

## How to build a near-isogenic line (NIL)

by Erik Andersen, May 18, 2017

We use NILs to both validate and narrow QTL genomic regions. For drug responses, it is often best to build the NIL in the direction where the genomic region that confers the resistant phenotype is crossed into the sensitive strain genetic background. In the Figure below, strain A (blue) is the resistant strain, and strain B (orange) is the sensitive strain. Only one chromosome is shown in the Figure. Remember that the other chromosomes need to be considered and eventually converted to the sensitive genetic background.

1. Start with a bulk cross of 7-10 L4 males with 3-4 L4 hermaphrodites (Figure, cross#1). If you are building the NIL on the X chromosome or you care about cytoplasmic factors, then you should keep track of which strain is the female/hermaphrodite parent. Otherwise, it does not matter. In the Figure, strain A is the male parent and strain B is the hermaphrodite parent.

2. To ensure that you have cross progeny and not self progeny in the next generation, let the males mate with the hermaphrodites for two days. Then, pick the hermaphrodites singly to individual plates. If you see male offspring on those new plates in three days, then you know you have heterozygous cross progeny.

3. Pick 3-4 heterozygous cross progeny L4 hermaphrodites to single plates (Figure, cross #2, a self cross). Recombination occurs in the germline of this heterozygous mother. The number of plates is dependent on how many recombinant offspring you want to score. With a 1 cM distance between your markers, you expect to find one recombinant out of 100 offspring. Many times, we score 1000 recombinant offspring to find the rare recombinants that we want.

The protocol splits into left recombinants (a) and right recombinants (b).

4a. To identify recombinants to the left of the confidence interval (denoted as vertical dotted lines in the Figure), we score all offspring from the heterozygous mother's self cross. These offspring are scored using molecular markers (like indels and differential PCR product sizes or snip-SNPs). The regions scored by PCR are denoted by horizontal red bars. Each red bar is composed of a left and right primer pair that amplifies a genomic region of 100-1000 bp to be scored for an indel or restriction site. To identify recombinants to the left of the CI, you use two PCR products. The left-most PCR will look heterozygous (strain A and strain B). The right-most PCR will look homozygous (strain A). It is possible that the other homolog will be from strain B instead of A like in the Figure, but you should avoid these recombinants, which means that the right-most PCR will look heterozygous. I know that means we are throwing away half of the recombinants, but it is possible that another recombination event in a subsequent generation could occur within the confidence interval. We will not be able to detect it until whole-genome sequencing later.

5a. Recombinant offspring should be picked to individual plates or to individual wells of a 96-well plate (with food). These recombinants can self and grow to starve out the plate or well.

6a. Once starved out either wash off of each individual plate (be sure to leave some progeny behind) or take half of the progeny from each well of the 96-well plate, lyse the worms.

7a. Perform the two PCRs described in step 4a and identify recombinant offspring in the region of interest.

8a. Go back to the original plate or the original well and recover the surviving worms to a fresh 6 cm plate. Wait a day and then pick 8-16 individual animals from that plate singly and let them self (Figure, cross #3, a self cross). It is best to pick a few times over the course of two days to ensure that you can identify your desired recombinant if it has a developmental delay.

9a. Score the 8-16 hermaphrodite parents as soon as they have laid embryos to identify a homozygote for your recombinant chromosome (strain B at the left PCR and strain A at the right PCR).

4b. To identify recombinants to the right of the confidence interval (denoted as vertical dotted lines in the Figure), we score all offspring from the heterozygous mother's self cross. These offspring are scored using molecular markers (like indels and differential PCR product sizes or snip-SNPs). The regions scored by PCR are denoted by horizontal red bars. Each red bar is composed of a left and right primer pair that amplifies a genomic region of 100-1000 bp to be scored for an indel or restriction site. To identify recombinants to the right of the CI, you use two PCR products. The right-most PCR will look heterozygous (strain A and strain B). The left-most PCR will look homozygous (strain A). It is possible that the other homolog will be from strain B instead of A like in the Figure, but you should avoid these recombinants. I know that means we are throwing away half of the recombinants, but it is possible that another recombination event in a subsequent generation could occur within the confidence interval. We will not be able to detect it until whole-genome sequencing later.

