# Retrospective genotyping: PCR, lethality or infertility

### Introduction:

This procedure is usually performed to select for recombinant flies that have two or more genetic Elements Of Interest (EOI) such as a mutation, insertion, etc. on the same chromosome, in which one or more cannot be selected using a visible marker. Note: recombinants are on the same chromosome, but need not be on the same arm. To isolate the recombinant chromosome and make flies carrying it into a stock, a standard recombination scheme is performed and individual males that may or may not carry the recombined chromosome are mated to a balancer. Potential stocks are then screened retrospectively to determine which have the element(s) of interest. The number of single-male crosses set up depends on how far apart the regions to be combined are from each other. Map distances for genes can be found on the flybase website.

### Preparation:

1. Expand the stocks for collecting virgins
2. If possible, determine the map positions of the genetic elements to be recombined.

### Procedure:

A typical crossing scheme is as follows:

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| --- | --- | --- | --- | --- | --- |
| P0 | w-; EOI (1)/CyO | x | w-; EOI (2)/CyO | 🡪 | select for straight-winged virgin females |
|  |  |  |  |  |  |
| F1 | w-; EOI (1)/EOI (2) | x | w-; sco/CyO | 🡪 | select curly-winged males |
|  |  |  |  |  |  |
| F2 | w; EOI (1), EOI (2)/CyO (single male1) | x | w; sco/CyO (virgin female) | 🡪 Screen single males by PCR, if applicable2 | Select curly-winged, normal-bristled males and females3  |
|  |  |  |  |  |  |
| F3 | w; EOI (1), EOI (2)/CyO | x | w; EOI (1), EOI (2)/CyO | 🡪 | Stock; screen stock for lethality or infertility, if applicable4, 5 |

1. At the F2 cross, set up 5-20 individual crosses between a single male and 5-10 virgin females. If the genetic elements to be recombined are 40 or more map units apart, 5 crosses should be sufficient; if they’re 20-40 map units apart or if the map distance is unknown, 10-15 crosses is better; if they’re less than 20 map units apart, 20 (or more) crosses may be necessary.
2. If screening by PCR, once the cross has been established, remove the F2 male, extract DNA from the whole fly and perform PCR to screen for the presence of the element(s) of interest. When planning the PCR reaction, be sure to include a positive and negative control. The lab has standard primers for amplifying rps that work well as a positive control. A good negative control is DNA harvested from the parent stock that should not have the element of interest.
3. If the PCR screen is completed before the F3 flies eclose, only those crosses that were sired by recombinant males need be carried forward to the F3 step; all others can be thrown away.
4. If screening by lethality, examine 30 or more flies in each stock. If there are no flies that lack the balancer, it is very likely that the stock has the lethal mutation of interest. If even one fly does not have the balancer, it should be assumed that the lethal mutation is not present and the stock should be discarded.
5. If screening by infertility, put 5-10 females that lack the balancer together in a vial with wildtype males (they do not need to be virgins). If they do not produce viable progeny, it is very likely that the stock has the infertile mutation of interest. f even a few progeny are produced, it should be assumed that the infertile mutation is not present and the stock should be discarded.