The Autosomal FLP-DFS Technique for Generating Germline Mosaics in Drosophila melanogaster

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Manuscript received July 10, 1996
Accepted for publication September 17, 1996

ABSTRACT

The production of female germline chimeras is invaluable for analyzing the tissue specificity of recessive female sterile mutations as well as detecting the maternal effect of recessive zygotic lethal mutations. Previously, we developed the "FLP-DFS" technique to efficiently generate germline clones. This technique uses the X-linked germline-dependent dominant female sterile mutation ovI as a selection for the detection of germline recombination events, and the FLP-FRT recombination system to promote site-specific chromosomal exchange. This method allows the efficient production of germline mosaics only on the X chromosome. In this paper we have built chromosomes that allow the use of this technique to the autosomes. We describe the various steps involved in the development of this technique as well as the properties of the chromosomes utilized.

THE dominant female sterile (DFS) technique allows the detection of female germline chimeras that is invaluable for analyzing the tissue specificity (germline vs. somatic) of recessive female sterile mutations (Wieschaus et al. 1981; Perrimon and Gans 1983), as well as detecting the maternal effect of recessive zygotic lethal mutations (Perrimon et al. 1984, 1989; Chou and Perrimon 1992). This technique has been used extensively to identify on the X-chromosome genes that play critical roles in embryonic patterning. It has allowed the identification of two distinct classes of loci: (1) loci that play specific roles during embryogenesis that could not be identified from screens for embryonic lethal mutations because these genes are also expressed during oogenesis (the maternally stored products being sufficient to rescue the absence of zygotic product) and (2) loci with specific maternal effect lethal phenotypes that could not be isolated from screens for female sterile mutations because loss of gene activity during zygotic development leads to lethality.

The DFS technique consists of the production of germline clones (GLCs) in females heterozygous for the X-linked germline-dependent DFS mutation ovI (Busson et al. 1983; Perrimon and Gans 1983; Perrimon 1984). ovI allows a positive selection for the detection of germline recombination events since only germ cells that have eliminated the DFS mutation, and thus become homozygous for the homologous chromosome, will lead to formation of eggs. To increase the frequency of germline recombination events, we have taken advantage of the properties of the yeast flipase recombinase target (FLP) site-specific recombinase and its recombination targets (FRTs) to develop the "FLP-DFS" technique (Chou and Perrimon 1992) that is almost 100% efficient. The heat-inducible FLP-recombinase gene, under the control of an hsp70 promoter, recognizes and catalyzes site-specific recombination between homologous chromosomes at the level of the FRT sequences (Golic and Lindquist 1989; Golic 1991).

To extend the DFS technique to the autosomes, which represent four-fifths of the Drosophila genome, we previously reported the recovery of P elements that carry the ovI gene (P[ovI]) on each autosomal arm (Chou et al. 1993). Transposition of the X-linked mutant gene was necessary because no autosomal DFS mutations with properties similar to ovI are available on all chromosomal arms (see Introduction of Chou et al. 1993). These P[ovI] chromosomes allow the easy detection of germline clone recombination events following X-ray-induced mitotic exchange (Chou et al. 1993). However, because the frequency of these events is very low we decided to develop the "autosomal FLP-DFS technique," which involves the construction of both second and third chromosomes that contain both FRT and P[ovI] insertions. We describe the construction and properties of these chromosomes that allow the production of germline chimeras for 95% of all loci on the second and third chromosomes. Finally, we have constructed "double FRT chromosomes," which contain FRT elements located at the base of each chromosomal arm to facilitate the large scale GLC analysis of autosomal zygotic lethal mutations. The search for this class of loci has only been conducted systematically on
the X chromosome (Perrimon et al. 1984, 1989). It is therefore critical for our global understanding of embryonic development to develop the tools necessary to conduct similar searches on the autosomes.

MATERIALS AND METHODS

Generation of P(>w+) > FRT: P(>w+) > FRT insertions were isolated following the transposition of the X-linked FRT<sup>69A</sup> insertion as previously described (Chou and Perrimon 1992) using the Δ2-3 "jumpstarter" strain (Robertson et al. 1988). The two transposable stocks, γ<sup>568</sup> P(γ<sup>7</sup>, Δ2-3.2<sup>3</sup>) and γ<sup>568</sup> Sb P(γ<sup>7</sup>, Δ2-3)/Tm6, Ubx, were obtained from the Bowling Green Stock Center.

