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 ovo^{D1} 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 ovo^{D1} (BUSSON *et al.* 1983; PERRIMON and GANS 1983; PERRIMON 1984). ovo^{D1} 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 ovo^{D1} gene ($P[ovo^{D1}]$) 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 ovo^{D1} are available on all chromosomal arms (see Introduction of CHOU *et al.* 1993). These $P[ovo^{D1}]$ 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 $P[ovo^{D1}]$ 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

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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^hs>, FRT]*: *P[>w^hs>, FRT]* insertions were isolated following the transposition of the *X*-linked FRT^{101} insertion as previously described (CHOU and PERRIMON 1992) using the *Δ2-3* "jumpstarter" strain (ROBERTSON *et al.* 1988). The two transposase stocks, ry^{506} *P[ry^+, Δ2-3]* and ry^{506} *Sb P[ry^+, 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 *pP[>w^hs>]* plasmid (GOLIC and LINDQUIST 1989).

Neomycin selection of fly strains 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 dH_2O) was added to standard fly medium.

***FRT* chromosomes with dominant markers:** The dominant markers are described in LINDSLEY and ZIMM (1992). Chromosomes carrying the *FRT* elements in *cis* with dominant markers were constructed to allow recombination with specific mutations. $FRT^{2L\text{-}40A}$ was marked with *Sco*, $FRT^{2R\text{-}G13}$ with *L*, $FRT^{3L\text{-}2A}$ with *D*, and $FRT^{3R\text{-}82B}$ with *Sb*. The following stocks were built to facilitate the construction of additional strains: *w/w; Sco* $FRT^{2L\text{-}40A}$*/CyO, w/w;* $FRT^{2R\text{-}G13}$ *L/CyO, w/w; D* $FRT^{3L\text{-}2A}$*/TM3, Sb* and *w/w;* $FRT^{3R\text{-}82B}$ *Sb/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 LINDQUIST (1989) carries an *hsp70-FLP* fusion gene and the *rosy* (*ry*) gene. Using the *Δ2-3* transposase, we mobilized a *hsp70-FLP P*-element transgene, FLP^{38}, located on the second chromosome (CHOU and PERRIMON 1992), onto an *X* chromosome that carries the ovo^{S1} mutation. ovo^{S1} 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^D1]* 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^{S1} was recombined away from these *FLP* insertions because the *P[ovo^D1]* autosomal insertions that we ultimately recovered were fully penetrant for DFS in the presence of two wild-type copies of *ovo*.

The jumps were generated as follows: *w/Y; CyO/+; MKRS/+* males were crossed with *FM3/*ovo^{S1} v^{24} females. Subsequently, ovo^{S1} v^{24}*/Y; CyO/+; MKRS/+* males were crossed with *C(1)DX, y f/Y;* FLP^{38}*/+; Δ2-3/+* females and their male progeny of genotype ovo^{S1} v^{24}*/Y; CyO/*FLP^{38} *(or +); MKRS/Δ2-3 (or +)* crossed with *C(1)DX, y f/Y; ry/ry* females. *X*-linked jumps of *P[ry^+, hsFLP]* were identified among ovo^{S1} v^{24} *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 w FLP/y w* females were crossed with *w/Y;* FRT^{75A}*/*FRT^{75A} males and their progeny heat shocked for 2 hr at 37° in a water bath during the first instar larvae. The efficiency of the flipase was determined by examining the frequency of *y w FLP/y w;* FRT^{75A}*/+* progeny that show mosaic eyes for the *white* marker. FRT^{75A} contains the *mini-white* gene (GOLIC and LINDQUIST 1989). FLP^{12} and FLP^{22} 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^{12}*; CyO/Sco* and *y w* FLP^{22}*; TM3, Sb/CxD* 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^{12}*/Y; CyO/P[*ovo^{D1}*] FRT* or FLP^{22}*/Y; TM3, Sb/P[*ovo^{D1}*] FRT*. These males were generated by crossing females from the *y w* FLP^{12}*; CyO/Sco* and *y w* FLP^{22}*; TM3, Sb/CxD* stocks with the appropriate *P[*ovo^{D1}*] 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° 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[*ovo^{D1}*]* 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^hs>, 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^{D1}*]* insertions. We recombined *P[*ovo^{D1}*]* and *FRT* elements following mitotic recombination in the male germline. Subsequently, the *P[*ovo^{D1}*] 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[*ovo^{D1}*]*: The four autosomal *P[*ovo^{D1}*]* 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^{D1}*]*$^{2L\text{-}13X13}$, *P[w^+,* ovo^{D1}*]*$^{2R\text{-}32X9}$, *P[w^+,* ovo^{D1}*]*$^{3L\text{-}2X48}$ and *P[w^+,* ovo^{D1}*]*$^{3R\text{-}C13X3}$.

