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

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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 $ova^{DF}$ 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 (Wierschus 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 $ova^{DF}$ (Busson et al. 1983; Perrimon and Gans 1983; Perrimon 1984). $ova^{DF}$ 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 recombination 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 $ova^{DF}$ gene ($P[ova^{DF}]$) on each autosomal arm (Chou et al. 1993). Transposition of the X-linked mutated gene was necessary because no autosomal DFS mutations with properties similar to $ova^{DF}$ are available on all chromosomal arms (see Introduction of Chou et al. 1993). These $P[ova^{DF}]$ 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 $P[ova^{DF}]$ 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 FRT⁶⁷ insertion as previously described (CHOU and PERRIMON 1992) using the Δ2-3 "jumpstrainer" strain (ROBERTSON et al. 1988). The two transposase stocks, ryo³⁰⁸ P[ryo⁺, Δ2-3] and ryo³⁰⁸ Sb P[ryo⁺, 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 LINDEQUST 1989).

**Neomycein selection of flies carrying P[ry⁺, hs-neo, FRT]**: Flies carrying the P[ry⁺, 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 dH₂O) was added to standard fly medium.

**FRT chromosomes with dominant markers**: The dominant markers are described in LINDEQUST and ZIMM (1992). Chromosomes carrying the FRT elements in cis with dominant markers were constructed to allow recombination with specific mutations. FRT⁹⁻⁸⁻ was marked with Sco, FRT⁹⁻¹⁰ with lα, FRT⁸⁻²⁴ with D, and FRT⁸⁻³₈ with Sh. The following stocks were built to facilitate the construction of additional strains: w/y; Sco FRT⁸⁻¹⁰/Cyo, w/y; FRT⁹⁻¹⁰ L/Cyo, w/y; D FRT⁸⁻²⁴/TM3, Sh and w/y; FRT⁸⁻³₈ Sh/TM6, Ubx.

**Flipase stocks**: X-linked flipase insertions were recovered following destabilization of an autosomal FLP insertion. The FLP element, P[ry⁺, hsFLP], constructed by GOLIC and LINDEQUST (1989) carries a hsFLP-FLP fusion gene and the ryo (ry) gene. Using the Δ2-3 transposase, we mobilized a hsFLP-FLP element transgene, FLP⁸⁻³₈, located on the second chromosome (CHOU and PERRIMON 1999) onto an X chromosome that carries the ovo⁹⁻¹ mutation. ovo⁹⁻¹ is a null mutation in the ovo gene (OLIVER et al. 1987). We initially decided to jump FLP elements onto this chromosome because some of the P[ovo⁻¹] 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 ovo in females (see CHOU et al. 1993). Subsequently, ovo⁻¹ was recombined away from these FLP insertions because the P[ovo⁻¹] autosomal insertions that we ultimately recovered were fully penetrant for DFS in the presence of two wild-type copies of ovo.

Two jumps were generated as follows: w/Y; CyO/+; MKRS/+ males crossed with FM3/ovo⁻¹ w⁻²⁴ females. Subsequently, ovo⁻¹ w⁺/Y; CyO/+; MKRS/+ males were crossed with C(1)DX, y¹/Y; FLP⁺/F⁺; Δ2-3/+ females and their male progeny of genotype ovo⁻¹ w⁺/Y; CyO/FLP⁺ (or +); MKRS/Δ2-3 (or +) crossed with C(1)DX, y¹/Y; ry⁺/ry⁺ females. X-linked jumps of P[ry⁺, hsFLP] were identified among ovo⁻¹ w⁺ FLP/Y; CyO/+; MKRS/ry⁺ males with ry⁺ eyes. We selected 11 independent X-linked P[ry⁺, hsFLP] jumps.

To select for the most efficient flipase, y FLP/y females were crossed with w/Y; FRT⁷⁵⁻⁴/FRT⁷⁵⁻⁴ males and their progeny heat shocked for 2 hr at 37°C in a water bath during the first instar larvae. The efficiency of the flipase was determined by examining the frequency of y FLP/y; FRT⁷⁵⁻⁴/+ progeny that show mosaic eyes for the white marker. FRT⁷⁵⁻⁴ contains the mini-white gene (GOLIC and LINDEQUST 1989). FLP⁺ and FLP⁻ 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⁺, CyO/Sco and y w FLP⁻, TM3, Sh/CyD 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 (Torrex 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⁺/Y; CyO/[ovo⁻¹] FRT or FLP⁻/Y; TM3, Sh/[ovo⁻¹] FRT. These males were generated by crossing females from the y w FLP⁺, CyO/Sco and y w FLP⁻, TM3, Sh/CyD stocks with the appropriate P[ovo⁻¹] 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 genotypes 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[ovo⁻¹] elements associated with tight DFS phenotypes that are appropriate for the identification and generation of germline mosaics (CHOU et al. 1993). Outcomes of 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; Xu and RUBIN 1993). Third, we built chromosomes with the FRT elements located proximally to the P[ovo⁻¹] insertions. We recombed P[ovo⁻¹] and FRT elements following mitotic recombination in the male germline. Subsequently, the P[ovo⁻¹] FRT chromosomes were maintained as stocks using dominant, male-sterile mutants. 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 conducted to conduct large scale GLC analysis of autosomal zygotic lethal mutations.