5b. Recombinant offspring should be picked to individual plates or to individual wells of a 96-well plate (with food). These recombinants can self and grow to starve out the plate or well.

6b. Once starved out either wash off of each individual plate or take half of the progeny from each well of the 96-well plate, lyse the worms.

7b. Perform the two PCRs described in step 4b and identify recombinant offspring in the region of interest.

8b. Go back to the original plate or the original well and recover the surviving worms to a fresh 6 cm plate. Use one plate for each potential recombinant strain. Keep them independent. Wait a day and then pick 8-16 individual animals from each recombinant plate singly and let them self (Figure, cross #3, a self cross). It is best to pick a few times over the course of two days to ensure that you can identify your desired recombinant if it has a developmental delay.

9b. Score the 8-16 hermaphrodite parents as soon as they have laid embryos to identify a homozygote for your recombinant chromosome (strain B at the right PCR and strain A at the left PCR).

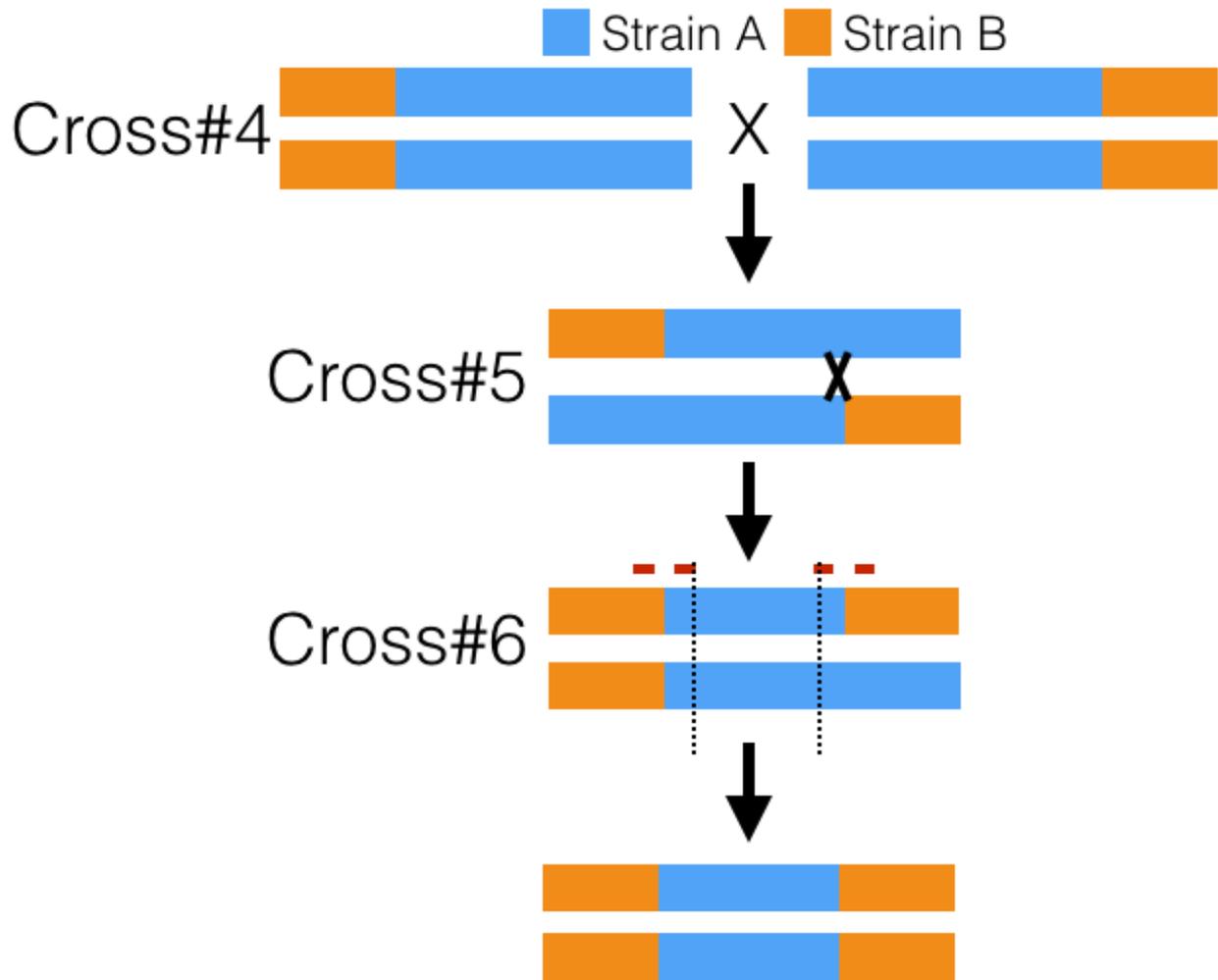
Now, you have (at least) two recombinant strains that are homozygous for the CI. These strains have to be crossed to get the interval isolated in the sensitive (strain B) chromosome background.

