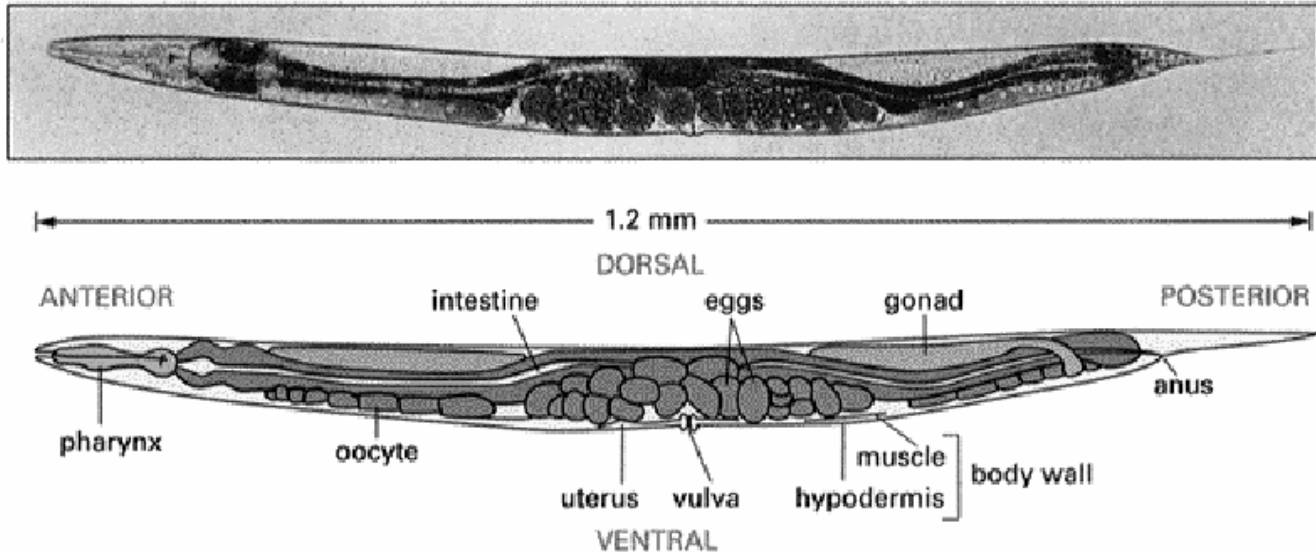


WORM LAB

BYS 219

February 2000

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Background:

The nematode, *Caenorhabditis elegans*, is a free-living, non-parasitic worm that normally lives in the soil. Researchers have been using this species as a model for development and neurobiology for the last few decades. The nematode is easy to grow in the laboratory and has a short generation time. Nematodes are grown on agar plates that have been seeded with a small dollop of *E. coli* bacteria. They eat the bacteria. The vast majority of these nematodes are hermaphrodites. They produce both male and female gametes and are capable of self fertilization. However, males do arise at a low frequency. In *C. elegans*, females have two X chromosomes and males have only one. Males arise from nondisjunction events that yield gametes with no X chromosome. Males are capable of fertilizing hermaphrodites, allowing for crosses to be done between different strains. Although it has a shorter history of use as a genetic model organism than *Drosophila melanogaster*, it is fast approaching the fruit fly in the number of research laboratories that choose the worm for research.

One valuable aspect of working with the worm is that the entire DNA sequence of the genome has been determined. This facilitates molecular genetic analysis of any gene. For example, screening for mutations can be performed using molecular techniques such as PCR.

In this laboratory investigation you will examine worms under the microscope to determine if the worms have a particular phenotype. The phenotype you will be looking for is referred to as *dumpy*. *Dumpy* worms are shorter than wild type worms. You will be working with a mutant allele of the *dpy-5* gene that is incompletely dominant and causes the worms to be *dumpy*. You will make an assessment of the phenotype of the worms with the microscope and make a prediction on the genotype of the worms. You will then test your prediction by using PCR to determine whether the worms have the wild type or mutant *dpy-5* allele. Since the mutations are incompletely dominant, you will be able to predict whether the worms are homozygous mutant, heterozygous or homozygous wild type. If the worms are heterozygous they should contain both a mutant copy and a wild type copy of the *dpy-5* gene.