Step 2: Selection of *FRT* insertions close to centromeres: We conducted a screen to isolate new autosomal *P[>w^hs>, 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

TABLE 1

Efficiency of the autosomal FLP-DFS technique

Genotype	Heat shock	N females with eggs/N females examined
2L		
$P[ovo^{D1}]^{2L}$ FRT^{2L}/*CyO*	No	0/120 (0)
$P[ovo^{D1}]^{2L}$ FRT^{2L}/+	No	0/122 (0)
Flp^{12}/+; $P[ovo^{D1}]^{2L}$ FRT^{2L}/FRT^{2L}	No	0/220 (0)
Flp^{12}/+; $P[ovo^{D1}]^{2L}$ FRT^{2L}/FRT^{2L}	Yes	86/86 (100)
Flp^{22}/+; $P[ovo^{D1}]^{2L}$ FRT^{2L}/FRT^{2L} FRT^{2R}	No	1/120 (1)
Flp^{22}/+; $P[ovo^{D1}]^{2L}$ FRT^{2L}/FRT^{2L} FRT^{2R}	Yes	96/98 (98)
2R		
$P[ovo^{D1}]^{2R}$ FRT^{2R}/*CyO*	No	0/110 (0)
$P[ovo^{D1}]^{2R}$ FRT^{2R}/+	No	0/180 (0)
Flp^{12}/+; $P[ovo^{D1}]^{2R}$ FRT^{2R}/FRT^{2R}	No	0/240 (0)
Flp^{12}/+; $P[ovo^{D1}]^{2R}$ FRT^{2R}/FRT^{2R}	Yes	102/102 (100)
Flp^{12}/+; $P[ovo^{D1}]^{2R}$ FRT^{2R}/FRT^{2R}	No	0/134 (0)
Flp^{12}/+; $P[ovo^{D1}]^{2R}$ FRT^{2R}/FRT^{2L} FRT^{2R}	Yes	46/48 (96)
3L		
$P[ovo^{D1}]^{3L}$ FRT^{3L}/*TM3, Sb*	No	0/125 (0)
$P[ovo^{D1}]^{3L}$ FRT^{3L}/+	No	0/220 (0)
Flp^{22}/+; $P[ovo^{D1}]^{3L}$ FRT^{3L}/FRT^{3L}	No	0/203 (0)
Flp^{22}/+; $P[ovo^{D1}]^{3L}$ FRT^{3L}/FRT^{3L}	Yes	89/90 (99)
Flp^{22}/+; $P[ovo^{D1}]^{3L}$ FRT^{3L}/FRT^{3L} FRT^{3R}	No	0/100 (0)
Flp^{12}/+; $P[ovo^{D1}]^{3L}$ FRT^{3L}/FRT^{3L} FRT^{3R}	Yes	46/48 (99)
3R		
$P[ovo^{D1}]^{3R}$ FRT^{3R}/*TM3. Sb*	No	0/160 (0)
$P[ovo^{D1}]^{3R}$ FRT^{3R}/+	No	0/110 (0)
Flp^{22}/+; $P[ovo^{D1}]^{3R}$ FRT^{3R}/FRT^{3R}	No	0/184 (0)
Flp^{22}/+; $P[ovo^{D1}]^{3R}$ FRT^{3R}/FRT^{3R}	Yes	68/68 (100)
Flp^{22}/+; $P[ovo^{D1}]^{3R}$ FRT^{3R}/FRT^{3L} FRT^{3R}	No	1/120 (1)
Flp^{22}/+; $P[ovo^{D1}]^{3R}$ FRT^{3R}/FRT^{3L} FRT^{3R}	Yes	96/100 (96)

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.

insertion lines that we recovered are FRT^{G31} at position 39E, FRT^{G13} at position 42B, FRT^{G5} at position 78E and FRT^{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^{40A} and FRT^{82B}, which contains the *hsneo* gene, located at 40A and 82B, respectively (XU and RUBIN 1993); FRT^{2A}, isolated by KENT GOLIC (unpublished results) and mapped to position 79D-F by M. SOTO (personal communication); and FRT^{G13}. The two *FRT* insertions FRT^{G13} and FRT^{2A} contain the *mini-white* (w^{hs}) gene. These insertions are referred to as $FRT^{2L\text{-}40A}$, $FRT^{2R\text{-}G13}$, $FRT^{3L\text{-}2A}$ and $FRT^{3R\text{-}82B}$.

