

FILE S1

Supporting Methods

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

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

Crosses to generate *inversion + attached-XY* chromosomes**Step 1. Placing the proximal *FRT*-bearing transposon insertions onto the *attached-XY* chromosome by meiotic recombination:**

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

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

G2: $C(1)RA, In(1)sc^{\bar{1}}, In(1)sc^{\bar{2}}, l(1)1Ac^1/Dp(1;Y)y^+ \text{♀} \times C(1;Y)N12, w^{1118} P\{w^{+mW.Scer\backslash FRT.hs=RS5}\} (f^1), B^S/Dp(1;Y)y^+ \text{♂}$

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

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

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

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

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

♂

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

$P\{RS3\}$ Set A

Forward primer: CAAAAACGCACCGGACTGTAAC

Reverse primer: CATTGTTTCAGATGCTCGGCAG

$P\{RS3\}$ Set B

Forward primer: CGCACATACAGCTCACTGTTTAC

Reverse primer: GATAGCCGAAGCTTACCGAAGT

$P\{RS5\}$ Set C

Forward primer: CAAAAACGCACCGGACTGTAAC

Reverse primer: GATAGCCGAAGCTTACCGAAGT

$P\{RS5\}$ Set D

Forward primer: AAGCATGCTGCGACGTGAAC

Reverse primer: GATAGCCGAAGCTTACCGAAGT

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

G0: $FM7j, y^{93j} w^l B^+ \text{♀} \times w^{1118}/Y; noc^{Sc}/SM6b, P\{y^{+17.2}=70FLP\}7 \text{♂}$

G1: $C(1;Y)N12, P\{w^{+mW.Ser\backslash FRT.hs}=RS3\}CB-5805-3 w^{1118} P\{w^{+mW.Ser\backslash FRT.hs}=RS5\} (f^l), B^S/FM7j, y^{93j} w^l B^+ \text{♀} \times FM7j, y^{93j} w^l B^+/Y; +/SM6b, P\{y^{+17.2}=70FLP\}7 \text{♂}$

G2: $C(1;Y)N12, P\{w^{+mW.Ser\backslash FRT.hs}=RS3\}CB-5805-3 w^{1118} P\{w^{+mW.Ser\backslash FRT.hs}=RS5\} (f^l), B^S/FM7j, y^{93j} w^l B^+; +/SM6b, P\{y^{+17.2}=70FLP\}7 \text{♀}$
(heat shocked as larvae at 37° for one hour three days after cultures established) $\times FM7j, y^{93j} w^l B^+/Dp(1;Y)y^+ \text{♂}$

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

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

Step 4. Inducing inversions by FLP-mediated recombination:

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

G0: *winscy* ♀ x *w¹¹¹⁸/Y*; *noc^{Scn}/SM6b*, *P{y⁺17.2=70FLP}7* ♂

G1: *winscy/C(1;Y)N12*, *P{w^{RS3r}=RS3r}CB-5805-3 w¹¹¹⁸ P{w^{RS5r.hs}=RS5r} (fl)*, *B^S ♀* x *winscy/Y*; *+/SM6b*, *P{y⁺17.2=70FLP}7* ♂

G2: *C(1;Y)N12*, *P{w^{RS3r}=RS3r}CB-5805-3 w¹¹¹⁸ P{w^{RS5r.hs}=RS5r} (fl)*, *B^S/winscy*; *+/SM6b*, *P{y⁺17.2=70FLP}7* ♀ (heat shocked as larvae at 37° for one hour five days after cultures established) x *winscy/Dp(1;Y)⁺* ♂

G3: *C(1;Y)N12*, *In(1)BSC*, *P{w^{+mW.Scr\FRT.hs3}=3'.RS5+3.3'}BSC w¹¹¹⁸ (fl)*, *B^S/winscy* (red-eyed) ♀ x *winscy/Dp(1;Y)⁺* ♂

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

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

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

G0: *C(1)M3, y²/0* ♀ x *C(1;Y)N12*, *In(1)BSC*, *P{w^{+mW.Scr\FRT.hs3}=3'.RS5+3.3'}BSC w¹¹¹⁸ (fl)*, *B^S/Dp(1;Y)⁺* ♂

G1: *C(1)M3, y²/0* ♀ x *C(1;Y)N12*, *In(1)BSC*, *P{w^{+mW.Scr\FRT.hs3}=3'.RS5+3.3'}BSC w¹¹¹⁸ (fl)*, *B^S/0* ♂

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

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

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

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

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

G0: $w^{1118} P\{w^{+mW.Scer\setminus FRT.hs}=RS\} \text{♀} \times w^{1118}/Y; noc^{Sco}/SM6b, P\{\gamma^{+17.2}=70FLP\}7 \text{♂}$

G1: $C(1)RA, In(1)sc^{\bar{1}}, In(1)sc^{\bar{8}}, l(1)lAc^l/Dp(1;Y)^+ \text{♀} \times w^{1118} P\{w^{+mW.Scer\setminus FRT.hs}=RS\}/Y; +/SM6b, P\{\gamma^{+17.2}=70FLP\}7 \text{♂}$ (heat shocked as larvae at 37° for one hour three days after cultures established)

