Genetic linkage and SNP mapping

Overview

Single nucleotide polymorphism (SNP) mapping is routinely used to narrow down the known physical location of mutations. Geographical separation and several million years of evolutionary drift have led to a sizeable number of genetic differences (DNA polymorphisms) between the Hawaiian (CB4856) and English (N2) *C. elegans* isolates. On average, there is one SNP, or single base-pair difference, every 1000 bp of genome sequence. In some cases, a SNP may result in either the creation or elimination of a restriction enzyme site within either the N2 genome or the HW genome. Thus, a DNA fragment containing such a SNP could be distinguished as coming from either the N2 background or the HW background based on whether or not the DNA fragment can be digested by that restriction enzyme. The location of every SNP difference between HW and N2 worms is known, allowing us to localize a mutation of interest to a specific chromosomal location in the *C. elegans* genome.

For all standard SNP mapping, HW males are crossed into a mutant strain to generate heterozygous cross progeny that are allowed to self, leading to the regeneration of homozygous mutant worms. An assessment of any SNP requires that the chromosomal region containing the polymorphism be amplified from worm genomic DNA using PCR. The amplified DNA is then digested using the appropriate restriction enzyme and agarose gel electrophoresis is performed to determine the sizes of the fragments. The closer your mutation lies to the given SNP being tested, the less likely that a homozygous mutant will harbor a HW allele of that polymorphism, and the more likely that there will be a significant over-representation of N2 homozygous loci among mutant animals. In fact, if the SNP you are testing lies very close to your mutation, you may observe nearly 100% of mutant animals to be homozygous for the N2 locus at that SNP.

We will map the genetic location of a recessive mutation that causes a dpy phenotype. Dpy worms are shorter and stouter than wild-type. You and your classmates will analyze 24 SNPs located in each of the six *C. elegans* chromosomes. The different SNPs you will examine either create or eliminate a *Dra*I restriction site in the genome, depending on whether the SNP comes from the N2 or HW background. You will digest the PCR fragments with *Dra*I – this will allow you later to determine which genomic backgrounds (N2 and/or HW) are present at each SNP location that was amplified from worm lysates. We will work together as a group to analyze the data and determine which SNPs are linked to the mutation.

Materials

Worm lysates from F3 generation, dpy x HW cross

PCR reagents (Taq DNA Polymerase, 10X reaction buffer, dNTPs)

5 mM SNP Mapping primer pair mixtures (5 mM each of forward and reverse primers)

10X NEB CutSmart Buffer (commercially available from New England BioLabs)

*Dra*I restriction enzyme (20 units/µL)

