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 throughout the day)

♂♂’s on plates?

Yes

Genotype

At least one plate with positive ♂♂

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

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.

No

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.