Sixty-seven independent autosomal insertions were generated and mapped by in situ hybridization to polytene chromosomes as described in Chou et al. (1993). The probe used was the P(>w+) plasmid (Golic and Lindquist 1989).

Neomycin selection of flies carrying P(γ<sup>7</sup>, hs-neo, FRT): Flies carrying the P(γ<sup>7</sup>, hs-neo, FRT) element can be selected by their resistance to G418 (Geneticin, Gibco laboratory) following the protocol described by Xu and Rubin (1993). G418 (0.25 g geneticin/40 ml dH<sub>2</sub>O) was added to standard fly medium.

FRT chromosomes with dominant markers: The dominant markers are described in Lindley and Zimm (1992). Chromosomes carrying the FRT elements in cis with dominant markers were constructed to allow recombination with specific mutations. FRT<sup>9</sup>-3<sup>24</sup> was marked with So, FRT<sup>-3</sup> G1<sup>13</sup> with L, FRT<sup>9</sup>-<sup>N24</sup> with D, and FRT<sup>-3</sup> R<sup>2</sup> with Sb. The following stocks were built to facilitate the construction of additional strains: w/w; So FRT<sup>-3</sup>-<sup>R2</sup>/CyO, w/w; FRT<sup>-3</sup> G1<sup>13</sup>/CyO, w/w; D FRT<sup>-3</sup>-<sup>N24</sup>/TM3, Sb and w/w; FRT<sup>-3</sup> R<sup>2</sup>/TM6, Ubx.

Flipase stocks: X-linked flipase insertions were recovered following destabilization of an autosomal FLP insertion. The FLP element, P(γ<sup>7</sup>, hs-FLP), constructed by Golic and Lindquist (1989) carries an hs-70-FLP fusion gene and the rosy (γ<sup>7</sup>) gene. Using the Δ2-3 transposase, we mobilized a hs-70-FLP element transgene, FLP<sup>0</sup>, located on the second chromosome (Chou and Perrimon 1999), onto an X chromosome that carries the w<sup>9</sup> mutation. w<sup>9</sup> is a null mutation in the w<sup>9</sup> gene (Oliver et al. 1987). We initially decided to jump FLP elements onto this chromosome because some of the P(ovd<sup>9</sup>) autosomal insertions that we originally isolated were not fully penetrant and their leaky expressivity could be strengthened in the presence of a single copy of w<sup>9</sup> in females (see Chou et al. 1993). Subsequently, w<sup>9</sup> was recombined away from these FLP insertions because the P(ovd<sup>9</sup>) autosomal insertions that we ultimately recovered were fully penetrant for DFS in the presence of two wild-type copies of w<sup>9</sup>

The jumps were generated as follows: w/Y; CyO/+; MKRS/+ males were crossed with FM3/ovo<sup>9</sup> w<sup>24</sup> females. Subsequently, ovo<sup>9</sup> v<sup>24</sup>/Y; CyO/+; MKRS/+ males were crossed with C(1)DX, y<sup>1</sup>/Y; FLP<sup>-</sup><sup>9</sup>/+; Δ2-3/+ females and their male progeny of genotype ovo<sup>9</sup> v<sup>24</sup>/Y; CyO/FLP<sup>-</sup> (or +); MKRS/Δ2-3 (or +) crossed with C(1)DX, y<sup>1</sup>/Y; ry/ry females. X-linked jumps of P(γ<sup>7</sup>, hs-FLP) were identified among ovo<sup>9</sup> v<sup>24</sup> FLP/Y; CyO/+; MKRS/ry males with ry females. We selected 11 independent X-linked P(γ<sup>7</sup>, hs-FLP) jumps.