Sterile dH_2O

2% agarose gel

6X loading dye

Overview of SNP mapping

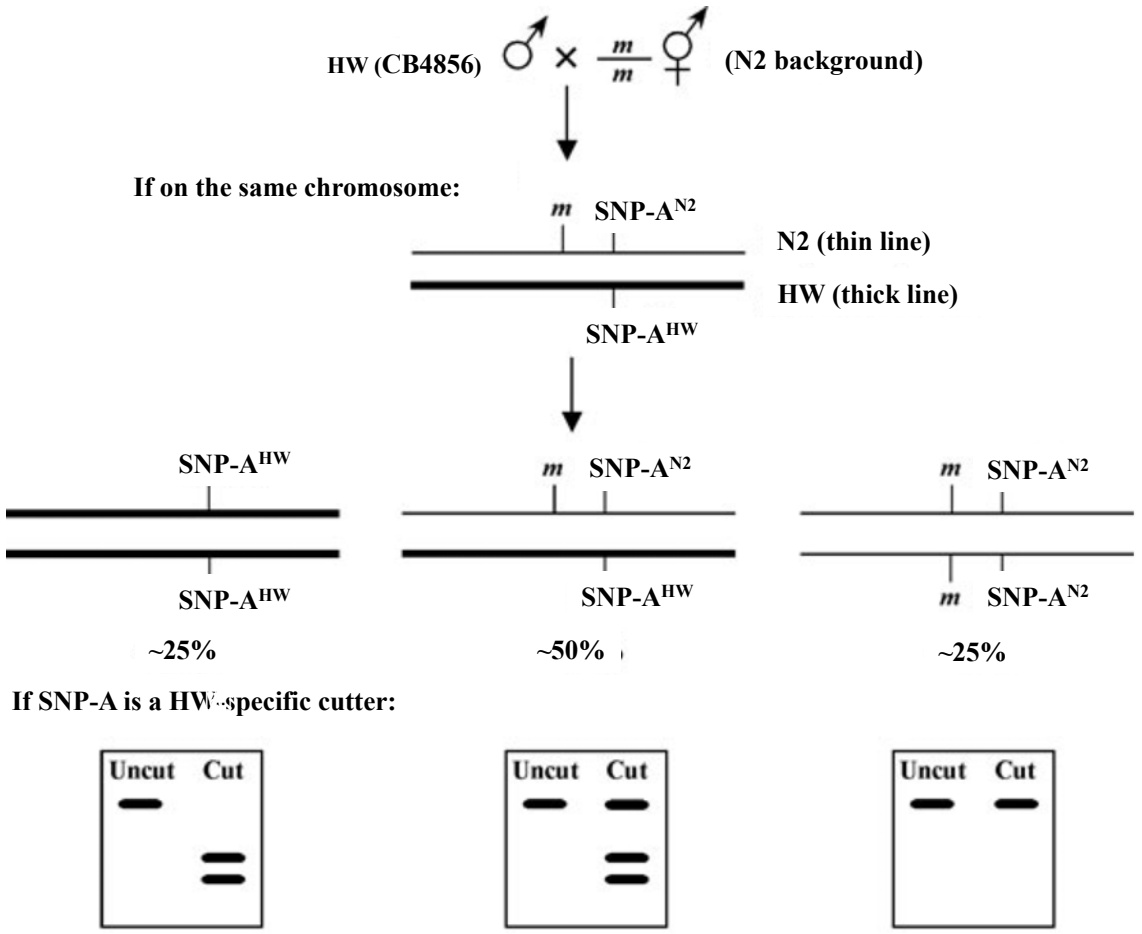


Figure 1. Overview of SNP mapping. In the case that your mutation is unlinked from the SNP being tested, homozygous mutants will segregate N2/N2: N2/HW : HW/HW animals in the standard 1:2:1 ratio. Thus 75% of the homozygous mutant animals will be either N2/HW (50%) or HW/HW (25%), whereas only 25% will be N2/N2. However, if the mutation is very close to the SNP being tested, near 100% of mutant animals will be homozygous for the N2 locus at that SNP. Adapted from doi/10.1895/wormbook.1.93.2, http://www.wormbook.org.

Procedure

A. PCR

1) Assemble the following reaction components for each 15 µL PCR reaction:
Note: To minimize pipetting errors, it is recommended to create a master mix (dH20, Buffer, dNTPs, Taq) for the total number of samples and dispense the PCR reaction mixture into each tube. Then add the corresponding lysate and primers to each tube.

Component	For 15 µL reaction	Final concentration
dH2O	10 µL	-
10X PCR Buffer	1.5 µL	1X
10 mM dNTPs	0.3 µL	200 µM
5 uM F/R Primer mix	2 µL	.666 µM
Worm Lysate	1 µL	Variable
Taq Polymerase	0.2 µL	1 U

2) Vortex the master mix briefly and centrifuge for 20 seconds.

3) Dispense 15 µL of the master mix into each 0.2 mL PCR tube.

4) Use the following thermocycling conditions for a routine PCR (500bp - 1kb amplicon):

Step	Temp.	Time
Initial Denaturation	95°C	2 minutes
35 cycles	95°C 60°C 68°C	15 seconds 30 seconds 1 minute
Final Extension	68°C	5 minutes
Hold	4°C	

5) Run 5 µL of the PCR product in a 2% agarose gel before proceeding with digests to check if the PCR reaction was successful. (Please refer to the **Agarose gel electrophoresis** protocol for instruction on how to prepare and run gels).

B. Digests

1) To digest the PCR product for SNP genotyping, combine the following reaction components. First, make a master mix containing the dH2O, 10X Buffer and Enzyme. Mix by vortexing, spin briefly and then add 5 µL of the master mix to 10 µL of the PCR product.

Component	For 15 µL reaction	Final concentration
dH2O	3.2 µL	-
10X Buffer	1.5 µL	1X
PCR product	10 µL	-
Restriction enzyme DraI	0.3 µL	6 U

2) Make sure that all the reaction components are well mixed and incubate the digest reaction 30 minutes at 37 °C. Alternatively, samples can be digested overnight at 37°C.

C. Agarose gel electrophoresis

Perform agarose gel electrophoresis to determine the sizes of the products of the *Dra*I restriction enzyme digestion. Use a 2% agarose gel to get good resolution of the fragments.

