CHAPTER 4
Synthesizing Specific Genotypes

Virtuoso fly genetics reaches its pinnacle with the art of synthesizing strains and generating new rearrangements. The Stradivarius of this art, Ed Novitski, set a standard that has yet to be equalled with his synthesis of an entire diploid set of autosomes attached to a single centromere. Not surprisingly, meiotic segregation was not a happy event in this strain and it did not survive very well, but the principle was established.

For most purposes, the extent of complex stock construction only goes as far as putting a few chromosomes together in the same fly. This can be more complicated than it sounds and always requires some planning. Many are the times when I thought I could whip off a new strain in a few crosses starting from stocks I happened to have handy, only to find two generations later that I couldn't distinguish genotypes clearly and had to start over again doing it the right way.

The ability to distinguish genotypes unambiguously is the heart and soul of fly chromosome manipulation. It is the feature that sets fly genetics apart from all others and that is essential to exploiting the full potential of the organism.

Principles

The strategies for stock construction take advantage of the following facts, most of which have been said before but which bear repeating:

1. Homologous chromosomes segregate from each other reliably during meiosis; thus, if a progeny received a particular chromosome from its mother, it did not also receive that chromosome's homolog.
2. There are dominant markers and balancers for each of the chromosomes; this tells which homolog from each parent a progeny received, either by presence of the marker or by its absence (see 1 above).
3. There is no recombination in males and a balancer chromosome effec-
tively suppresses recombination with its homolog in females; this means that a dominant marker that starts out on a particular homolog will stay on that homolog through meiosis and thus serve as a reliable marker for it.

As a result of these facts, all chromosomes can be faithfully followed.

Designing schemes for fly matings is a little like doing problems in organic chemistry. You have to figure out how to combine the available starting materials in the most economical and reliable steps to reach a final product, and you have to be able to "purify" the product of each step before proceeding with the next. It is often helpful to work backward from the final product. (If the organic chemistry analogy summons up bad memories, don't worry; fly mating schemes are more fun and ultimately more satisfying to carry out. In addition, you can work on them while sitting in boring seminars or lab meetings.)

**SIMPLE MANIPULATIONS OF A SINGLE CHROMOSOME**

This is the baseline of fly manipulations and is essentially the same as outlined in Chapter 2 on isolating mutants and performing complementation tests. The principle of identifying progeny by absence of dominant markers is illustrated in this complementation test between hunchback (hb) and a new third-chromosome lethal:

\[
\begin{align*}
\text{Tm5, Ser} & \quad \times \quad \text{Tm6, Ubx} \\
\ell & \quad \times \quad \text{hb} \\
\downarrow \\
\ell \quad \text{Survival of this class of progeny that are} \\
\text{hb} \quad \text{non-Ubx and non-Ser indicates complementation}
\end{align*}
\]

The key feature of this cross is that each genotype is distinguished by a unique combination of markers—a goal to be aspired to always.

**Linking Mutations**

Another common manipulation, slightly more involved, is recombining two mutations (or a P-element insertion and a mutation) onto the same chromosome. This is often necessary for carrying out double mutant tests (see Chapter 5) and for linking markers to mutations for mosaic analysis (see Chapter 6).

In its simplest form, the process requires females that are doubly heterozygous on the same chromosome for the two mutations to be linked.
One then recovers individual, putative recombinant chromosomes in the progeny of these females and tests them for the presence of both mutations. An example is shown below for making a double mutant of odd-skipped and even-skipped on the second chromosome:

\[
\frac{\text{In}(2LR)O, Cy}{\text{add}} \times \frac{\text{In}(2LR)O, Cy}{\text{eye}}
\]

Collect \(Cy^+\) virgins

\[
\frac{\text{add} +}{\text{eve}} \times \frac{\text{In}(2LR)O, Cy}{\text{Sco}}
\]

Collect male progeny and mate individually

\[
\frac{\text{In}(2LR)O, Cy \text{ or Sco}}{\text{add? eve?}} \times \frac{\text{In}(2LR)O, Cy}{\text{Sco}}
\]

Set up vial stocks of balanced, putatively recombinant lines by collecting \(Cy\) but not \(Sco\) virgins and males; then test-cross males of each line to each of the two starting stocks, \(\text{In}(2LR)O, Cy/\text{add}\) and \(\text{In}(2LR)O, Cy/\text{eve}\) in a standard complementation test.

The question immediately comes up of how many vial stocks to set up of putatively recombinant chromosomes. The answer is, it depends on how far apart the two mutations are. The farther apart, the more likely you are to recover the product you want. In this case, \(\text{add}\) and \(\text{eve}\) are very far apart, approximately 50 map units, which is equivalent to being unlinked. Therefore, you will probably get it in 10 lines, but ought to do 20 just in case.

For loci that are closer together, you need to use correspondingly higher numbers of lines to be certain of recovering your recombinant. The expectation is a simple function of map distance: if there are 5 map units between loci, there is a 5% chance of recombination. You might recover it in 100 lines, but would be better advised to do 200.