Laboratory #1

- 1- You will be given a petri dish with worms on it. Look at the worms and get familiar with determining the *dumpy* phenotype.
- 2- The dish contains worms that are homozygous *dpy-5* mutant, homozygous wild type and heterozygous. Transfer 5 heterozygous worms to a new dish that has some *E. coli* bacteria on it. Put this dish at 20° C until the next lab. (20° C environmental chamber in room 243.)
- 3- Next you will design the primers that you will use for your PCR reactions next lab. (see below)

Design primers for the *dpy-5* gene.

The goal is to design primers that will allow you to amplify DNA from the *dpy-5* gene. You want to be able to amplify DNA from both wild type and mutant alleles. You will do PCR of whole worms to distinguish between worms that have the *dpy-5* mutation and ones that do not. The DNA sequence of the *dpy-5* gene is listed below. One strand of the wild type sequence is shown. The *dpy-5* mutation is a deletion of a part of the wild type DNA sequence. The region of the deletion is indicated with brackets.

Here are some tips for designing your primers.

- design primers that will produce a PCR product that is between 200 and 1000 bp long
- design primers that have melting points around 65°C
(calculate the melting point with this equation:
$$2(\#A + \#T) + 4(\#G + \#C) = \text{melting temp.}$$
)
- primers should be 20 to 21 nucleotides long
- primers should have a G or C at the 5' end
- primers should have no complementary regions within or between them

After your group has designed suitable primers, write them down and clearly indicate the 5' and 3' ends. Calculate what size product will be made during PCR from both the wild type and the mutant allele. Fill in the table below.

Primer #1 sequence	
# of nucleotides of primer #1	
T _m of primer #1	
Primer #2 sequence	
# of nucleotides of primer #2	
T _m of primer #2	
Size of wild type PCR product	
Size of mutant PCR product	

Compare the primers you have designed with other groups. Which set do you think will work the best? Show the instructor the primer sequences you have designed. The instructor will place an order for the primers. They will be synthesized by a company and will be available for you next time you come to lab.

>Nco I dpy-5 genomic fragment

5'ccatggaattgaattgaacatccccgaaggccattgtttcaattggtgtgttttacatata
aaaattaaacaattccacgtaaggaaaaaaagaacctttcaataactatctaggtaa
aatcattctttcagaaaggatggcacaacttcattctccttcattctttgccacttattccaca
acttcattctttccacaataagtgtcatgtgccggtgaatcgtcttcattcagagagttca
cttgcaaagatcagttatcaagaagcagagaaaaattattgaaaatttcagtgaccttctg
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tgtggaacactatacttttcaaattagaagcttttcctttttcttttga [acttgctaactttatct
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GATTCGGCGCTGCATGCGGAATCTCTGCTATCGTTGCTTGCCTT
TGGGCTGCACTTGTTCATCACAATGACATCAATGACATGTATG
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TGACACTTGGGGAACCCTTCTCGACGTTTCGTCACGGAGCCGGA
GAGTCTGCTGAGCAATACGTTTCGTGGAATCTTCGGACGTCACA
AGCGTTCCAACAGCCAATGCTCTTGCGGACTTCCATCTCAAGG
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GCCAGGAGGCACTGGACCAGACGGAAAGAACGGACCAACTG
GACTTCCAGGACTTAACATTCCAATTCCAATGACTTCCCTAAG
GAGTGCATCAAGTGCCCAGCTGGACCACCAGGACAAGATGGA
CTTCCAGGACAAGAAGGATTCCAAGGACTTCCAGGAGACGCT
GGAAAGCGTGGAACCCCAGGAAAGGACGGAGAGCCAGGACG
TGTTGGAGATATTGGAGATCAAGGAACTCCAGGACAAGACGG
ACAACCAGGACTTGCTGGACCACCAGGACGCGATGGACTTAC
CGGAAAGGGACAACCAGGAGTCGCTGGACGCCAGGAATGCC
AGGACCACGTGGAGAGCCAGGAAACAACGGAAATCCAGGAG
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GCTGGACCACAAGGAGCCGTTGGAGCCGATGCCGAATACTGC
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TATTCAAAATAAATATTTATGTATTCAATTGAAGTTTATCACAT
TTTTTGAATAATTCTTTTTGAAAATACGATGGCAGGTCTCGACG

