# Neomycin-based selection for FRT recombinants

### Introduction:

This procedure is usually performed to select for recombinant flies that have a mutation in a Gene Of Interest (GOI\*) on the same arm as a commonly used FRT. Neomycin is normally toxic to larvae, but FRTs 19A, 40A, 42D, 80B and 82B carry neomycin resistance genes that confer survival. The neomycin gene is under the control of a heat shock promoter, so heat shocking the larvae at regular intervals improves survival rates. A typical crossing scheme is as follows:

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| --- | --- | --- | --- | --- | --- |
| Po | w-; GOI(\*, mW+)/CyO | x | w-; FRT 40a/CyO | 🡪 | select for straight-winged virgin females |
|  |  |  |  |  |  |
| F1 | w-; GOI(\*, mW+)/FRT 40a(females) | x | w-; sco/CyO(males) | 🡪on neo+ media | select for survivors with curly wings and pink or red eyes |
|  |  |  |  |  |  |
| F2\* | w; GOI(\*, mW+), FRT 40a/CyO(single male) | x | w; sco/CyO(5-10 females) | 🡪 | Select for curly wings and normal bristles |
|  |  |  |  |  |  |
| F3 | w; GOI(\*, mW+), FRT 40a/CyO | x | w; GOI(\*, mW+), FRT 40a/CyO | 🡪  | Stock |

\*This cross is recommended to homogenize the recombinant chromosome, but not strictly necessary.

### Materials:

Vials with regular food

Neomycin (also called Geneticin or G418), liquid stock 25 mg/ml

Wooden applicator stick

### Preparation:

1. Get one vial with food for each cross plus two more, one for a positive control and one for a negative control.
2. Thaw one or more aliquots of G418, as needed. You will need 100 µl of stock solution per 10 ml of food.
	1. To determine food volume, measure the height of the food in the vial and use the following conversion: 17 mm = 10 ml)
	2. If no stock is available, add 1 ml ddH20 to a new 100 mg vial. Close cap and vortex to mix thoroughly. Transfer solution to a larger tube and bring up to 4 ml total. Put 0.5 ml aliquots into microfuge tubes, label, and store in freezer.
3. Poke 4-6 holes in the food by pushing the wooden applicator into food, down to the bottom of the vial.
4. Add the appropriate volume of G418 stock solution to each vial:
	1. 100 µl of 25 mg/ml G418 per 10 ml of food in the vial
5. Put vials, without plugs, in the chemical hood so that the excess liquid can absorb into food and/or evaporate. It usually takes 1-2 hours for vials to dry. Be careful not to over dry the vials!

### Procedure:

1. Set up a cross on the neo-containing media using 5-10 virgin heterozygous females and 3-6 males with the appropriate balancer.
2. Put flies from a stock that has hs-neor into one vial with food for the positive control and flies from a stock that lacks hs-neor for the negative control.
	1. The cleanest controls to use are the parents of the original cross. DO NOT use FRT 101, 42B (also called G13), or 2A as positive controls as these FRTs do not contain a neo resistance gene.
3. Put cross at 25°C.
4. After 2-3 days, once many eggs are visible on the surface of the food in the vials, heat shock the vials (with or without the adults present) at 37°C for 1 hour 1-2 times per day everyday until you see pupae form on the sides of the vials.
5. Transfer adults to a new vial before the next generation begins to eclose.
6. When the next generation ecloses, check to make sure there was good survival in the positive control and no survival in the negative control.
7. Collect the adults that eclose and select for the mutant phenotype (in many cases, the mutant allele will be marked with mW+, allowing you to select for pink or red-eyed flies).
8. If flies eclosed in the negative control vial, you cannot be confident that the selection worked efficiently. Therefore, you either have to set it up again, or select for the FRT by PCR. The second option can be a good choice if your mutation is more than ~20 map units from the FRT because it is likely that many of the flies recovered are recombinants. (Map unit positions of genes can be found in the gene reports on flybase).