**Step 1: Selection of autosomal P[ovo⁻¹]**: The four autosomal P[ovo⁻¹] 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⁺, ovo⁻¹], P[w⁺, ovo⁻¹] in situ, P[w⁺, ovo⁻¹] in situ, P[w⁺, ovo⁻¹] in situ, and P[w⁺, ovo⁻¹] in situ.

**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
insertion lines that we recovered are FRT\textsuperscript{G3} at position 39E, FRT\textsuperscript{G7} at position 42B, FRT\textsuperscript{G9} at position 78E and FRT\textsuperscript{G6} at position 84D. Because some of these insertions were not as proximal as others isolated in different screens (K. Golic, personal communication; Xu and Rubin 1993), we selected a combination of FRT insertions from our screen and others for further experiments. We chose FRT\textsuperscript{G6} and FRT\textsuperscript{G2}, which contains the hsneo gene, located at 40A and 82B, respectively (Xu and Rubin 1993); FRT\textsuperscript{G4}, isolated by Kent Golic (unpublished results) and mapped to position 79D-F by M. Soto (personal communication); and FRT\textsuperscript{G3}. The two FRT insertions FRT\textsuperscript{G3} and FRT\textsuperscript{G2} contain the mini-white (w\textsuperscript{m}) gene. These insertions are referred to as FRT\textsuperscript{G2-G6}, FRT\textsuperscript{G4-G3} and FRT\textsuperscript{G2-G2}.

**Step 3: Construction of autosomal P[ovo\textsuperscript{D1}] FRT chromosomes:** The structure of the P[ovo\textsuperscript{D1}] 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[ovo\textsuperscript{D1}] FRT\textsuperscript{G2}:** We first marked P[w\textsuperscript{+}, ovd\textsuperscript{G1}]\textsuperscript{2-13X13} with the Lobe (L) dominant marker that is located at 51A2-B1. w/Y; P[w\textsuperscript{+}, ovd\textsuperscript{G1}]\textsuperscript{2-13X13}/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\textsuperscript{+}, ovd\textsuperscript{G1}]\textsuperscript{2-13X13} L were obtained. To recombine P[w\textsuperscript{+}, ovd\textsuperscript{G1}]\textsuperscript{2-13X13} and the FRT element, w/Y; P[w\textsuperscript{+}, ovd\textsuperscript{G1}]\textsuperscript{2-13X13} L/CyO males were mated to w/w; Sco FRT\textsuperscript{G4-G6}/CyO females and their progeny irradiated at the second larval instar stage. Emerging w/w; P[w\textsuperscript{+}, ovd\textsuperscript{G1}]\textsuperscript{2-13X13} L/Sco FRT\textsuperscript{G4-G6} adult males were mated to w/w; CyO/Sco females. Forty Cy males that did not have the Sco and L markers were recovered as putative P[w\textsuperscript{+}, ovd\textsuperscript{G1}]\textsuperscript{2-13X13} FRT\textsuperscript{G4-G6} recombinants. Two of these chromosomes were associated with a complete DFS phenotype and led to a high frequency of autosomal FLP-DFS technique

