

Flow Chart for a Cross

Set up a bulk pair mating
(7-10 ♂ and 3-5 ♀)



Set up a bulk pair mating
(7-10 ♂ from first mating and 3-5 ♀ from strain with the background you want to maintain)



Set up single-pair matings

(set up using ♂ from cross plates and ♀ from the strain with the background you want to maintain; set up at least 8 and it might be best to set them up at different times through out the day)



♂'s on plates?

Yes

No

Genotype

At least one plate with positive ♂

No plates with positive ♂

Go back the the last plate with positive ♂, chunk that plate and set up a new round of single-pair matings using ♂ from that chunk.

Set up the next set of single-pair matings using ♂ from that positive plate and ♀ from the strain with the background you want to maintain

Go back the the last plate with positive ♂, chunk that plate and set up a new round of single-pair matings using ♂ from that chunk.

- Continue with the above for a minimum of six rounds of outcrossing. This ensures that your newly built strain has as much of the ♂ strain background as possible.
- After the strain has been outcrossed, the genome needs to be homozygosed.
 - Pick single hermaphrodites to eight different plates.
 - Genotype the offspring from each plate.
 - Ideally, you want to genotype for both the allele you want and the allele you don't want.
 - If you get an offspring that is homozygous for the allele you want, you are done! Freeze the strain!
 - If you don't, continue putting single ♂ on eight plates and genotyping the offspring until you get a plate that shows offspring that are homozygous for your allele.
 - If you are not able to genotype both alleles, genotype for the allele you want. Go through enough rounds of homozygosing (putting eight single ♂ on plates from a plate shown to have positive animals) such that each plate shows animals with the allele you want. This process will probably take 6-8 rounds of homozygosing.