(To be more rational about this, you can use Mather’s [1951] formula for calculating how many progeny \(N\) you need for a 95% probability \(p\) of obtaining a recombinant if \(f\) is the expected fraction of progeny that will be recombinant:

\[
N = -\log(1-p)/\log(1-f)
\]
If the expected recombination frequency is 1%, to be 95% certain of obtaining the desired recombinant, you need to score ~300 progeny.

As mentioned earlier, exchange is temperature-sensitive. Both high and low temperatures increase it (see figure), so the probability of obtaining a recombinant chromosome for two closely linked markers can be somewhat increased (~twofold) by raising the doubly heterozygous females at 30°C (Plough 1917). It is crucial that these double heterozygotes are raised at high temperature, rather than simply placed there as adults for the next cross, because the critical period for this effect does not extend into adulthood. On the other hand, if they are raised at 30°C for their entire life cycle, their fecundity (egg-laying) will be reduced by approximately twofold. Therefore, the best compromise is to shift the bottles to 30°C for most of the larval period—that is, beginning when you see the food starting to be churned up and ending when you see pupae beginning to form on the sides of the bottle. The high-temperature effect is most pronounced near the centromere and the tips of the chromosome arms.

Another condition that will increase recombination is the presence of a heterozygous inversion on another chromosome. This “interchromosomal effect” increases recombination anywhere in the genome outside of the rearranged regions.

**PROBLEM 8** Work out how to recombine a P[hw' lacZ] insertion element located on 3L in cytological region 61B (meiotic map position approximately 3-0) with a mutation at the hairy (h) locus (3-26.5).

Sometimes it helps to use additional, visible markers to aid in the recognition of recombinants for invisible, recessive mutations. This is very easy to do if, for instance, you have carried out meiotic mapping of your mutation and therefore already have a chromosome with your invisible mutation and some visible markers on it.

**MANIPULATING TWO CHROMOSOMES**

To control the genotype on two different chromosomes at once, it is necessary to start planning the mating schemes. Here we will make use of balancers, dominant markers, the lack of recombination in males, and the reliable segregation of homologs. As an example, we'll consider how to make a stock to produce double mutant embryos for f(z) on the third chromosome and eve on the second chromosome.

Most mutation-bearing stocks contain the mutation and a balancer for that chromosome, e.g., TM3, Ser/fz. This is the healthiest way to keep them,
since the presence of other dominant markers and balancers on other chromosomes only detracts from the stock's viability. To manipulate two chromosomes at once, it is necessary to introduce additional balancers and dominants, but to do so without losing track of the original chromosome. For this reason, it is useful to keep on hand a stock with two chromosomes' worth of balancers and markers, such as:

\[ \frac{\text{FM}7\alpha; \text{T}M_6, \text{Ubx}}{\text{Sb}} \text{ or } \frac{\text{In}(2LR), \text{O}, \text{Cy}}{\text{Sca}}; \frac{\text{T}M_3, \text{Ser}}{\text{Sb}} \text{ or } \frac{\text{In}(2LR), \text{O}, \text{Cy}^O}{\text{Sca}}; \frac{\text{T}M_6, \text{Ubx}}{\text{Sb}} \]

However, the deleterious effects of multiple balancers on female meiosis indicate that males of these genotypes should be used whenever possible. The trick is to mate the starting mutant stock with another that uses different markers. That way, one can exploit the obligatory segregation of homologs to trade markers:

**Cross #1**

\[ \frac{\varphi \varphi \text{T}M_6, \text{Ubx}}{\text{flz}} \times \frac{\text{In}(2LR), \text{O}, \text{Cy}}{\text{Sca}}; \frac{\text{T}M_3, \text{Ser}}{\text{Sb}} \]

\[ \frac{\text{F}1}{\varphi \varphi} \]

\[ \frac{\text{In}(2LR), \text{O}, \text{Cy}}{\text{flz}}; \frac{\text{T}M_3, \text{Ser}}{+} \]

\[ \text{flz} \text{ is now recoverable in the same flies with the balancer In}(2LR), \text{O}, \text{Cy} \text{ in the Ser, Ubx}^+ \text{ progeny. These are not particularly healthy and happy females, however, due to the presence of two balancers.} \]

In this first step, you build out from one chromosome onto another. In parallel, make an analogous genotype from the other mutant line:

**Cross #2**

\[ \frac{\varphi \varphi \text{In}(2LR), \text{O}, \text{Cy}}{\text{eve}} \times \frac{\text{In}(2LR), \text{O}, \text{Cy}}{\text{Sca}}; \frac{\text{T}M_6, \text{Ubx}}{\text{Sb}} \]

\[ \frac{\text{F}1}{\varphi \varphi} \]

\[ \frac{\text{Sca}, \text{TM}_6, \text{Ubx}}{\text{eve}}; \frac{+}{+} \]

ev is now recoverable heterozygous with the dominant Sca in the same flies with the balancer Tm6, Ubx in the Sca, Cy+ males. The flies of this genotype that are used in any subsequent cross must be males, however, to prevent any exchange on the second chromosome that could put eve and Sca on the same homolog.
Now the F1 virgins from Cross #1 and the F1 males from Cross #2 can be mated to yield a stock for producing double mutants:

\[
\begin{align*}
\text{♀♀ } \text{In}(2L)O, C_y \times \frac{TM3, S_{er}}{c^{16}} \times \frac{S_c}{e_{ve}} \times \frac{TM6, U_b}{c^{16}} \times \frac{O}{c^{16}} \\
\text{↓} \\
\text{In}(2L)O, C_y \times \frac{TM6, U_b}{c^{16}} \times \frac{O}{c^{16}} \\
\end{align*}
\]