Step 3: Construction of autosomal $P[ovo^{D1}]$ *FRT* chromosomes: The structure of the $P[ovo^{D1}]$ *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 $P[ovo^{D1}]^{2L}$ FRT^{2L}: We first marked $P[w^+, ovo^{D1}]^{2L\text{-}13X13}$ with the *Lobed* (*L*) dominant marker that is located at 51A2-B1. *w/Y;* $P[w^+, ovo^{D1}]^{2L\text{-}13X13}$*/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 $P[w^+, ovo^{D1}]^{2L\text{-}13X13}$ *L* were obtained. To recombine $P[w^+, ovo^{D1}]^{2L\text{-}13X13}$ and the *FRT* element, *w/Y;* $P[w^+, ovo^{D1}]^{2L\text{-}13X13}$ *L/CyO* males were mated to *w/w; Sco* $FRT^{2L\text{-}40A}$*/CyO* females and their progeny irradiated at the second larval instar stage. Emerging *w/Y;* $P[w^+, ovo^{D1}]^{2L\text{-}13X13}$ *L/Sco* $FRT^{2L\text{-}40A}$ 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 $P[w^+, ovo^{D1}]^{2L\text{-}13X13}$ $FRT^{2L\text{-}40A}$ recombinants. Two of these chromosomes were associated with a complete DFS phenotype and led to a high frequency of GLCs when tested for their abilities to undergo FLP-induced mitotic exchange (see below). The $P[ovo^{D1}]^{2L}$ FRT^{2L} line we use is kept as $P[w^+, ovo^{D1}]^{2L\text{-}13X13}$ $FRT^{2L\text{-}40A}$*/S Sp Ms(2)M* bw^D*/CyO. Ms(2)M* is a dominant, male-sterile mutation that was obtained from D. LINDSLEY (personal communication).

Construction of $P[ovo^{D1}]^{2R}$ FRT^{2R}: Progeny from the cross between *w/Y;* $P[w^+, ovo^{D1}]^{2R\text{-}32X9}$*/CyO* males with *w/w;* $FRT^{2R\text{-}G13}$ *L/CyO* females were irradiated at the