10. Set up a bulk cross of 7-10 L4 males from the left recombinant strain with 3-4 L4 hermaphrodites from the right recombinant strain (Figure, cross#4). You could also set the cross up in the other direction. You might have to generate male stocks by heat shock (follow the making male stocks protocol).

11. To ensure that you have cross progeny and not self progeny in the next generation, let the males mate with the hermaphrodites for two days. Then, pick the hermaphrodites singly to individual plates. If you see male offspring on those new plates in three days, then you know you have heterozygous cross progeny.

12. Pick 3-4 heterozygous cross progeny L4 hermaphrodites to single plates (Figure, cross #5, a self cross). Recombination occurs in the germline of this heterozygous mother.

13. The progeny from cross #5 need to be scored for a rare recombinant that makes the final NIL region from strain A in the strain B background. Using the same four PCRs from 4a and 4b, identify that recombinant using the same procedure as before (either on plates or in wells of a 96-well plate). This time, you want to identify a recombinant who is strain B homozygous, strain A homozygous, strain A homozygous, and strain B heterozygous (from left to right across the PCRs) (Shown in the figure) OR a recombinant who is strain B heterozygous, strain A homozygous, strain A homozygous, and strain B homozygous (from left to right across the PCRs) (the other homolog is from the right recombinant parent).



14. As before, recover this rare recombinant once identified. Wait a day and then pick 8-16 individual animals from each recombinant plate singly and let them self (Figure, cross #6, a self cross). It is best to pick a few times over the course of two days to ensure that you can identify your desired recombinant if it has a developmental delay.

15. Score the 8-16 hermaphrodite parents as soon as they have laid embryos to identify a homozygote for your NIL genomic confidence interval (strain B at the right-most and left-most PCRs and strain A at the inner two PCRs).

You have that chromosome constructed. Now, you have to consider the other five chromosomes. They are a mix of strain A and B because we haven't been following them. You want to convert those other chromosomes to strain B genetic background.

16. If your NIL region is not on X, then use X exchange to swap out the X chromosome. Make a male stock of your strain with the NIL genomic region. Cross those males to strain B parent stock. Take male cross progeny from that cross and cross back to the strain B parent.

17. Identify 8-16 cross progeny L4 hermaphrodites from the last cross and clone them singly onto 6 cm plates. Lyse and score to confirm that you maintained the intact NIL genomic confidence interval (strain B at the right-