Collected as males and virgin females (C_y, S_c^+ and U_bx, S_e r^+), these flies will constitute a true-breeding (albeit unhealthy) stock, 1/16 of whose progeny would be doubly homozygous for e_{ve} and f_{tz}. If, perchance, this e_{ve} mutation suppressed the lethality of this f_{tz} mutation (see Chapter 5), even if only 1% of the time, it could be detected and scored unambiguously by the markers in this stock. A unique combination of markers permits each genotype to be distinguished at each step.

If, on the other hand, all you wanted to do was produce doubly mutant embryos, these adult markers would be useless, and the desired result could have been obtained much more simply and with better viability by crossing the two original stocks:

\[
\begin{align*}
\text{In}(2L)O, C_y \times \frac{TM6, U_b}{c^{16}} \times \frac{O}{c^{16}} \\
\text{↓} \\
\frac{e_{ve}}{c^{16}} \times \frac{f_{tz}}{c^{16}} \\
\end{align*}
\]

Collect C_y^+ U_bx^+ males and virgins, mate them to each other and collect embryos, 25% of which will be doubly homozygous for e_{ve} and f_{tz}.

The drawback of this simple approach is that there is no way of knowing if the double mutant phenotype is different from either of the single mutant phenotypes. To solve this problem, one makes use of balancer chromosomes that have P-elements expressing lacZ in them. Then, when progeny are collected from the stock consisting of In(2LR)O, C_y P[lacZ] e_{ve}; TM6, U_bx P[lacZ] f_{tz}, they can be stained for β-galactosidase. Only those progeny failing to stain will be double mutants.
The value of markers becomes apparent whenever you want to score viability, detect small numbers of progeny of a given genotype, and recover individual progeny of known genotype.

An additional tool that is helpful for manipulating the second and third chromosomes simultaneously is a reciprocal translocation between \( \text{In(2LR)O,Cy} \) and \( \text{TM9} \) called \( T(2;3) \text{CyO; TM9} \). As a reciprocal translocation between both balancers, it ensures that all pieces of the translocation must be present in a fertilized egg for it to be viable—if not, the zygote will be aneuploid and will die. Since it contains complete sequences for both chromosomes, it effectively balances all of chromosomes 2 and 3 and allows one to keep a stock such as:

\[
T(2;3) \text{CyO; TM9} \\
eve; ftz
\]

Manipulations of two chromosomes are necessary when producing P-element transformants to see if a genomic clone rescues a mutant phenotype. Then you can use the visible marker in the P-element (e.g., \( w^+ \)) as a dominant marker, provided the X chromosomes in the scheme all carry a mutant allele of white.

For example, if you have produced a transformed line carrying a \( P[w^+, \text{add}^+] \) insert on the third chromosome and you want to construct flies that are homozygous for \( \text{add} \) on the second chromosome and also carry this P-element on their third chromosome, it helps to start with stocks that are mutant for \( w \). This will generally be true already of the P-element-containing stock, since it will probably have been made with \( w \) in it:

\[
w^+;\frac{P[w^+, \text{add}^+]}{+} \quad \text{but will need to be made for} \quad \text{add} \quad w;\frac{\text{In(2LR)O,Cy}}{\text{add}}
\]

Similarly, all the balancer stocks used in the scheme must be \( w \). These are stocks that one would generally have on hand anyway to do linkage analysis of newly derived transformants (see Chapter 3).

Since \( P[w^+, \text{add}^+] \) will be scorable as dominant on a \( w \) background, the scheme is somewhat simpler than the previous example for making a double mutant:
Cross #1

\[
\frac{w}{w' \text{ P}[w^+, \text{odd}^+]} \times \frac{w}{Y' \text{ In}(2L)O, Cy \text{ Sco}} \times \frac{\text{In}(2L)O, Cy \text{ odd}}{\text{add}} \rightarrow \text{P}
\]

Select \(w^+\) Cy Sco^+ virgins from Cross #1 and \(w^+\) Sco^+ males from Cross #2

Cross #2

\[
\frac{w}{w' \text{ P}[w^+, \text{odd}^+]} \times \frac{w}{Y' \text{ In}(2L)O, Cy \text{ Sco}} \times \frac{\text{In}(2L)O, Cy \text{ odd}}{\text{add}} \rightarrow \text{F1}
\]

Select \(w^+\) Cy Sco^+ virgins and males

\[
\frac{w}{w' \text{ P}[w^+, \text{odd}^+]} \times \frac{w}{Y' \text{ In}(2L)O, Cy \text{ Sco}} \times \frac{\text{In}(2L)O, Cy \text{ odd}}{\text{add}} \rightarrow \text{F2}
\]

See if there are any \(w^+\) Cy^+ adults

The first line of this scheme is shorthand for the fact that two crosses were set up separately but simultaneously in the parental (P) generation, using males of the same genotype for each. The last line shows that you get both males (\(w^+/Y\)) and females (\(w^+/w^+\)) that test the efficacy of the P-element rescue. If \(\text{odd}/\text{odd}\); \(P[w^+, \text{odd}^+]/+\) flies survive, the insert rescues the mutation.