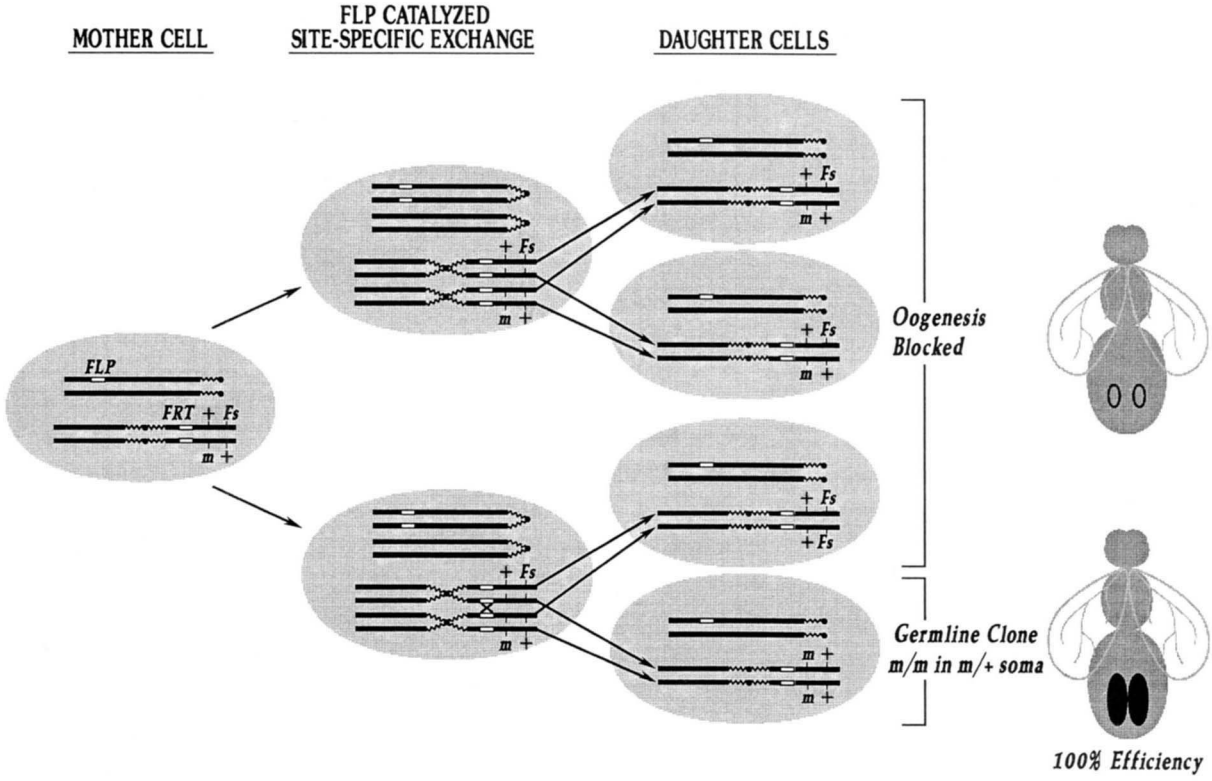

FIGURE 1.—The autosomal FLP-DFS technique. FLP-recombinase induced site-specific exchange. A chromosomal exchange that occurs in the euchromatin of a fly of genotype *Flp/Flp/+; FS + FRT/+ m FRT* is shown. The *X*-linked *hsp70-FLP* can provide sufficient recombinase activity following heat induction 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.

second larval instar stage to induce male germline recombination. Male progeny of genotype *w/Y; P[w+, ovo^{D1}]$^{2R\text{-}32X9}$/FRT$^{2R\text{-}G13}$ L* 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+, ovo^{D1}]$^{2R\text{-}32X9}$ FRT$^{2R\text{-}G13}$* recombinants because the eye color associated with either *P[w+, ovo^{D1}]$^{2R\text{-}32X9}$* and

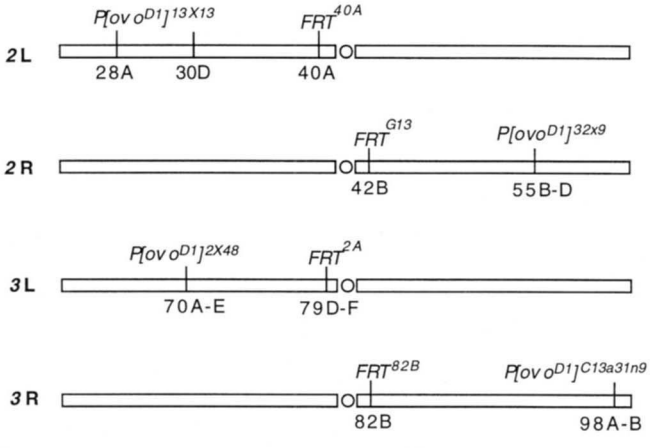

FIGURE 2.—Structure of the *P[ovo^{D1}] FRT* chromosomes. The location of both *FRT* and *P[ovo^{D1}]* insertions are shown respectively. On 2L, *P[ovo^{D1}]$^{2L\text{-}13X13}$* contains *P*-element insertions on 28A and 30D (see CHOU *et al.* 1993). The location of *P[ovo^{D1}]^{C13a31n9}* is based on the location of the *P[ovo^{D1}]^{C13}* insertion (for details see RESULTS AND DISCUSSION).

FRT$^{2R\text{-}G13}$ is dark orange. These putative *P[ovo^{D1}]^{2R} FRT^{2R}* 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[ovo^{D1}]^{2R} FRT^{2R}* line we use is kept as *P[w+, ovo^{D1}]$^{2R\text{-}32X9}$ FRT$^{2R\text{-}G13}$/S Sp Ms(2)M bw^{D}/CyO.*