To select for the most efficient flipase, y w FLP<sup>-</sup>/+ females were crossed with w/Y; FRT<sup>9</sup> FRT<sup>75A</sup> males and their progeny heat shocked for 2 hr at 37°C in a water bath during the first larval instar. The efficiency of the flipase was determined by examining the frequency of y w FLP<sup>-</sup>/+ progeny that show mosaic eyes for the white marker. FRT<sup>75A</sup> contains the mini-white gene (Golic and Lindquist 1989). FLP<sup>-</sup> and FLP<sup>75</sup> were found to provide an efficient source of flipase activity when tested for mosaic eye production as well as when tested for production of GLCs. The two strains y w FLP<sup>-</sup>; CyO/Sco and y w FLP<sup>75</sup>; TM3, Sco/Dd were built to facilitate the generation of germline mosaics on the second and third chromosomes, respectively. These two FLP insertions are comparable in their efficiency to generate GLCs.

X-ray-induced mitotic recombination: To induce male germline mitotic recombination, second instar larvae (48–72 hr old) from the appropriate cross were irradiated at a constant dose of 1500 rad (Torrrex 120D X-ray machine; 100 KV, 5 mA, 3-mm aluminum filter).

Production of germline mosaics using the autosomal FLP-DFS technique: To generate homozygous GLCs, females were crossed with males of genotype FLP<sup>-</sup>/Y; CyO/P(ovd<sup>9</sup>) FRT or FLP<sup>75</sup>/Y; TM3, Sco/P(ovd<sup>9</sup>) FRT. These males were generated by crossing females from the y w FLP<sup>-</sup>; CyO/Sco and y w FLP<sup>75</sup>; TM3, Sco/Dd stocks with the appropriate P(ovd<sup>9</sup>) FRT males. Females of the appropriate genotypes (see Table 1) were allowed to lay eggs for 1 day in glass vials and the progeny heat shocked twice for 2 hr at 37°C in a circulating water bath over a period of 2 days when they reached late L2 to L3 larval stages. Subsequently, females of the appropriate genotype were analyzed for the presence of GLCs.

RESULTS AND DISCUSSION

The autosomal FLP-DFS technique: A number of steps were designed to develop the tools necessary to extend the FLP-DFS technique to the autosomes (Figure 1). First, we selected autosomal chromosomes carrying P(ovd<sup>9</sup>) elements associated with tight DFS phenotypes that are appropriate for the identification and generation of germline mosaics (Chou et al. 1993). Second, we generated P(>w+) > FRT insertion lines on wild-type chromosomes with the goal to identify a subset that localized near the centromeres of each chromosomal arm. Subsequently, the most appropriate FRT insertions were selected either from our collection or others (K. Golic, personal communication; X. and Rubin 1993). Third, we built chromosomes with the FRT elements located proximally to the P(ovd<sup>9</sup>) insertions. We recombined P(ovd<sup>9</sup>) and FRT elements following mitotic recombination in the male germline. Subsequently, the P(ovd<sup>9</sup>) FRT chromosomes were maintained as stocks using dominant, male-sterile mutations. Fourth, X-linked FLP insertions were recovered and tested for their abilities to generate GLCs. Fifth, chromosomes that carry FRT insertions on each chromosomal arm were constructed to conduct large scale GLC analysis of autosomal zygotic lethal mutations.

Step 1: Selection of autosomal P(ovd<sup>9</sup>): The four autosomal P(ovd<sup>9</sup>) lines we selected are described in Chou et al. (1993). They are associated with tight DFS phenotypes and have been shown to promote GLC production following X-ray irradiation. These are P(w<sup>9</sup>, ovd<sup>9</sup>, ovd<sup>11</sup>-<sup>3</sup> X<sup>3</sup>, P(w<sup>9</sup>, ovd<sup>9</sup>, ovd<sup>11</sup>-<sup>26.3X</sup>, P(w<sup>9</sup>, ovd<sup>11</sup>-<sup>3.2X</sup>, and P(w<sup>9</sup>, ovd<sup>11</sup>-<sup>3</sup> X<sup>3</sup>)

Step 2: Selection of FRT insertions close to centromeres: We conducted a screen to isolate new autosomal P(>w+) > FRT insertions (see MATERIALS AND METHODS). A total of 67 independent autosomal insertions were recovered and localized by in situ hybridization to polytene chromosomes. The four most proximal
were not as proximal as others isolated in different screens. We constructed is shown schematically in Figure 2.