**Problem 9** Construct a stock for testing the phenotype of four doses of \(P[w^+, \text{odd}^+]\) on an odd mutant background, starting with P inserts on the X and third chromosomes. (Note: a \(P[w^+, \text{odd}^+]\) insert on the X creates some problems for using \(w^+\) to score the presence of the P-element.)

**Pitfalls**

*How Many Flies?*

The viability problems that come from using multiple dominants and balancers have been alluded to already. In general, the more that are piled into the same fly, the sicker the fly. The sicker the fly, the more you need to start with at the beginning of the scheme. Otherwise, you may find yourself at the end of a 3-month, multigenerational scheme, only to be left with one male of the correct genotype who turns out to be sterile.
You can get a rough idea of how hard it will be to obtain the flies you need by figuring out how few of the progeny from each cross are the ones you want. Usually, they are theoretically 1/8 or 1/16. Poor viability of markers and nonhomologous segregation of multiple balancers reduce the number further. Unlike cloning, the products cannot be amplified at each step, so you must start with enough to get you through—as in organic synthesis.

A simple rule of thumb is to aim for enough flies to do at least one full bottle cross for the last generation of a scheme. Given the viability problems discussed, a bottle cross will require roughly 40–50 virgins. Working backward, this suggests that the initial crosses in a scheme for manipulating two chromosomes should be started with several bottles each and will thus require 100–150 virgins.

**Which Sex?**

The choice of which sex to use at each step of a scheme is influenced by several factors. One already mentioned is the lack of exchange in males. This makes it possible to use a dominantly marked chromosome as if it were a balancer; a technique used many times in the examples in this book (e.g., *Sco/odd*). A second factor is the trouble associated with multiple balancers in females, to which males are oblivious (so what else is new?).

A third factor in choosing the sex at each step is the problem of non-virginity. Despite our best efforts, non-virgins do sometimes sneak through our fine net. The presence of a few non-virgins in a cross can totally subvert the scheme, since a given set of markers can apply to more than one genotype. By taking a careful look at each cross, it will be clear that in some cases non-virginity will not be a problem because the desired progeny will be uniquely marked whether or not all of the mothers are virgin.

This is not an argument against collecting virgins for your crosses; there is still the matter of how tiny a fraction of the progeny are the ones you want. It is simply another consideration for making the schemes work in the end. Take an example from an earlier cross (p. 68):

$$\phi \phi \frac{\text{In}(2L)O, Cy}{frz} \times \frac{\text{TM}3, \text{Ser}}{e} \frac{\text{TM}6, \text{Ubx}}{e} \frac{\text{Sco}}{e} \sigma \sigma$$

$$\downarrow$$

$$\frac{\text{In}(2L)O, Cy}{frz} \times \frac{\text{TM}6, \text{Ubx}}{e}$$

To determine whether you are at risk, consider the markers you are seeking in the desired progeny (Cy and Ubx) and see if it is possible to get the same
combination of markers from a non-virgin. In order to determine this, you must consider the cross that produced the virgins and what rogue males might have been present in the bottle with them. Could the In(2LR)0,Cy/+, TM3, Ser/ftz virgins have mated with males carrying TM6, Ubx such that you could potentially get Cy with Ubx in the progeny of a non-virgin? In this case, the earlier cross was:

\[
\begin{align*}
\text{♀♀ } TM6, Ubx^\text{ftz} & \times \frac{\text{In(2LR)0,Cy}, \text{TM3, Ser}}{\text{ScA}, \text{Sb}} \text{♂♂} \\
\downarrow \\
\text{♀♀ } \frac{\text{In(2LR)0,Cy}, \text{TM3, Ser}}{\text{ScA}, \text{Sb}} \\
\end{align*}
\]

so it is clear that the virgins could have mated with TM6, Ubx males and thus produce misleading progeny with the appropriate markers but the wrong genotype on other chromosomes. Hence, you are at risk and should be scrupulous about ensuring virginity.

**MANIPULATING THREE CHROMOSOMES**

Just as in juggling, where there is a major gap in going from three balls to four, so in chromosome manipulation the great divide is between two chromosomes and three. Fortunately, three chromosomes are rarely needed. Even rarer, in fact nearly unheard of, is the need to maneuver all four chromosomes at once. The principles are the same as in two-chromosome schemes, but the problems are magnified. It gets harder to make genotypes that are uniquely distinguishable, and the likelihood of ending up with one sterile male (at most) is greater. If you are not likely to need such esoteric techniques, or are uninterested in such matters, you may want to skip to the next section.