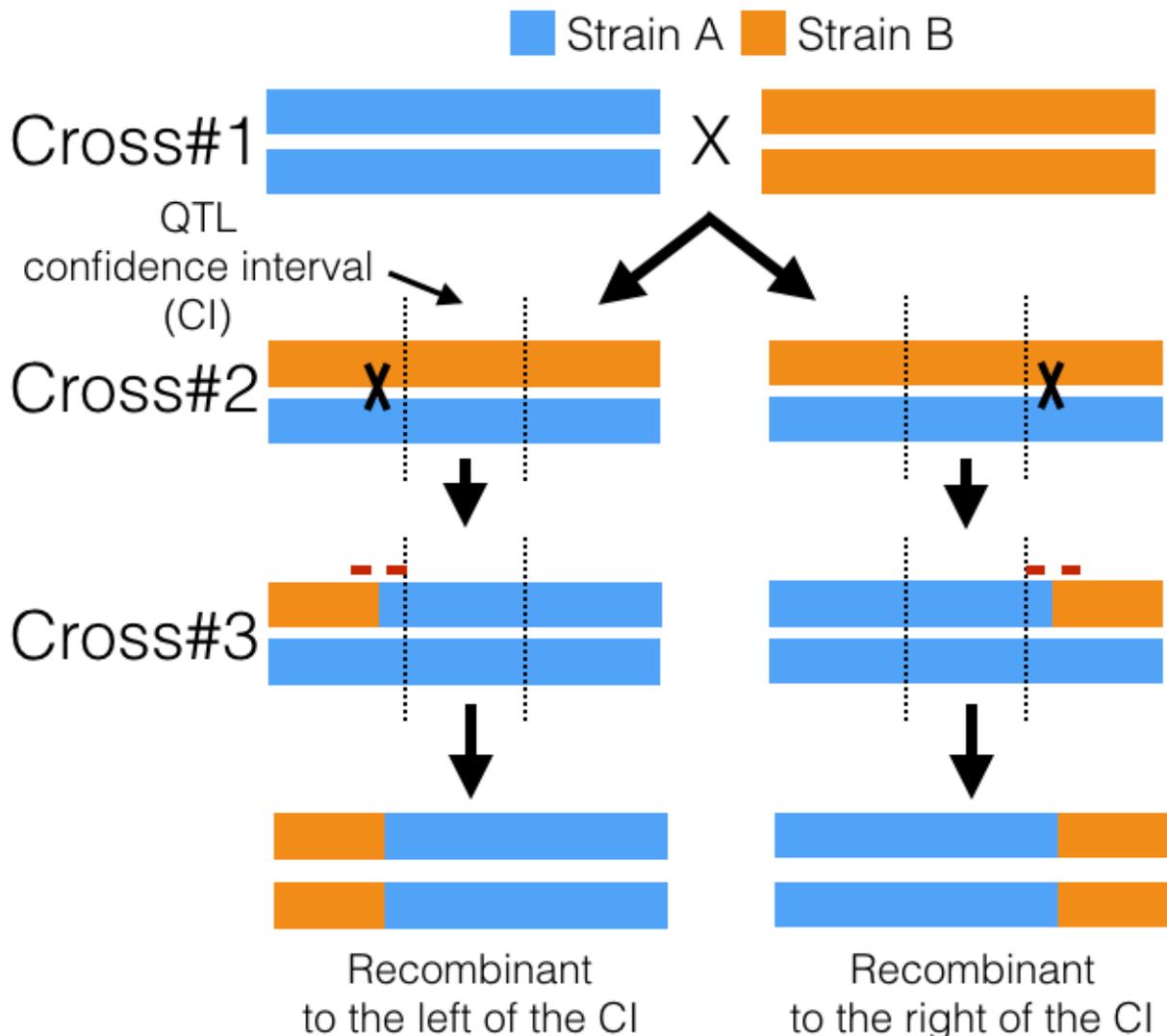
most and left-most PCRs and strain A at the inner two PCRs). When identified, homozygous. That strain has a X chromosome from strain B and the NIL interval you constructed.

18. Take males from that strain and cross to strain B (bulk cross). Take cross progeny males from that cross and again cross to strain B (bulk cross).

19. The progeny from that cross are either a heterozygote for your NIL genomic confidence interval or homozygous for a strain B chromosome. Because each male is unique, set up cross progeny males as single-pair crosses to strain B hermaphrodite mothers (L4 single pair crosses). Set up 8-16 single-pair crosses at different times over at least one day.

20. Once these males have mated for two days, lyse and score them to find which males had the NIL interval. Keep those crosses and throw out all the others.

21. From those crosses, set up 8-16 single-pair crosses with those cross progeny males crossed to strain B hermaphrodite mothers (L4 single pair crosses). Each generation of this type of introgression cross removes more of strain A from unlinked chromosomes and converts the genetic background to strain B. You can also see and follow the flow chart for a introgression cross protocol.



22. Perform at least six introgression crosses following the NIL interval using the inner PCRs each generation.
23. After six introgression crosses, homozygose the NIL interval and self two more generations.
24. In parallel, chunk the strain to freeze and to collect DNA for whole-genome sequencing.