2. The various steps involved in the construction of these chromosomes are described in detail below. Insertions from our screen and others for further experiments. We chose FRT60A and FRT82B, which contains the hsmem gene, located at 40A and 82B, respectively (Xu and Rubin 1993); FRT24, isolated by Kent Golic (unpublished results) and mapped to position 79D-F by M. Soto (personal communication); and FRT132. The two FRT insertions FRT13 and FRT24 contain the mini-white (w) gene. These insertions are referred to as FRT24, FRT26, FRT28, and FRT82B.

Step 3: Construction of autoosomal P[w+; ovO] FRT chromosomes: The structure of the P[w+; ovO] FRT chromosomes we constructed is shown schematically in Figure 2. The various steps involved in the construction of these chromosomes are described in detail below.

Construction of P[w+; ovO] FRT24: We first marked P[w+, ovO] with the Lodged (L) dominant marker that is located at 51A2-B1. w/Y; P[w+, ovO] FRT24/CyO males were mated to w/w; L/CyO females and their progeny irradiated at the second larval instar stage to induce male recombination events in their germline. Four independent lines of P[w+; ovO] FRT24 and the FRT element, w/Y; P[w+, ovO] FRT24/CyO males were mated to w/w; Sco FRT3640A/CyO females and their progeny irradiated at the second larval instar stage. Emerging w/w; P[w+, ovO] FRT24/CyO males were mated to w/w; Sco FRT3640A/CyO females. Forty Cy males that did not have the Sco and L markers were recovered as putative P[w+, ovO] FRT24 recombinants. Two of these chromosomes were associated with a complete DFS phenotype and led to a high frequency of FRT132 and FRT24.

TABLE 1
Efficiency of the autoosomal FLP-DFS technique

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Heat shock</th>
<th>N females with eggs/N females examined</th>
</tr>
</thead>
<tbody>
<tr>
<td>P[w+; ovO] FRT24/CyO</td>
<td>No</td>
<td>0/120 (0)</td>
</tr>
<tr>
<td>P[w+; ovO] FRT24/+</td>
<td>No</td>
<td>0/122 (0)</td>
</tr>
<tr>
<td>FlpF22/+; P[w+; ovO] FRT24/CyO</td>
<td>No</td>
<td>0/200 (0)</td>
</tr>
<tr>
<td>FlpF22/+; P[w+; ovO] FRT24/FRT24</td>
<td>Yes</td>
<td>102/102 (100)</td>
</tr>
<tr>
<td>FlpF22/+; P[w+; ovO] FRT24/FRT24</td>
<td>No</td>
<td>0/134 (0)</td>
</tr>
<tr>
<td>FlpF22/+; P[w+; ovO] FRT24/FRT24</td>
<td>Yes</td>
<td>46/48 (96)</td>
</tr>
</tbody>
</table>

Heat shock treatments were performed as described in the MATERIALS AND METHODS. To determine the number of females with eggs, ovaries were dissected and examined for the presence of vitellogenic eggs. Females were examined 5 days following eclosion. Numbers in parentheses are percentages.
second larval instar stage to induce male germline recombination. Male progeny of genotype \( w/Y; \) \( P[w^+, \text{ovd}^{D1}]^{2X:32X9}/\text{FRT}^{2X:G13} \) were mated to \( w/w; \) CyO/ \( Sco \) females. Nine males with dark red eyes that did not have the \( L \) marker were recovered as putative \( P[w^+, \text{ovd}^{D1}]^{2X:32X9}/\text{FRT}^{2X:G13} \) recombinants because the eye color associated with either \( P[w^+, \text{ovd}^{D1}]^{2X:32X9}/\text{FRT}^{2X:G13} \) and

\[ \text{FRT}^{2X:G13} \text{ is dark orange. These putative } P[w^+, \text{ovd}^{D1}]^{2X}/\text{FRT}^{2X} \text{ chromosomes were tested for their DFS and ability to generate FLP-induced GLCs. Two independent lines were associated with a complete DFS phenotype and high frequency of FLP-induced mitotic exchange. The } P[w^+, \text{ovd}^{D1}]^{2X}/\text{FRT}^{2X:G13} \text{ line we use is kept as } P[w^+, \text{ovd}^{D1}]^{2X:32X9}/\text{FRT}^{2X:G13}/\text{S} \text{ Sp Ms(2)M ba d}/\text{CyO}. \]