In the past, such elaborate schemes were mainly needed for certain kinds of mosaic experiments—the generation of individuals with a mixture of mutant and wild-type cells (see Chapter 6). The stocks thus generated were used to produce gynandromorphs (X-chromosome mosaics) under the influence of an autosomal mutation that induces chromosome loss. In addition, such stocks make use of a duplication on the X to cover the mutation being studied, which is recessive and lies on an autosome. Thus, when X-chromosome mosaicism is induced, the individual is also mosaic for the autosomal locus duplicated on the X. Kankel and Hall (1976) used this kind of system in their fate map study of the nervous system with an enzyme
marker, acid phosphatase (Acp). The challenging stock they had to make consisted of:

\[
X-Acp^+ \text{ pal; } Acp^-
\]

\[
\text{Xy}^2 \text{ pal; } Acp^+
\]

The designation \(X-Acp^+/Xy^2/Y\) indicates that males in the stock have the \(X-Acp^+/Y\) and females have \(Xy^2/Y\). \(Acp^+\) is the mutation being made mosaic. It is a mutation in the enzyme alkaline phosphatase (n is for null, actually allele number \(n11\)) and it served as a histochemical marker for lineage analysis during development. \(X-Acp^+\) is an \(X\) chromosome with a duplication of the \(Acp\) locus on it, and \(pal\) is the mosaic-producing mutation paternal loss. To produce mosaics, males of this stock were mated to \(y; Acp^+\) females (for details, see Chapter 6). For the sake of posterity, Jeff Hall’s mating scheme used to generate it is included here. It is long and almost as difficult to understand as to carry out. Consider it a challenge and a test of your growing fly genetic acumen. If you are still baffled after studying it, don’t despair—schemes of this complexity are like a path to Zen.

\[
\frac{Cy}{pm}, \frac{TM6}{Sb} \times ^* \frac{Xy^2}{Y} \times ^* \frac{Y}{y^2Y} \frac{pm}{pal} \frac{pm}{pal}
\]

*Same cross, so instead of discarding the parents, transfer them to fresh bottles

\[
\frac{Xy^2}{Y}, \frac{Cy}{pal'}, \frac{TM6}{y^2Y} \times ^* \frac{Cy}{pm}, \frac{TM6}{pal'}
\]

\[
\frac{Xy^2}{Y}, \frac{Cy, Sb}{pal} \times ^* \frac{X-Acp^+}{y^2Y}, \frac{Cy, Sb}{pal'}
\]

\[
\frac{Xy^2}{Y}, \frac{Cy}{pal}, \frac{TM6}{pal'} \times ^* \frac{X-Acp^+}{y^2Y}, \frac{Cy}{pal'}, \frac{Sb}{Acp^+}
\]

\[
\frac{Xy^2}{Y}, \frac{Cy}{pal'}, \frac{TM6}{Acp^+} \times ^* \frac{X-Acp^+}{y^2Y}, \frac{Cy}{pal'}, \frac{TM6}{Acp^+}
\]

† Unhealthy females; use lots of them.
MAKING REARRANGEMENTS

There have been a few virtuoso practitioners of chromosome rearranging in the history of Drosophila genetics. H.J. Muller was the first (in this, as in so many other things), Ed Novitski the grand master, and Loring Craymer the most recent (for details and references, see Ashburner 1989). Deletions, duplications, and translocations are the classes of rearrangements that you are likely to make, and of these, deletions are the most commonly needed. In general, rearrangements can be made from scratch, i.e., from normal chromosomes, or from preexisting rearrangements. Usually the process requires some kind of agent, such as radiation or chemical mutagens. In some cases, they can be made by exchange events between rearrangements. Above all, it is important to consult the Red Book (Lindsley and Zimm 1992), FlyBase, and the stock collections to make sure the chromosome you want does not exist already.

The universal rule for making rearrangements is that it is always easier to start from some preexisting rearrangement.

Inducing Deletions

The best way to induce deletions is to call (or e-mail) the Bloomington Stock Center and request that they be sent to you. When this is not possible, producing a deletion by radiation or chemical mutagenesis is very much like inducing a new recessive allele of a gene (see Chapter 2): You carry out a complementation test of the treated chromosomes. If you start with a wild-type chromosome and want to delete a region containing an easily scored, visible marker, simply treat normal males with the agent (radiation or chemicals), mate to females homozygous for the marker, and recover progeny displaying the marker phenoype.