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

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

Step 2. Recovering recombinant chromosomes by meiotic recombination and inducing inversions by FLP-mediated

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

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

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

G2: $P\{w^{RS3r}=RS3r\} w^{1118}/w^{1118} P\{w^{RS5r.hs}=RS5r\} \text{♀} \times w^{1118}/Y; noc^{Sco}/SM6b, P\{\gamma^{+17.2}=70FLP\}7 \text{♂}$

G3: $P\{w^{+mW.Scer\setminus FRT.hs}=RS3\}l(1)CB-6411-3^l, w^{1118}/FM7h, y^{93j} w^l B^l \text{♀} \times P\{w^{RS3r}=RS3r\} w^{1118} P\{w^{RS5r.hs}=RS5r\}/Y; +/SM6b,$

$P\{\gamma^{+17.2}=70FLP\}7 \text{♂}$ (heat shocked as larvae at 37° for one hour for three days beginning three days after cultures established; males carrying recombinant chromosomes recognized from w^+ clonal eye sectoring)

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

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

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

G0: $In(1)BSC, P\{w^{+m}W.Scer\backslash FRT.hs3=3'.RS5+3.3'\}BSC w^{1118}/FM7h, y^{93j} w^l B^l \text{♀} \times C(1;Y)N12, In(1)BSC25,$

$P\{w^{+m}W.Scer\backslash FRT.hs3=3'.RS5+3.3'\}BSC w^{1118} fl, B^S/0 \text{♂}$

G1: $In(1)BSC, P\{w^{+m}W.Scer\backslash FRT.hs3=3'.RS5+3.3'\}BSC w^{1118}/C(1;Y)N12, In(1)BSC25, P\{w^{+m}W.Scer\backslash FRT.hs3=3'.RS5+3.3'\}BSC w^{1118} fl, B^S \text{♀} \times$

$C(1;Y)1, y^1/0 \text{♂}$

G2: $C(1)M3, y^2/0 \text{♀} \times C(1;Y)N12, In(1)BSC, P\{w^{+m}W.Scer\backslash FRT.hs3=3'.RS5+3.3'\}BSC w^{1118}, B^S/0 \text{♂}$

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

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

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

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

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

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

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

Rescuing female-specific phenotypes in XXY females

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

$P\{w^{+mW.Scer\FRT.hs3}\}BSC77 w^{1118}$, B^S females. Similar crosses were undertaken with $Dp(1;Y)BSC79, y^+ P\{w^{+mW.Scer\FRT.hs3}\}BSC79 w^{1118}$, B^S . In the third set of crosses, $winscy, y^1 w^1/winscy, y^1 w^1/Dp(1;Y)BSC15, y^+ P\{w^{+mW.Scer\FRT.hs3}\}BSC15 w^{1118}$, B^S females recovered directly from the stock were crossed to $f^1 fu^1/Y$ males to produce $f^1 fu^1/winscy, y^1 w^1/Dp(1;Y)BSC15, y^+ P\{w^{+mW.Scer\FRT.hs3}\} BSC15 w^{1118}$, B^S females. These females were crossed to $f^1 fu^1/Y$ males to produce $f^1 fu^1/f^1 fu^1/Dp(1;Y)BSC15, y^+ P\{w^{+mW.Scer\FRT.hs3}\}BSC15 w^{1118}$, B^S females.

Male nondisjunction was assayed in the cross f^1 females x $winscy, y^1 w^1/Dp(1;Y)BSC182, y^+ P\{w^{+mW.Scer\FRT.hs3}\}BSC182 w^{1118}$, B^S males. The frequency of male nondisjunction was calculated as the fraction of exceptional progeny arising from XY or nullo- X male gametes: $(XXY + X0)/(XX + XY + XXY + X0)$. Exceptional XXY and $X0$ progeny arising from nondisjunction in f^1 females were included only in the total progeny count. We showed that nondisjunction was not elevated in homozygous $winscy$ females by measuring nondisjunction in $winscy, y^1 w^1/winscy, y^1 w^1$ females crossed to $C(1;Y)2, y^1 B^1/0$ males.

LITERATURE CITED IN SUPPORTING METHODS

- ASHBURNER, M., K. G. GOLIC and R. S. HAWLEY, 2005 *Drosophila : a laboratory handbook*. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
- HAZELRIGG, T., P. FORNILI and T. C. KAUFMAN, 1982 A cytogenetic analysis of X-ray induced male steriles on the Y chromosome of *Drosophila melanogaster*. *Chromosoma* **87**: 535-559.
- KENNISON, J. A., 1981 The Genetic and Cytological Organization of the Y Chromosome of *Drosophila melanogaster*. *Genetics* **98**: 529-548.
- RYDER, E., F. BLOWS, M. ASHBURNER, R. BAUTISTA-LLACER, D. COULSON *et al.*, 2004 The DrosDel collection: a set of P-element insertions for generating custom chromosomal aberrations in *Drosophila melanogaster*. *Genetics* **167**: 797-813.
- WOODRUFF, R. C., SLATKO, B.E., THOMPSON, J.N., 1983 Factors affecting mutation rates in natural populations, pp. 37-124 in *The Genetics and Biology of Drosophila*, edited by M. ASHBURNER, CARSON, H.L., THOMPSON, J.N. Academic Press.