### Efficiency of the autosomal 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[ovo\textsuperscript{D1}]\textsuperscript{8L} FRT\textsuperscript{A}/CyO</td>
<td>No</td>
<td>0/120 (0)</td>
</tr>
<tr>
<td>P[ovo\textsuperscript{D1}]\textsuperscript{8L} FRT\textsuperscript{G}/+</td>
<td>No</td>
<td>0/122 (0)</td>
</tr>
<tr>
<td>FLP\textsuperscript{G}/+; P[ovo\textsuperscript{D1}]\textsuperscript{8L} FRT\textsuperscript{A} FRT\textsuperscript{G}</td>
<td>No</td>
<td>0/220 (0)</td>
</tr>
<tr>
<td>FLP\textsuperscript{G}/+; P[ovo\textsuperscript{D1}]\textsuperscript{8L} FRT\textsuperscript{G}</td>
<td>Yes</td>
<td>86/86 (100)</td>
</tr>
<tr>
<td>FLP\textsuperscript{G}/+; P[ovo\textsuperscript{D1}]\textsuperscript{8L} FRT\textsuperscript{A} FRT\textsuperscript{G}</td>
<td>No</td>
<td>1/120 (1)</td>
</tr>
<tr>
<td>FLP\textsuperscript{G}/+; P[ovo\textsuperscript{D1}]\textsuperscript{8L} FRT\textsuperscript{A} FRT\textsuperscript{G}</td>
<td>Yes</td>
<td>96/98 (98)</td>
</tr>
</tbody>
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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+, ovoD1]32K-2Xs9/FRT32K-X13 L were mated to w/w; CyO/ Sco females. Nine males with dark red eyes that did not have the 1 marker were recovered as putative recombinants because the eye color associated with either P[w+, ovoD1]32K-2Xs9 and P[w+, ovoD1]32K-X13 recombinants is dark orange. These putative P[w+, ovoD1]32K-FRT32K 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+, ovoD1]32K-FRT32K line we use is kept as P[w+, ovoD1]32K-FRT32K/S Tp Ms(2)M bcd/CyO.

Construction of P[w+, ovoD1]32K-FRT32K: Recombinants between P[w+, ovoD1]32K-2Xs9 and FRT32K were obtained by crossing w/Y; P[w+, ovoD1]32K-2Xs9/TM3, Sb males with w/w; FRT32K-2Xs9/FRT32K females. Their progeny were irradiated to induce germline recombination in males. Emerging w/y; P[w+, ovoD1]32K-2Xs9/FRT32K males were crossed to y w/y w females. Eighty males with dark red eyes were recovered as candidate P[w+, ovoD1]32K-2Xs9/FRT32K recombinants because the eye color associated with either P[w+, ovoD1]32K-2Xs9 and FRT32K is dark orange. Four of them turned out to be appropriate for the induction of GLCs. The P[w+, ovoD1]32K-FRT32K line we use is kept as w; P[w+, ovoD1]32K-2Xs9/FRT32K/ru h st βTub85D1; e'/TM3, Sb. The dominant, male-sterile mutation, βTub85D1, was obtained from K. MATTHEWS and T. KAUFMAN (KEMPFLIES et al. 1980).

Construction of P[w+, ovoD1]32K-FRT32K: To construct the P[w+, ovoD1]32K-FRT32K chromosome, we first marked the P[w+, ovoD1]32K-X13 chromosome with the dominant marker Roughened (R), which maps at 62B8-12. Following male

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**Figure 1.**—The autosomal FLP-DFS technique. FLP recombinase induced site-specific exchange. A chromosomal exchange that occurs in the euchromatin of a fly of genotype FLP/FLP+/+; FSC + FRT+/+ m FRT is shown. The X-linked hsp70-FLP can provide sufficient recombinase activity following heat induction to catalyze site-specific chromosomal exchange at the positions of the FRT sequences. FLP-catalyzed recombination can result in the recovery of 100% of females with m/m GLCs. Atrophic ovaries are shown as empty ovals and developed ovaries as black ovals. FLP recombinase target sequences (FRT); FS, dominant female sterile; m, recessive zygotic lethal mutation; FLP, hsp70-FLP.