The scheme outlined below takes a shortcut that was eschewed in Chapter 2, namely screening in the F1 generation. The rationale for doing it here is that deletions generally span more than one locus, do not arise with great frequency, and are almost always homozygous lethal. Thus, you can afford to take the trouble of separating out the chromosomes in the F2 generation because it allows you to throw out so many of the F1s. It may be necessary to screen 10,000 or more mutagenized chromosomes. The limitation is that this approach only works with visible markers, but since deletions are hard to get, it's worthwhile trying to find a way to do it with visible markers.
Mutagenized males: $\frac{+}{+} \times \frac{en^1}{en^1}$ en$^1$ is a viable allele of the engrailed locus, a locus that can mutate to lethality.

\[ \frac{en^1}{-(-)} \quad \text{Any progeny that look engrailed will have a newly induced allele or deletion - symbolized } (-(-)). \text{ If it is a deletion, it is likely to be homozygous lethal, which is testable by separating the two chromosomes and testing each for homozygous lethality and then retesting for failure to complement engrailed. For many loci, including en, new alleles are likely to be lethal themselves and can be handled the same way.} \]

\[ \frac{en^1}{-(-)} \times \frac{\text{In}(2LR)O,Cy}{\text{Sco}} \]

Mate males individually to $\text{In}(2LR)O,Cy/Sco$, collect Cy, non-Sco progeny, and mate sibs together. Vials producing no Cy$^+$ progeny are possible deletions. Confirm first by seeing that they uncover en and ultimately by cytology to determine breakpoints.

A dominant allele at or near the locus you want to delete works just as well. Here you screen for reversion of the dominant phenotype. This can also be done as an F1 screen. (Warning! Not all dominant mutations are revertible. Dominants that are due to haplo-insufficiency [e.g., Ubx or the class of mutants known as Minutes] are not. Those that are revertible represent gain-of-function mutations, hypermorphs, neomorphs, or antimorphs, discussed further in Chapter 5.) Note that all Ph$^+$ inserts are dominant if the fly is mutant at the w locus. Since these inserts are just about ubiquitous, it is now possible to "revert a dominant" almost anywhere.
Of the mutagens described in Chapter 2, radiation (specifically X-rays) has been used most commonly to induce deletions and is generally preferable to chemical agents in its reliability, although the frequency of recovering such deletions is on the order of 1–5/10,000 using 4000r. Up to half of the recovered X-ray-induced alleles at a locus are multi-locus deletions (Pastink et al. 1987, 1988). Large ones are rarer than small ones. As in other kinds of mutagenesis, mature sperm are the most sensitive. EMS mutagenesis, traditionally thought to be a point mutagen, has been found over the years to produce many deletions as well. Many of these are small and intragenic, which is an ideal way to generate a null allele (see Chapter 5) without taking out other genes.

P-element excision has also been widely used for producing small deletions. Imprecise excisions produce deletions that are generally small (up to 2 kb) and thus usually intragenic, but larger deletions also occur (for references and details, see Ashburner 1989). The frequency of producing such deletions is as unpredictable as any P-element transposition event, depending in large part on the site of insertion.

Excision schemes are set up to detect loss of the wild-type allele of an eye-color marker such as \( w^+ \) in the P-element, following the same general principles outlined earlier for induction of new mutations by P-elements. For a lethal, \( w^+ \) insertion on chromosome 3, the scheme would go like this:

\[
\begin{align*}
\frac{w}{Y'} \quad P[w^+] \times \frac{w}{w'} \quad Sb \quad P[ry^+ \Delta 2-3] \quad TM6, Ubx
\end{align*}
\]

Select \( Ser^+ Sb \) males

\[
\begin{align*}
\frac{w}{Y'} \quad Sb \quad P[ry^+ \Delta 2-3] \quad \times \quad \frac{w}{w'} \quad TM6, Ubx
\end{align*}
\]

Select \( w, Sb^+, Ubx \) males and mate to balancer stock to test for lethality

\[
\begin{align*}
w \quad \frac{-(\_)}{Y'} \quad TM6, Ubx \quad \times \quad \frac{w}{w'} \quad TM6, Ubx
\end{align*}
\]

The frequency of obtaining such P-element excisions depends on both the sequences in the P-element and the site of insertion. The ratio of precise to imprecise excisions, which will influence the recovery of deletions, also varies but tends to favor those that are imprecise. Of those that are imprecise, however, most are not deletions but are excisions that have left be-
hind a piece of the P-element. Thus, the induction of new deletions may be as rare as 0.1% and as common as 10% of chromosomes screened. The frequency of imprecise excisions can be increased if the chromosome bearing the P-element is unable to pair with its homolog and use it as a template for repair (Engels et al. 1990). The virtue of the genetic screen outlined above is that it allows one to score simultaneously for excision (loss of w*) and lethality.

The ability to recover a deletion will depend on what loci are being uncovered. If the region you want to delete contains a haplo-lethal locus, you are in big trouble. If it contains some other kind of haplo-insufficient locus, such as sterility, you are also in trouble. Even if it only contains a locus that is unhealthy in one dose, such as a Minute locus, you will have a hard time. (Minutes are a class of loci, encoding ribosomal proteins, sprinkled around the genome that have a similar hemizygous phenotype: thin bristles, retarded development, small size. In addition to being haplo-insufficient, they are also recessive lethal. For detailed discussion, see Ashburner [1989].)

**Synthesizing Stable Deletions from T(Y;A)s**

In keeping with the principle that it is better to make rearrangements from preexisting ones, the T(Y;A)s described above (Chapter 3, Synthesis of Deletions and Duplications) are sometimes a good starting point for making deletions.