cga]ctgaaagacgtaagggagttcgcgcctttaatgtatttctaacgctccgcccctga

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tgacacctcatatgtggcgccataatgaaaatagcagcacacgataaaagcatacct
gaagagttgttaaattaactaacagttaagaataaactgaattgaataatgaaaatctt
accgaaaagtgaatccgtcctccaaaatcgaatagaataacttcttcaaatcctccatg
g 3'

Laboratory #2

Today you will prepare worms for PCR reactions. In the next lab period, you will do the PCR reactions.

Below is a published protocol for PCR reactions with single whole worms (B.D. Williams in *Caenorhabditis elegans: Modern biological analysis of an organism*, Academic Press). The solutions will be provided for you, but, the recipes are listed here for your information. Follow the procedure for DNA purification today.

Set up 3 tubes according to the procedure below. In each tube put one or two worms.

Tube	Genotype of worm
#1	Homozygous <i>dpy</i>
#2	Heterozygous
#3	Homozygous wild type

DNA preparation:

A single worm is transferred directly from the culture plate to a 2.5 μ l drop of lysis buffer in the bottom of a 0.5 ml tube suitable for PCR. Care is taken to avoid transferring a large amount of bacteria with each worm. Try picking worms from a region of the plate where there is no bacterial lawn. Observe that the worm is in the tube using the microscope. Close the tube and freeze at -70°C until the next lab period. Make sure that your tubes are labeled accurately.

Laboratory #3

Remove your tubes from the freezer and add a drop of mineral oil as an overlay. Heat tube to 60°C for 1 hour, followed by 95°C for 15 minutes. Keep tube on ice until PCR cocktail is added.

Polymerase Chain Reaction :

PCR cocktail (22.5 μ l) is added on top of the mineral oil overlay that covers the DNA sample. When all the samples are ready, the tubes are centrifuged for a few seconds to move the PCR cocktail through the oil, mixing it with the 2.5 μ l DNA sample to bring the reaction volume to 25 μ l. The tubes are immediately transferred to the thermocycler and cycled 30 times with the following conditions: 94°C for 30 seconds, 58°C for 1 minute, and 72°C for 1 minute.

Recipes

1. Lysis buffer: 50 mM KCl, 10 mM Tris (pH8.3), 2.5 mM MgCl₂, 0.45% Nonidet P-40, 0.45% Tween 20, 0.011% (w/v) gelatin. Autoclave and then store in aliquots at -20°C ; just before use add proteinase K to 60 $\mu\text{g}/\text{ml}$.

2. Proteinase K stock: 10 mg/ml proteinase K (Boehringer-Mannheim) in water. Store in aliquots at -20°C.
3. 10X Taq Buffer: 100mM Tris (pH8.3), 500 mM KCl, 15 mM MgCl₂, 0.01% (w/v) gelatin. Autoclave and store in aliquots at -20°C.
4. PCR cocktail: 1.1X Taqbuffer, 5 nmole each dNTP per reaction , 25 pmole each specific primer per reaction, 0.6 units of Taq DNA polymerase (Promega) per reaction. Final amplification reaction should be 1X Taq buffer. PCR cocktail is freshly made before use.

Laboratory #4

Today, you will analyze your PCR products via agarose gel electrophoresis.

- 1- Prepare your samples for the gel by adding 5 µl of 6X loading dye to each tube. Use the centrifuge or flick the tube to move the loading dye through the mineral oil into the aqueous phase.
- 2- Load 20 µl from each sample onto an agarose gel and electrophorese the samples at an appropriate voltage.
- 3- Stain the gel with ethidium bromide and take a picture of the gel.

Are the results from the gel consistent with your predictions about the genotypes of the worms?