Construction of P[ovo^{D1}]^{3L} FRT^{3L}: Recombinants between *P[ovo^{D1}]^{3L}* and *FRT^{3L}* were obtained by crossing *w/Y; P[w+, ovo^{D1}]$^{3L\text{-}2X48}$/TM3, Sb* males with *w/w; FRT$^{3L\text{-}2A}$/FRT$^{3L\text{-}2A}$* females. Their progeny were irradiated to induce germline recombination in males. Emerging *w/Y; P[w+, ovo^{D1}]$^{3L\text{-}2X48}$/FRT$^{3L\text{-}2A}$* males were crossed to *y w/y w* females. Eighty males with dark red eyes were recovered as candidate *P[w+, ovo^{D1}]$^{3L\text{-}2X48}$ FRT$^{3L\text{-}2A}$* recombinants because the eye color associated with either *P[w+, ovo^{D1}]$^{3L\text{-}2X48}$* and *FRT$^{3L\text{-}2A}$* is dark orange. Four of them turned out to be appropriate for the induction of GLCs. The *P[ovo^{D1}]^{3L} FRT^{3L}* line we use is kept as *w; P[w+, ovo^{D1}]$^{3L\text{-}2X48}$ FRT$^{3L\text{-}2A}$/ru h st βTub85D^{D} ss e^{s}/TM3, Sb.* The dominant, male-sterile mutation, *βTub85D^{D}*, was obtained from K. MATTHEWS and T. KAUFMAN (KEMPHUES *et al.* 1980).

Construction of P[ovo^{D1}]^{3R} FRT^{3R}: To construct the *P[ovo^{D1}]^{3R} FRT^{3R}* chromosome, we first marked the *P[w+, ovo^{D1}]$^{3R\text{-}C13X3}$* 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[ovo^{D1}]$^{3R\text{-}C13X3}$* recombinants. Since these lines showed a yellow eye color rather than the red eye color of the original *P[ovo^{D1}]$^{3R\text{-}C13X3}$* insertion, it suggested that *P[ovo^{D1}]$^{3R\text{-}C13X3}$*, which was not mapped by *in situ* hybridization (CHOU *et al.* 1993), might carry two *P[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[ovo^{D1}]$^{3R\text{-}C13X3}$* chromosome may contain the original *P[ovo^{D1}]^{C13}* insertion (CHOU *et al.* 1993) instead of two *P[ovo^{D1}]* insertions. This putative *R P[ovo^{D1}]^{C13}* line was used for generating *P[ovo^{D1}]^{3R} FRT^{3R}*: *w/Y; R P[ovo^{D1}]^{C13}/CxD* males were crossed with *w/w; FRT$^{3R\text{-}82B}$ Sb/TM6, Ubx* females. Their progeny were irradiated and the emerging *w/Y; R P[ovo^{D1}]^{C13}/FRT$^{3R\text{-}82B}$ Sb* males were crossed to *y w/y w* females. Seventy putative *P[ovo^{D1}]^{C13} FRT^{3R}* recombinant lines that have lost both *R* and *Sb* were established and one line, *P[ovo^{D1}]^{C13a31} FRT^{3R}*, was kept following two rounds of neomycin selection (to select for the presence of *FRT$^{3R\text{-}82B}$*). Since females heterozygous for *P[ovo^{D1}]^{C13a31} FRT^{3R}* were not associated with full DFS sterility, presumably due to the presence of a single *P[ovo^{D1}]* insertion, males of genotype *w/Y; FRT$^{3R\text{-}82B}$ P[ovo^{D1}]^{C13a31}/ry^{506} Sb P[ry^{+},Δ2-3]* were created and crossed with *y w/y w* females to duplicate the original *P[ovo^{D1}]^{C13}* 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$^{3R\text{-}82B}$ P[ovo^{D1}]$^{3R\text{-}C13a31n9}$* was designated as *P[ovo^{D1}]^{3R} FRT^{3R}* and is kept as *w; P[w^{+}, ovo^{D1}]$^{3R\text{-}C13a31n9}$ FRT$^{3R\text{-}82B}$/ru h st βTub85D^{D} ss e^{s}/TM3, Sb.*

Step 4: 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. As shown in Table 1, the *P[ovo^{D1}] 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[ovo^{D1}] FRT* chromosomes is almost as severe as the mutant phenotype of *ovo^{D1}/+* females (Figure 3B). These chromosomes most likely carry two *P[ovo^{D1}]* elements (see CHOU *et al.* 1993), both of which are necessary to confer a tight DFS phenotype. A single copy of these *P[ovo^{D1}]* 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 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[ovo^{D1}] FRT* recombinant chromosomes are associated with a fully penetrant DFS phenotype such that all eggs laid by these females are derived from germline 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.