One generates flies carrying a deletion by crossing two T(Y;A) stocks bearing different breakpoints (different points along the autosome and different arms of the Y so that the y* and B* markers are oppositely arranged):

Both of these stocks look y* and B*. The deletion-bearing progeny will lack B* and will have two doses of y*.
It is possible to collect males and virgin females of this \( y^+ B^+ \) genotype and set up a stock. Such a stock, however, does not thrive, because many aneuploid progeny are produced. Since one usually wants a deletion for screening new mutations, or some other use that requires large numbers of flies, the poor viability of the stock is a liability.

It is possible to reattach the autosomal pieces in this stock by irradiation to produce an intact, stable deletion. An event of this sort will occur at a much higher frequency than induction of a deletion de novo, because the arms of the \( Y \) present a large target and because one is inducing an exchange event between them. Since it is necessary to have both autosomal pieces of the \( T(Y;A) \) present in the cell that receives the radiation, this is done in females and at a much lower dose than used in males, 1500r, so as not to produce damage in the oocyte.

The recovery of such reattachments can sometimes be made easier by using a selection scheme based on the restoration of fertility with the reattached chromosome to an otherwise sterile genotype when it is unattached (Lytte 1984).

Selection of the reattachment is done easily by scoring loss of \( y^+ \) in flies otherwise mutant for yellow on the \( X \) (which is already true for the \( T(Y;A) \) stocks). Thus, one simply collects females from the unhealthy, synthetic deletion stock, irradiates them, mates them, and looks for progeny. Since the stock is balanced anyway, the new chromosome comes out balanced. It can then be tested to confirm that it is truly reattached by mating it to one of the parental \( T(Y;A) \) stocks and seeing that no aneuploids are recovered, i.e., no separation of \( y^+ \) and \( B^+ \).

**Synthesizing Deletions from P-element Insertions**

P-elements have brought a new level of rationality into the generation of deletions. Since their chromosomal position can be accurately determined, they provide identified targets for rearrangement events. Since they can be
mobilized to insert at new sites easily by a simple cross (see Chapter 2 on mutagenesis), new insertions can be readily obtained. More importantly, there is a nonrandom probability that transposition will occur to a site relatively near the original site. This means that if a mechanism existed to catalyze chromosome breakage and rejoicing at these P-element insertions, a technique could be developed for the rational synthesis of deletions and duplications by unequal crossing-over.

Golic (1994) devised a strategy for making P-elements into sites of interchromosomal recombination. He introduced target sites for the yeast flip recombinase target (FRT) sequence into P-elements and made transformants carrying these sequences at various sites. (Other uses of this technique for analyzing time and place of gene action are discussed in Chapters 5 and 6.) He then made use of the fact that when an inserted P-element is transposed, it has a high probability of moving to a new site in the same general vicinity of the chromosome. When two homologs have such inserts in the same vicinity and the FLP recombinase is induced, it can catalyze an unequal recombination event between the homologs. The result is a deletion and the reciprocal duplication. (Induction of the recombinase is accomplished by heat shock of the hsp70 promoter driving the FLP gene.)

The frequency of obtaining transpositions varies with the location and composition of the starting insert. Golic obtained a transposition rate ranging from 18/97 \( G_1 \) males (F1 progeny of the cross between the initial P-element and the \( \Delta 2-3 \) transposase) to 97/104 \( G_1 \) males. Of those, 86% went to nearby regions of the chromosome. When set up as heterozygotes with inserts at different but nearby sites (~50 bands apart), the frequency of obtaining deletions and duplications was 0.7%. Although not overwhelming, it is significant and has the advantage of producing rearrangements with known, nearby breakpoints.
Inducing Duplications

Stable duplications sometimes arise when a piece of chromosome is cut out during the X-ray induction of deletions. The excised fragment is sometimes inserted into a site on another chromosome (or elsewhere on the same one) and when the chromosome with the inserted piece segregates away from the excised chromosome, a reciprocal duplication and deletion are formed. This approach, however, is not one to use when looking deliberately for a particular duplication.

Free duplications of the X are the easiest to induce. These are small pieces of the X, retaining its centromere and the distal tip with the \( y^+ \) locus on it. The technique is basically to chop out a piece of the X to create a freely segregating mini-chromosome that is detectable by the dominance of \( y^+ \) on a \( y \) background (i.e., in a female with \( X^yY \)). By retaining this marker, it is easy to select for such "free duplications" since flies better tolerate large duplications of the X chromosome, especially in females, than of autosomes. The basic idea is illustrated below:

\[
\begin{align*}
X \text{-ray} & \quad y^+pn^+ \quad y^+pn^+ \quad \nu^+ \\
& \quad \gamma \quad \gamma \\
\text{♀}^+ & \quad y^+pn^+ \quad \nu^+ \\
& \quad \nu \quad \nu \\
\text{♀}^+ & \quad y^+pn^+ \quad \nu^+ \\
& \quad \nu \quad \nu \\
\end{align*}
\]

\( \text{♀} \) attached-X with free duplication

\( \text{♀} \) attached-X with free duplication

Synth

For details as well as for specific breaks, however reliable, the help of the chromosomal homozygous stocks (1/100 Mcgregor, linked) stocks will be very formidable.
Two of many possible outcomes are illustrated here, a y<sup>+</sup> duplication and a y<sup>+</sup>p<sup>+</sup> duplication. These each represent possible female genotypes in progeny. The free duplications survive if they are not full-sized X chromosomes. They can then be assayed for other loci they cover by mating to males with a multiply marked X.

