Mapping the allyl alcohol resistant *bet21* in *C. elegans*

SPUR Program
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Dr. Bettinger’s Laboratory

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Alcoholism

• Alcoholism is a severe disorder that has lasted for centuries and yet we have not found any cure or a clear explanation of how it works.
• Some research has shown that variability in genes encoding ADH enzymes in humans alter the likelihood to become alcoholic.
• Thus, we are interested in determining the exact genes that affect ADH functions.
Alcohol Metabolism

- ADH = alcohol dehydrogenase = an important enzyme to metabolize ethanol into acetaldehyde which will be further metabolized by aldehyde dehydrogenase ALDH into acetic acid.

ADH also metabolize allyl alcohol into acrolein, the volatile substance that can kill the animals.

- Comparing to ethanol, allyl alcohol exposure allows us to identify whether the animals have ADHs or not.
Overview of C. elegans Model

- *C. elegans = Caenorhabditis elegans*
- Size of the adult ≈ 1.5 mm
- Life cycle ≈ 3 days
- Progeny ≈ 300
- Nervous system ≈ 302 neuron cells for hermaphrodite and 381 neurons cells for male *C. elegans*
- Well mapped wild type circuit diagram (by White)
- Fertilization types:
  1. Inbreeding by self fertilizing hermaphrodite
  2. Out-breeding by crossing with male *C. elegans* which will give 1:1 ratio of male vs. hermaphrodite

* These stand out points suggest that *C. elegans* is an excellent model to study in Biology especially in Neuronal Biology.
Wild types and *bet21*

- CB4856 from Hawaii.
- These two strains’ genomic sequence are different in about every 1000 base pairs with a single nucleotide polymorphisms or SNPs. Hence, some of these SNPs are recognition sites for endonuclease restriction enzymes to cut. These are referred as snip SNPs.
- *bet21 C. elegans* were made by mutating N2 *C. elegans* with EMS (Ethyl Methansulfonate), a carcinogenic compound. They exhibit an insensitivity on Allyl Alcohol
  - Hypothetically, *bet21* must carry a mutated gene that leads to a nonfunctional ADH enzyme.
- Where is the gene?
Mapping strategy using SNPs (single nucleotide polymorphisms)

Where are these animals ALWAYS homozygous N2?

(pick only animals with resistant phenotype)

the chromosome of interest
bet2 ♀ × CB4856 ♂

F1: 100% Bet21/CB4856

F2
- 25% Bet21/Bet21
  - All survive
- 50% Bet21/CB4856
  - F2: ½ survive
- 25% CB4856/CB4856
  - All die

F3
- All survive
- F3: ½ survive

F4
- All survive
- Discarded

Isolate DNA
Materials and Methods

• Cross 5 bet21 hermaphrodites with 5 CB4856 male.
• Next day, move each mated bet21 hermaphrodite to new plates and let them produce F1 progeny which will be all heterozygote of bet21/CB4856
• Move 5 L4 F1 hermaphrodites to new plate.
• Let them to produce F2 progeny.
• Pick about 30-40 L4 F2 to prepare for testing on allyl alcohol the next day.
• 50% of all F2 progeny being tested die because bet21 is not totally recessive to CB4856 or CB4856 is semi-dominant to bet21.
• Each survivor is moved onto new regular NGM plate and let it reproduce F3 progeny.
• Testing F3 progeny again on allyl alcohol and only pick out plates with full survival rate.
• Retest F4 generation.
• Isolate DNA from the survivors of F4 generation for PCR.
PCR 18 point mapping

- *C. elegans* have 6 chromosome 1 to 5 and X chromosome. There are 3 points on each chromosome (left, right, and middle) to be mapped → 18 point mapping
- The genomic sequence of the recombinants will be easily mapped by using specific restriction enzyme at the different SNPs points.
- Areas on chromosomes that only carry *N2* genes are the interests because the mutation only lies in *N2* genes. From that area we can narrow down the interesting interval and locate the specific location of the gene causing nonfunctional ADH by sequencing within the interval.
## PCR results

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<th>Chromosome I</th>
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<td>N2: 345</td>
<td>N2: 377, 121</td>
<td>N2: 486</td>
<td>N2: 376</td>
<td>N2: 435, 70</td>
<td>CB: 398, 300, 78</td>
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<td>N2: 500</td>
<td>N2: 206, 189</td>
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Discussion

• *N2* only intervals were not clearly found because they were overlapped with either *CB4856* or Het (heterozygote).

• Explanations:
  1. Too few recombinants to suggest the correct result
  2. The *N2* only areas may lie on some other places that were not mapped
Future goals

• Make more recombinants
• Narrow down the interesting interval by PCR
• Sequence the interesting interval.
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