1) Pipette 3 uL of 6X Loading Dye directly into each digest sample. Use a new tip for each tube to avoid cross-contamination. Mix all tubes by slowly pipetting up-and-down several times (avoid making bubbles).

2) Load the first lane of the 2 % agarose gel with 10 µL of the 100-bp DNA Ladder.

3) Load 15 µL of each of your *Dra*I digest samples in the remaining lanes of the gel in the following order:

Ladder	A N2	A HW	A Mut	B N2	B HW	B Mut	C N2	C HW	C Mut	D N2	D HW	D Mut

4) Run the gels at constant voltage (~130V) until the dye-front has run about two-thirds of the way along the gel (about 30-45 minutes). Take a photograph of your gel using the UV Transilluminator.

D. Data analysis: determine restriction fragment sizes.

1) Using the 100-bp DNA Ladder (see ladder band sizes below), examine your agarose gel photograph and estimate the sizes of the restriction fragments present in each lane.

2) Compare the sizes of the restriction fragments you observed experimentally to the known expected sizes of the restriction fragments generated from N2 and HW SNP backgrounds as indicated in the attached table. (*Note: it may be difficult to clearly visualize DNA fragments smaller than 100 bp*). I Based on the sizes of your restriction fragments, determine if the SNPs are homozygous N2, homozygous HW or heterozygous (both N2-sized and HW-sized fragments are present in the same lane for heterozygous). Indicate in your table the genotypes for the SNPs you analyzed.

3) If both N2-sized and HW-sized fragments are present in the same lane, indicate in your notebook if there are significant differences in abundance of N2 and HW fragments relative to each other as observed on your gel.

4) Share your results with the other groups and collect their data for the other chromosomes to complete your table.

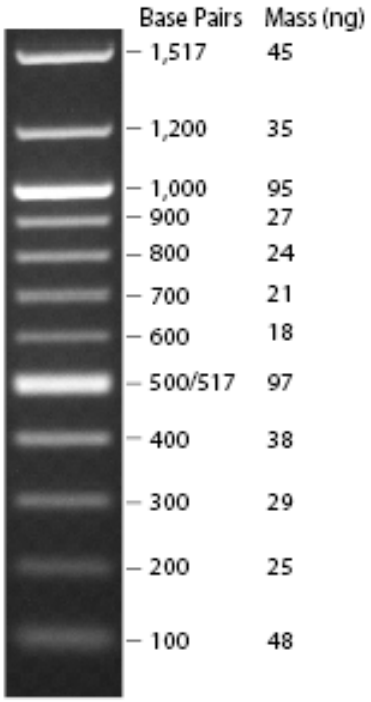
5) Based on the results, determine if any SNP are closely linked to your mutation (*Note: the genetic background for a linked SNP will be N2*). Use the information provided to assign the

gene to one of the *C. elegans* chromosomes and to identify the general region of that chromosome to which the gene maps.

Reference

Fay, D. and Bender, A. SNPs: Introduction and two-point mapping (September 25, 2008), *WormBook*, ed. The *C. elegans* Research Community, WormBook, doi/10.1895/wormbook.1.93.2, http://www.wormbook.org.

100 bp ladder



Scoring SNP mapping

Primers	Chromosome location	Expected N2 Fragment Sizes (bp)	Expected HW Fragment Sizes (bp)	Mutant genotype (N2, HW or Het?)
I, A	169,017	354, 146	500	
I, B	2,818,973	395, 144	538	
I, C	10,722,146	494	365, 129	
I, D	12,729,812	236, 99, 78	335, 78	
II, A	176,720	263, 112	375	
II, B	3,828,599	516	387, 129	
II, C	12,605,350	483	352, 132	
II, D	13,235,564	500	368, 132	
III, A	939,698	206, 189	395	
III, B	2,599,699	368, 105	473	
III, C	7,320,107	486	354, 132	
III, D	11,656,188	339, 156	495	
IV, A	1,799,032	187, 304	491	
IV, B	3,347,952	295, 124	419	
IV, C	4,991,851	376	300, 76	
IV, D	16,085,085	241, 108, 78, 48	319, 108, 48	
V, A	2,726,662	288, 167	455	
V, B	4,550,757	454	307, 147	
V, C	13,235,564	500	348, 152	
V, D	17,610,508	282, 205	487	
X, A	4,161,493	422, 72, 40	326, 96, 72, 40	
X, B	10,637,922	409, 133	542	
X, C	13,339,566	318, 191, 37	509, 37	
X, D	15,500,013	358, 134	492	

7.15 Experimental Molecular Genetics

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