Selection schemes can be designed for duplications based on their ability to rescue the phenotype of a haplo-insufficient locus. A balanced haplo-insufficient locus will appear in all non-balancer progeny unless another wild-type dose of the locus is present. Thus, irradiation of wild-type flies and mating to a balanced haplo-insufficient locus will reveal the presence of a new duplication in any non-balancer, non-haplo-insufficient progeny.

Tandem duplications—side by side on the original chromosome—can be obtained with a reasonable probability (~1/10,000) by irradiating normal chromosomes in females instead of males with a lower dose of radiation, 1500 r (Ashburner 1989). This presumably results from the induction of an unequal exchange event between the two homologs, both of which are present in the irradiated oocyte.

**Synthesizing Stable Duplications from T(Y;A)s**

For duplications, the counterpart to resealing of T(Y;A) pieces that works well for deletions exists in principle, but has not been tried. T(Y;A)s whose breakpoints are relatively near the end of a chromosome arm can be used, however, to attach an autosomal piece onto the X chromosome in a fairly reliable manner.

This technique relies on detaching an attached-X, an exchange event between the Y-derived heterochromatic portion of a T(Y;A) and the heterochromatic regions near the centromere of an attached-X chromosome, helped along by a little radiation. Heterochromatic regions have rough homology with each other and will recombine with a low probability (1/1000) that can be boosted by radiation.

Most attached-Xs are not suitable for this maneuver, since they are generally deleted or rearranged for most or all of their centric (centromere-linked) heterochromatin. There is good reason for this: Since attached-X stocks are usually kept with a free Y chromosome, they would detach spontaneously if heterochromatic pairing and exchange could occur. The particular attached-Xs that are suitable for this technique retain their centric heterochromatin and are thus not good for normal stock maintenance, e.g., C(1)RM, y p n v. One simply crosses the T(Y;A) into that attached-X stock, irradiates females, and males them to unmarked males.
The resulting product is an X chromosome linked to the terminal fragment of an autosome. It is recognizable because males will not receive the recessive markers on the attached-X unless there has been a breakdown. Thus, one looks for a male with \( y \) \( pn \) \( v \) but not \( B^5 \) (or \( y^v \), depending on the \( Y \) breakpoint). The size of duplication that will be viable depends on which autosomal arm it comes from (see above).

**Synthesizing Attached-Xs**

A new attached-X can be made in the same way just described for taking one apart. Why would you ever want to do that? If you wanted to make an
attached-X with, for instance, a temperature-sensitive lethal mutation on it so that the stock could be made to produce only males. The strategy is to start with an X chromosome with the desired mutation on it and then allow it to recombine with an X joined to a Y marked with B5 (called XYS•YL:B5 and carrying y2 su(w3) and w3);
binations of the differing markers. Here, one would again start strains from individual female progeny and test them for presence of the temperature-sensitive paralytic mutation shibire-temperature-sensitive (*shil*).

**Autosynaptic Chromosomes and the Joys of Gibberish**

One of the more esoteric backwaters of fly genetics is the synthesis of duplications and deletions from that class of inversions spanning the centromere, known as pericentric inversions:

\[
\begin{array}{cccccc}
  a & b & (d & c) & e & f \\
\end{array}
\]

The meiotic behavior of inversion chromosomes has held a strange fascination for chromosome mechanics aficionados ever since the classic and voluminous study of crossing-over in inversion heterozygotes by Sturtevant and Beadle (1936).

Pericentric inversions are relevant to the construction of aneuploids because exchange in a heterozygote produces reciprocally duplicated and deleted products:

\[
\begin{array}{cccccc}
  a & b & c & d & e & f \\
\end{array}
\]

\[
\begin{array}{cccccc}
  a & b & c & d & b & a \\
\end{array}
\]

\[
\begin{array}{cccccc}
  f & e & c & d & e & f \\
\end{array}
\]

If one has access to more than one pericentric inversion of this sort, where breakpoints of one are somewhat displaced from those of the other, it is possible to generate tandem duplications and deletions of the region of chromosome that is displaced between the two.

\[
\begin{array}{cccccc}
  a & b & (e & d & c) & f \\
\end{array}
\]

\[
\begin{array}{cccccc}
  a & (d & c & b) & e & f \\
\end{array}
\]

\[
\begin{array}{cccccc}
  a & b & (b & c) & d & e & f \\
\end{array}
\]

\[
\begin{array}{cccccc}
  a & (c & d & e & f) \\
\end{array}
\]
This occurs by a series of exchange events, the details of which are spelled out in Craymer (1981, 1984) and Ashburner (1989). The technique has the virtue of being rational and deliberate. It is limited by the availability of appropriate pericentric inversions. They too can be synthesized (see the same references), but not with great ease.