**Figure 2.**—Structure of the P[w+, ovoD1]32K-FRT32K chromosomes. The location of both FRT and P[w+, ovoD1]32K insertions are shown respectively. On 2L, P[w+, ovoD1]32K-X13 contains P-element insertions on 28A and 30D (see CHOW et al. 1993). The location of P[w+, ovoD1]32K-2Xs9 is based on the location of the P[w+, ovoD1]32K-X13 insertion (for details see RESULTS AND DISCUSSION).
germ line recombination induced by X-ray treatment of larvae, we recovered seven independent chromosomes as putative \( P[\text{ovd}^{Pl}] \) recombinants. Since these lines showed a yellow eye color rather than the red eye color of the original \( P[\text{ovd}^{Pl}] \) insertion, it suggested that \( P[\text{ovd}^{Pl}] \) was not mapped by in situ hybridization (Chou et al. 1993), might carry two \( P[\text{ovd}^{Pl}] \) insertions on each separate chromosomal arm, and that one of them had been lost as the result of the recombination event. The putative \( P[\text{ovd}^{Pl}] \) chromosome may contain the original \( P[\text{ovd}^{Pl}] \) insertion (Chou et al. 1993) instead of two \( P[\text{ovd}^{Pl}] \) insertions. This putative \( P[\text{ovd}^{Pl}] \) line was used for generating \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\), w/Y; R \( P[\text{ovd}^{Pl}]^{-} \) /CxD males were crossed with w/w; FRT\(^{R-82B}\) Sb/TM6, Ubx females. Their progeny were irradiated and the emerging w/y; R \( P[\text{ovd}^{Pl}]^{-} \) /FRT\(^{R-82B}\) Sb males were crossed to y w/y w females. Seventy putative \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\) recombinant lines that have lost both R and Sb were established and one line, \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\), was kept following two rounds of neomycin selection (to select for the presence of FRT\(^{R-82B}\)). Since females heterozygous for \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\) were not associated with full DFS sterility, presumably due to the presence of a single \( P[\text{ovd}^{Pl}]^{-} \) insertion, males of genotype w/Y; FRT\(^{R-82B}\) \( P[\text{ovd}^{Pl}]^{-} \) /y\(^{506}\) Sb \( P[\text{ovd}^{Pl}]^{-} \) were created and crossed with y w/y w females to duplicate the original \( P[\text{ovd}^{Pl}]^{-} \) insertion (see Chou et al. 1993). Two independent lines were associated with a complete DFS phenotype and produced a high frequency of FLP-induced GLCs. The line we use, FRT\(^{R-82B}\) \( P[\text{ovd}^{Pl}]^{-} \) was designated \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\) and is kept as w; \( P[\text{ovd}^{Pl}]^{-} \), \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\) /h st \( \beta \) tub85D\(^{P}\) ss e'/TM3, Sb.

**Step 4: Efficiency of the technique.** To test the efficiency of these chromosomes to generate germ line mosaics, we determined both their ability to confer a DFS phenotype and to generate high frequency of female germ line mosaics. As shown in Table 1, the \( P[\text{ovd}^{Pl}]^{-} \) FRT chromosomes are associated with tight DFS as no eggs are laid by females heterozygous for these chromosomes. Examination of the ovarian phenotypes associated with these chromosomes reveal that they block oogenesis before vitellogenesis (Figure 3, C–F). The severity of the ovarian phenotypes associated with the \( P[\text{ovd}^{Pl}]^{-} \) FRT chromosomes is almost as severe as the mutant phenotype of \( \text{ovd}^{Pl} \) + / females (Figure 3B). These chromosomes most likely carry two \( P[\text{ovd}^{Pl}]^{-} \) elements (see Chou et al. 1993), both of which are necessary to confer a tight DFS phenotype. A single copy of these \( P[\text{ovd}^{Pl}]^{-} \) transposon usually does not produce sufficient expression to lead to a fully penetrant DFS phenotype (Chou et al. 1993).

When these chromosomes were tested for their efficiency in generating germ line 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 \( P[\text{ovd}^{Pl}]^{-} \) FRT recombinant chromosomes are associated with a fully penetrant DFS phenotype such that all eggs laid by these females are derived from germ line recombination events. As previously 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 side of each centromere. To construct the FRT\(^{\text{X}^{-}040}\) FRT\(^{\text{X}^{-}040}\) chromosome, FRT\(^{\text{X}^{-}040}\) FRT\(^{\text{X}^{-}040}\) was first marked with Sco and FRT\(^{\text{X}^{-}040}\) with L. Progeny from Sco FRT\(^{\text{X}^{-}040}\)/FRT\(^{\text{X}^{-}040}\) \( L \) females crossed with w/Y; CyO/ScO males were grown on media containing G418 to select for the presence of the \( P[\text{ovd}^{Pl}]^{-} \) element. Potential recombinants were selected and tested for their abilities to promote the induction of GLCs in the presence of the \( P[\text{ovd}^{Pl}]^{-} \) FRT chromosomes. A similar strategy was used to build

![Figure 3.—Ovarian phenotypes associated with \( P[\text{ovd}^{Pl}]^{-} \) FRT chromosomes. The ovarian phenotypes are shown as follows: (A) Oregon R wild type, (B) \( \text{ovd}^{Pl} \) + /, (C) \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\) /+, (D) \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\) /+, (E) \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\) /+, and (F) \( P[\text{ovd}^{Pl}]^{-} \) FRT\(^{-}\) /+ females are shown. The ovaries of 5-day-old, well fed females were dissected in Ringer’s solution. In each picture a number of ovaries are shown: two in A, eight in B, C, D and F, six in E.]
1: Test 2L