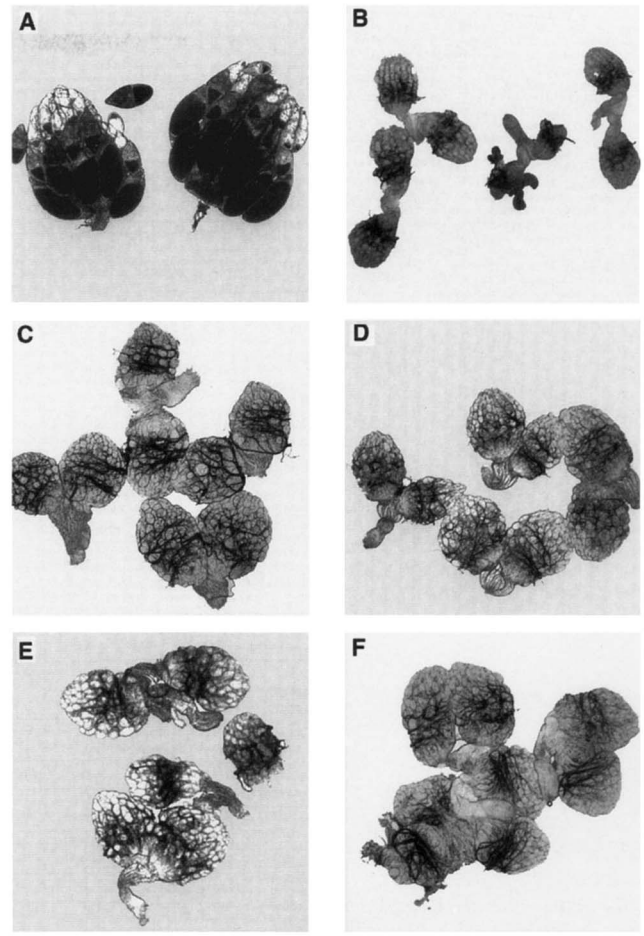

FIGURE 3.—Ovarian phenotypes associated with *P[ovo^{D1}] FRT* chromosomes. The ovarian phenotypes are shown as follows: (A) *Oregon R* wild type, (B) *ovo^{D1}/+*, (C) *P[ovo^{D1}]^{2L} FRT^{2L}/+*, (D) *P[ovo^{D1}]^{2R} FRT^{2R}/+*, (E) *P[ovo^{D1}]^{3L} FRT^{3L}/+*, and (F) *P[ovo^{D1}]^{3R} FRT^{3R}/+* 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.

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$^{2L\text{-}40A}$ FRT$^{2R\text{-}G13}$* chromosome, *FRT$^{2R\text{-}G13}$* was first marked with *Sco* and *FRT$^{2L\text{-}40A}$* with *L*. Progeny from *Sco FRT$^{2R\text{-}G13}$/FRT$^{2L\text{-}40A}$ L* females crossed with *w/Y; CyO/Sco* males were grown on media containing G418 to select for the presence of the *P[ry^{+}, hs-neo, FRT]* element. Potential recombinants were selected and tested for their abilities to promote the induction of GLCs in the presence of the *P[ovo^{D1}] FRT* chromosomes. A similar strategy was used to build

1: Test 2L

Step 1A:

5♀♀ *w/w; [FRT*^{2L}*, FRT*^{2R}*]*/ CyO* X 2♂♂ *w FLP/Y;P[ovo*^{D1}*]2L FRT*^{2L}*/CyO*

Heat shock the progeny at 37° for 2 hours

Step 2A:

Select 15 ♀♀ *w/w FLP; [FRT*^{2L}*, FRT*^{2R}*]*/P[ovo*^{D1}*]2L FRT*^{2L}

X 5♂♂ *w/Y; [FRT*^{2L}*, FRT*^{2R}*]*/P[ovo*^{D1}*]2L FRT*^{2L}

Determine the maternal effect

2: Test 2R

Step 1B:

5♀♀ *w/w; [FRT*^{2L}*, FRT*^{2R}*]*/ CyO* X 2♂♂ *w FLP/Y;FRT*^{2R} *P[ovo*^{D1}*]2R/CyO*

Heat shock the progeny at 37° for 2 hours

Step 2B:

