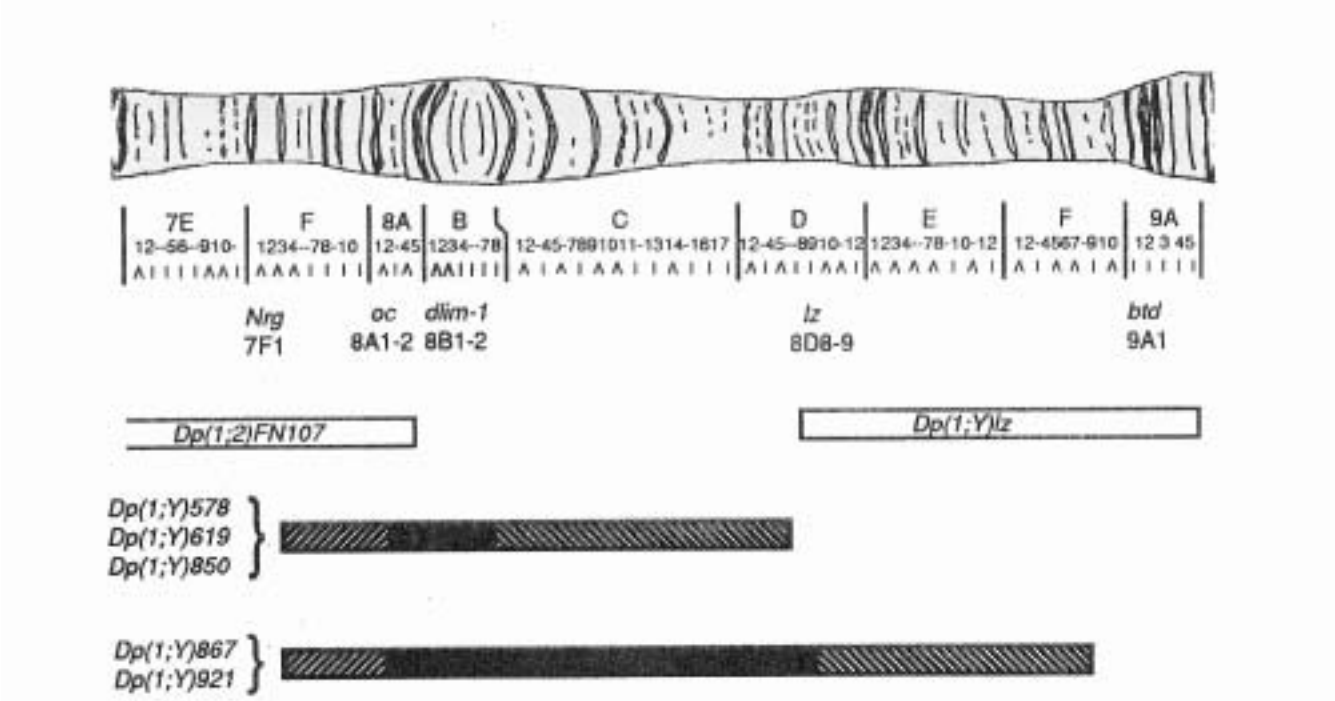


Figure 3. Cytogenetic map of the interval covered by the duplications. The breakpoints of the five new duplications were defined by complementation tests with alleles surrounding *oc*. The alleles used are shown with their map position along the chromosome. Two preexisting duplications are shown as open boxes. The distal duplication, *Dp(1;2)FN107* has breakpoints, 7A8-8A5, and covers *oc*. The proximal duplication, *Dp(1;Y)lz* has breakpoints, 8D(7-9)-9A(8-9), and covers *lz* and *btd*. The new duplications are represented as filled boxes. All have a distal breakpoint between *Nrg*, 7F1 and *oc*, 8A1. Three of these duplications have proximal breakpoints between *dlim1*, 8B(1-2) and *lz*, 8D(8-9). The two remaining duplications break proximally between *lz* and *btd* (9A1). The strips within the boxes represent the region of uncertainty in the breakpoints.

From this screen we generated five new Y-linked duplications that covered the 8B interval. Of these duplications, two span a gap of the 8B region that covers an uncharacterized region. The other three duplications break within this interval and cover smaller portions of the 8A-C region. Our method allowed us to generate duplications of the 8B region with great efficiency and specificity. By taking advantage of the lethality caused by hyperploidy and the translocation breakpoints near 8B, our screening process was highly selective. This scheme could be modified and used for other selected regions of the X chromosome. The duplications that were generated and described here provide us with valuable tools for dissecting out the genetics of the X chromosome. This study should greatly facilitate further genetic and molecular analysis of loci mapping within this region.

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