Step 1A:

\[ \varnothing \varnothing^{w/w; \{FRT^{2L}, FRT^{2R}\}^{+/\sim}/CyO} \times 2 \sigma \sigma^{w/w; FLP/Y; P[ovoD1]^{2L}/FRT^{2L}/CyO} \]

Heat shock the progeny at 37° for 2 hours

Step 2A:

Select 15 \varnothing w/w FLP; \{FRT^{2L}, FRT^{2R}\}^{+/\sim}/P[ovoD1]^{2L}/FRT^{2L} \times 5 \sigma \sigma^{w/Y; \{FRT^{2L}, FRT^{2R}\}^{+/\sim}/P[ovoD1]^{2L}/FRT^{2L}}

Determine the maternal effect

2: Test 2R

Step 1B:

\[ \varnothing \varnothing^{w/w; \{FRT^{2L}, FRT^{2R}\}^{+/\sim}/CyO} \times 2 \sigma \sigma^{w/w; FLP/Y; FRT^{2R} P[ovoD1]^{2R}/CyO} \]

Heat shock the progeny at 37° for 2 hours

Step 2B:

Select 15 \varnothing w/w FLP; \{FRT^{2L}, FRT^{2R}\}^{+/\sim}/\{FRT^{2R} P[ovoD1]^{2R}\} \times 5 \sigma \sigma^{w/Y; \{FRT^{2L}, FRT^{2R}\}^{+/\sim}/\{FRT^{2R} P[ovoD1]^{2R}\}}

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 \( P[ovo^{+/\sim}]^{2R} \) FRT chromosome as well as an X chromosome that contains the FLP recombinase. The other batch is mated to two males that carry the \( FRT^{2R} P[ovo^{+/\sim}]^{2R} \) 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 \( w/w \) FLP; \( \{FRT^{2R}, FRT^{2R}\}^{+/\sim}/FRT^{2R} P[ovo^{+/\sim}]^{2R} \) and 15 females of genotype \( w/w \) FLP; \( \{FRT^{2R}, FRT^{2R}\}^{+/\sim}/P[ovo^{+/\sim}]^{2R} \) 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 \( FRT^{2R}, FRT^{2R} \) 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).

CONCLUSION

In summary, we have developed a method that allows the efficient recovery of female germline mosaics for mutations localized on the autosomes. The \( P[ovo^{+/\sim}] \) 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 \( \sim 1–2\% \) (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 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.

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LITERATURE CITED


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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, y f/w ovo ^1/24 P[ mini w ]; FRT ^101/Y; P[ ry ]; FLP ^38/P[ ry ]; FLP ^38

#1843 C(1)DX, y f/w ovo ^1/24 P[ mini w ]; FRT ^92/Y; P[ ry ]; FLP ^38/P[ ry ]; FLP ^38

#1903 y w ^24 P[ mini w ]; FRT ^101

Flipase stocks to generate germine clones on the autosomes:

#1929 y w P[ ry ]; FLP ^12%; CyO/Sco

#1970 y w P[ ry ]; FLP ^22%; TM3, Sh/CyO

FRT ^2:

#1622 P[h+ neo; ry ]; FRT ^3.404/; ry

#1821 Tfl P[h+ neo; ry ]; FRT ^3.404/CyO

#2125 P[ mini w ]; ovo ^1/2.13X1 P[h+ neo; ry ]; FRT ^3.404/S Sp Ms(2)M bsd/; CyO

FRT ^3:

#1956 w; P[ mini w ]; FRT ^3.4G13

#1958 w; P[ mini w ]; FRT ^3.4G13 L/CyO

#2125 Tfl P[ mini w ]; FRT ^3.4G13 P[ mini w ]; ovo ^1/2.13X9/S Sp Ms(2)M bsd/; CyO

FRT ^4:

#1997 w; P[ mini w ]; FRT ^4.2A

#2024 w; D P[ mini w ]; FRT ^4.2A/TM3, Sh

#2139 w; P[ mini w ]; ovo ^1/2.24X6 P[ mini w ]; FRT ^4.2A/rf h st β-Tub85D ^1 ss e'/TM3, Sh

FRT ^5:

#2035 P[h+ neo; ry ]; FRT ^5.828/; ry

#2051 y w; P[h+ neo; ry ]; FRT ^5.828 Sh/TM6, Ubx

#2149 w; P[h+ neo; ry ]; FRT ^5.828 P[ mini w ]; ovo ^1/2.13X1G3331X9/rf h st β-Tub85D ^1 ss e'/TM3, Sh

APPENDIX