Construction of \( P[w^+, \text{ovd}^{D1}]^{2X}/\text{FRT}^{2X} \): Recombinants between \( P[w^+, \text{ovd}^{D1}]^{2X} \) and \( \text{FRT}^{2X} \) were obtained by crossing \( w/Y; \) \( P[w^+, \text{ovd}^{D1}]^{2X:2X48}/\text{TM3}, \text{S} \) males with \( w/w; \) \( \text{FRT}^{2X:2X48}/\text{FRT}^{2X:2X48} \) females. Their progeny were irradiated to induce germline recombination in males. Emerging \( w/Y; \) \( P[w^+, \text{ovd}^{D1}]^{2X:2X48}/\text{FRT}^{2X:2X48} \) males were crossed to \( y/y \) \( w/w \) females. Eighty males with dark red eyes were recovered as candidate \( P[w^+, \text{ovd}^{D1}]^{2X:2X48}/\text{FRT}^{2X:2X48} \) recombinants because the eye color associated with either \( P[w^+, \text{ovd}^{D1}]^{2X:2X48}/\text{FRT}^{2X:2X48} \) and \( \text{FRT}^{2X:2X48} \) is dark orange. Four of them turned out to be appropriate for the induction of GLCs. The \( P[w^+, \text{ovd}^{D1}]^{2X}/\text{FRT}^{2X} \) line we use is kept as \( w/Y; P[w^+, \text{ovd}^{D1}]^{2X:2X48}/\text{FRT}^{2X:2X48}/\text{ru h st } \beta \text{Tub85D}^{8} \text{ ss e'/TM3, S}. \) The dominant, male-sterile mutation, \( \beta \text{Tub85D}^{8}, \) was obtained from K. MATTHEWS and T. KAUFMAN (KEMPHUES et al. 1980).

Construction of \( P[w^+, \text{ovd}^{D1}]^{2X}/\text{FRT}^{2X} \): To construct the \( P[w^+, \text{ovd}^{D1}]^{2X}: \text{FRT}^{2X} \) chromosome, we first marked the \( P[w^+, \text{ovd}^{D1}]^{2X:2X48}/\text{FRT}^{2X:2X48} \) chromosome with the dominant marker Roughened (\( R \)), which maps at 62B8-12. Following male
germline recombination induced by X-ray treatment of larvae, we recovered seven independent chromosomes as putative $R[P_{\text{ovo}^{D1}}]$ recombinants. Since these lines showed a yellow eye color rather than the red eye color of the original $P_{\text{ovo}^{D1}}$ insertion, it suggested that $P_{\text{ovo}^{D1}}$ was not mapped by in situ hybridization (CHOU et al. 1993), might carry two $P_{\text{ovo}^{D1}}$ insertions on each separate chromosomal arm, and that one of them had been lost as the result of the recombination event. The putative $R[P_{\text{ovo}^{D1}}]$ chromosome may contain the original $P_{\text{ovo}^{D1}}$ insertion (CHOU et al. 1993) instead of two $P_{\text{ovo}^{D1}}$ insertions. This putative $R[P_{\text{ovo}^{D1}}]$ line was used for generating $P_{\text{ovo}^{D1}}$ FRT recombinants. When these chromosomes were tested for their efficiency of the technique: To test the efficiency of these chromosomes to generate germline mosaics, we determined both their ability to confer a DFS phenotype and to generate high frequency of female germline mosaics using the method depicted in Figure 1, we found that all of them, following the appropriate heat shock treatment, allowed the recovery of almost 100% of mosaic females (Table 1). All of the $P_{\text{ovo}^{D1}}$ FRT recombinant chromosomes are associated with a fully penetrant DFS phenotype such that all eggs laid by these females are derived from germine recombination events. As previous observed for the X chromosome FLP-DFS technique (CHOU and PERRIMON 1992), we did not detect any effects of these chromosomes on survival rates since all classes of females were recovered at the expected ratios.