Select 15 ♀♀ *w/w FLP; [FRT*^{2L}*, FRT*^{2R}*]*/FRT*^{2R} *P[ovo*^{D1}*]2R*

X 5♂♂ *w/Y; [FRT*^{2L}*, FRT*^{2R}*]*/FRT*^{2R} *P[ovo*^{D1}*]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 $P[ovo^{D1}]^{2L}$ FRT^{2L} 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} $P[ovo^{D1}]^{2R}$ 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;* $[FRT^{2L}, FRT^{2R}]^*/FRT^{2R}$ $P[ovo^{D1}]^{2R}$ and 15 females of genotype *w/w FLP;* $[FRT^{2L}, FRT^{2R}]^*/P[ovo^{D1}]^{2L}$ FRT^{2L} 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 $[FRT^{2L}, FRT^{2R}]^*$ 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).

the $FRT^{3L\text{-}2A}$ $FRT^{3R\text{-}82B}$ recombinant chromosome: $FRT^{3L\text{-}2A}$ was marked with *Sb* and $FRT^{3R\text{-}82B}$ with *D*. Then we determined whether these double *FRT* chromosomes work efficiently for the recovery of GLCs. GLCs were induced following the protocol described in MATERIALS AND METHODS. 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 $P[ovo^{D1}]$ *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.

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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^{D1} v^{24} $P[mini\ w^{+}; FRT]^{101}/Y$; $P[ry^{+}; FLP]^{38}/P[ry^{+}; FLP]^{38}$

#1843 *C(1)DX, y f/*ovo^{D2} v^{24} $P[mini\ w^{+}; FRT]^{9\text{-}2}/Y$; $P[ry^{+}; FLP]^{38}/P[ry^{+}; FLP]^{38}$

#1844 *y w* v^{24} $P[mini\ w^{+}; FRT]^{101}$

#1903 *y w* v^{24} $P[mini\ w^{+}; FRT]^{9\text{-}2}$

Flipase stocks to generate germline clones on the autosomes:

#1929 *y w* $P[ry^{+}; FLP]^{12}$; *CyO/Sco*

#1970 *y w* $P[ry^{+}; FLP]^{22}$; *TM3, Sb/CxD*

***FRT*^{2L}:**

#1622 $P[hs\ neo; ry^{+}; FRT]^{2L\text{-}40A}$; *ry*
#1821 *Tft* $P[hs\ neo; ry^{+}; FRT]^{2L\text{-}40A}$/*CyO*

***P*[*ovo*^{D1}]^{2L} *FRT*^{2L}:**

#2121 $P[mini\ w^{+}; ovo^{D1}]^{2L\text{-}13X13}$ $P[hs\ neo; ry^{+}; FRT]^{2L\text{-}40A}$/ *S Sp Ms(2)M* bw^{D}/*CyO*

***FRT*^{2R}:**

#1956 *w;* $P[mini\ w^{+}; FRT]^{2R\text{-}G13}$

#1958 *w;* $P[mini\ w^{+}; FRT]^{2R\text{-}G13}$ *L/CyO*

***P*[*ovo*^{D1}]^{2R} *FRT*^{2R}:**

#2125 $P[mini\ w^{+}; FRT]^{2R\text{-}G13}$ $P[mini\ w^{+}; ovo^{D1}]^{2R\text{-}32X9}$/*S Sp Ms(2)M* bw^{D}/*CyO*

***FRT*^{3L}:**

#1997 *w;* $P[mini\ w^{+}; FRT]^{3L\text{-}2A}$

#2024 *w; D* $P[mini\ w^{+}; FRT]^{3L\text{-}2A}$/*TM3, Sb*

***P*[*ovo*^{D1}]^{3L} *FRT*^{3L}:**

#2139 *w;* $P[mini\ w^{+}; ovo^{D1}]^{3L\text{-}2X48}$ $P[mini\ w^{+}; FRT]^{3L\text{-}2A}$/ *ru h st* $\beta Tub85D^{D}$ *ss* e^{s}/*TM3, Sb*

***FRT*^{3R}**

#2035 $P[hs\ neo; ry^{+}; FRT]^{3R\text{-}82B}$; *ry*

#2051 *y w;* $P[hs\ neo; ry^{+}; FRT]^{3R\text{-}82B}$ *Sb/TM6, Ubx*

***P*[*ovo*^{D1}]^{3R} *FRT*^{3R}:**

#2149 *w;* $P[hs\ neo; ry^{+}; FRT]^{3R\text{-}82B}$ $P[mini\ w^{+}; ovo^{D1}]^{3R\text{-}C13a31n9}$/*ru h st* $\beta Tub85D^{D}$ *ss* e^{s}/*TM3, Sb*