Step 5: Properties of the double FRT chromosomes: To facilitate the screening for zygotic lethal mutations with specific maternal effect phenotypes (see Figure 4), we built second and third chromosomes chromosomes that carry FRT elements on both sides of each centromere. To construct the $FRT^{3R-43}$/ $FRT^{3R-41}$ chromosome, $FRT^{3R-43}$ was first marked with $Sco$ and $FRT^{3R-40a}$ with $L$. Progeny from $Sco$ $FRT^{3R-41}$/ $FRT^{3R-40a}$ $L$ females crossed with $w/Y$; CyO/Sco males were grown on media containing G418 to select for the presence of the $P[\text{y}^{+}, \text{hs-neo}, \text{FRT}]$ element. Potential recombinants were selected and tested for their abilities to promote the induction of GLCs in the presence of the $P_{\text{ovo}^{D1}}$ FRT chromosomes. A similar strategy was used to build
FIGURE 4.—Use of the double FRT chromosomes for screening. To identify zygotic lethal mutations with specific maternal effect phenotypes, the following screen, in this case designed for the second chromosome, is proposed. This protocol is twice as efficient as screening with single FRT chromosomes. Ten virgin females heterozygous for the second chromosome carrying a lethal mutation(s) are collected and separated into two groups of five females each. One group is mated to two males that carry the P[ovo"]
\[FRT^{+}, FRT^{+}\] \[y\] chromosome as well as an X chromosome that contains the FLP-recombinase. The other batch is mated to two males that carry the FRT \[FRT^{+}, FRT^{+}\] chromosome as well as an X chromosome that contains the FLP-recombinase. Flies from this cross are allowed to lay eggs for a period of 3 days, after which the adults are discarded and the larva progeny are aged for 2 days and then heat shocked for 2 hr at 37°. Subsequently, 15 females of genotype \[w/ru \ FLP; \ [FRT^{+}, FRT^{+}\] */P[ovo"]
\[FRT^{+}\] and 15 females of genotype \[w/w \ FLP; \ [FRT^{+}, FRT^{+}\] */P[ovo"]
\[FRT^{+}\] are examined for the presence of GLCs. To allow the detection of mutations with paternally rescuable maternal effects, each group of females is mated with five sibling males heterozygous for the \[FRT^{+}, FRT^{+}\] chromosome. Since the FLP-DFS technique is so efficient, the analysis of 15 females of the appropriate genotype is sufficient for the recovery of at least 10 females with GLCs (if the lethal mutation is not associated with germ cell lethality). The analysis of 10 females with GLCs is sufficient to determine the maternal expression of a zygotic lethal mutation (PERRIMON et al. 1984, 1989).

PROTOCOL

1: Test 2L

Step 1A:

\[5 \phi \phi w/w; \ [FRT^{2L}, FRT^{2R}]^{\phi} /CyO \times 2 \sigma \sigma w \ FLP/Y; P[ovo\text{rD}1]^{2L} \ FRT^{2L}/CyO\]

Heat shock the progeny at 37° for 2 hours

Step 2A:

Select 15 \[\phi \phi \ w/w \ FLP; \ [FRT^{2L}, FRT^{2R}]^{\phi} /P[ovo\text{rD}1]^{2L}\]

\[\times 5 \sigma \sigma w/y; \ [FRT^{2L}, FRT^{2R}]^{\phi} /P[ovo\text{rD}1]^{2L} \ FRT^{2L}\]

Determine the maternal effect

2: Test 2R

Step 1B:

\[5 \phi \phi w/w; \ [FRT^{2L}, FRT^{2R}]^{\phi} /CyO \times 2 \sigma \sigma w \ FLP/Y; FRT^{2L} \ P[ovo\text{rD}1]^{2R}\]

Heat shock the progeny at 37° for 2 hours

Step 2B:

Select 15 \[\phi \phi \ w/w \ FLP; \ [FRT^{2L}, FRT^{2R}]^{\phi} /FRT^{2R} \ P[ovo\text{rD}1]^{2R}\]

\[\times 5 \sigma \sigma w/y; \ [FRT^{2L}, FRT^{2R}]^{\phi} /FRT^{2R} \ P[ovo\text{rD}1]^{2R}\]

As shown in Table 1, these chromosomes allowed the recovery of GLCs at the same frequency as single FRT chromosomes indicating that if intrachromosomal FRT recombination events occur, it is not a major problem for screening purposes.

CONCLUSION

In summary, we have developed a method that allows the efficient recovery of female germline mosaics for mutations localized on the autosomes. The P[ovo"] FRT chromosomes are associated with tight DFS phenotypes and under the appropriate heat shock conditions lead to 100% recovery of germline mosaics. The frequency of GLCs recovered following X-ray treatment at the same developmental larval stage is ~1–2% (CHOU et al. 1993) allowing us to conclude that the autosomal
FLP-DFS technique is at least 50 times more efficient than X-rays to induce germline recombination. The FRT elements we used are located near the centromeres and allow the analysis of mutations in ~95% of the loci located on either the second or third chromosomes.

The availability of this technique, in combination with the previous X-linked FLP-DFS technique, now permits the production of GLCs for almost the entirety of the mutations in the Drosophila genome. These tools should greatly contribute to our analysis of Drosophila development.

We are indebted to E. NOLL and A. LANJUN for excellent technical assistance. We thank Drs. T. Xu and G. RUBIN for providing the FRT^9,24 and FRT^9,24 lines, Dr. K. GOLIC for providing the FRT^9,24 stock before publication and Dr. M. SOTO for informing us of its location, Drs. D. LINDSEY for Ms(2)M and T. KAUFMAN for β Tub85D^P. Various dominant marker stocks were obtained from the Bowling Green and Bloomington Stock Centers. We are grateful to LIZ PERRETT for providing the X chromosome of Drosophila melanogaster.

LITERATURE CITED


APPENDIX

The following stocks are available individually or as a kit from the Bloomington Stock Center. The stock numbers and the genotypes are indicated. The kit also includes the X-linked stocks to generate GLCs.

**X-linked stocks:**

- #1813 C(1)DX, γ / γ ovo^D1 γ / γ ^24 P[mini w^+; FRT]^101 / Y; P[ry^+; FLP]^38 / P[ry^+; FLP]^38
- #1843 C(1)DX, γ / γ ovo^D2 γ / γ ^24 P[mini w^+; FRT]^62 / Y; P[ry^+; FLP]^38 / P[ry^+; FLP]^38
- #1844 γ w γ / γ P[mini w^+; FRT]^101
- #1903 γ w γ / γ P[mini w^+; FRT]^92

*Flipase stocks to generate germine clones on the autosomes:*

- #1929 γ w γ P[ry^+; FLP]^12; CyO/Sco
- #1970 γ w γ P[ry^+; FLP]^22; TM3, Sh/CyD
- #1622 P[hs neo; ry^+; FRT]^A-40A / CyO
- #1821 Tfl P[hs neo; ry^+; FRT]^A-40A/CyO
- P[ovo^D1^L P[ovo]XR:
  - #2121 P[mini w^+; ovo^D1]^A-13X13 P[hs neo; ry^+; FRT]^A-40A / Sp Ms(2)M btl / CyO
  - P[ovo^D1^L P[ovo]XR:
    - #2125 P[mini w^+; FRT]^A-12X13 P[mini w^+; ovo^D1]^A-12X9 / Sp Ms(2)M btl / CyO
  - P[ovo^D1^L P[ovo]XR:
    - #1956 w; P[mini w^+]; FRT]^A-12G13
    - #1958 w; P[mini w^+]; FRT]^A-12G13 L/CyO
    - P[ovo^D1 L P[ovo]XR:
      - #2139 w; P[mini w^+; ovo^D1]^A-2X6 P[mini w^+; FRT]^A-2X6 / ru h st β Tub85D^P ss e^+ / TM3, Sh
      - P[ovo^D1 L P[ovo]XR:
        - #2035 P[hs neo; ry^+; FRT]^A-82B; ry
        - #2051 γ w; P[hs neo; ry^+; FRT]^A-82B Sh/TM6, Ubx
        - P[ovo^D1 L P[ovo]XR:
          - #2149 w; P[hs neo; ry^+; FRT]^A-82B P[mini w^+; ovo^D1]^A-82B / ru h st β Tub85D^P ss e^+ / TM3, Sh