

Some tips concerning Lab Exercise 4-2 Molecular Gene Mapping

You will be using canned data for this experiment which can be found below as part of exercise 4.2. The procedures used to generate this canned data are very similar to what you've been doing in Lab exercise 4.1. However, in order for you to have enough data to analyze, we are providing this set of canned data. The canned data is complex and real and should be approached with caution (;-).

Your job is to determine whether either of the two markers described below is linked to the disease trait. Recall from the previous lab exercise what it means to say that two traits are linked. In a sense, the pattern of bands seen on a gel are a 'trait' and the disease is another trait. To help you analyze this data and answer this question, we have prepared a series of questions at the end of this exercise that essentially unpack this problem. If you work through these questions one by one you will find that you will be able to easily determine whether the disease gene is linked to either of these markers. You are not to turn in answers to these questions. They are simply to help you solve the problem. *Please be advised - many students have told us that simply working through the unpacking questions is all they need to solve this problem.* Try doing this before asking questions. You will be impressed with how smart you really are if you just give yourself a chance.

Concerning the lab report

You will describe how you prepared your gel in the Methods section but you will use the canned data for the results and conclusion section. Your lab report should state clearly whether either of these two traits is linked (essentially **Question 1**) and you should describe how you know that (The "unpacking the problem" questions should help you explain this). **Question 2** is also to be done and the answer (how far the disease gene maybe from this VNTR marker gene) should be included in your lab report. **You do not need to write out answers to any of the questions contained in Lab Exercise 4.1("Detection of a VNTR polymorphism by polymerase chain reaction").**

FYI: While the gel you prepared itself will not be discussed in the results section of your Lab report, you may still be interested in its analysis. Page 369 (number 8) of Lab Exercise 4.1 ("Detection of a VNTR polymorphism by polymerase chain reaction") has several helpful hints for how to interpret your gel. Review these as you look at your gel results to learn more about what happened with your particular PCR. You may also find it useful to look at problem 2 on page 370 in the same exercise to see how the frequency of the different polymorphisms vary across populations in the US. Approximately where does your polymorphism fall? (each allele number refers to the size of the allele in terms of the number of multiples of the 16 basepair repeat are present. For example, allele 14 is $14 \times 16 (=224)$ basepairs in size.

Below is a picture of the classroom data from Fall 2000.

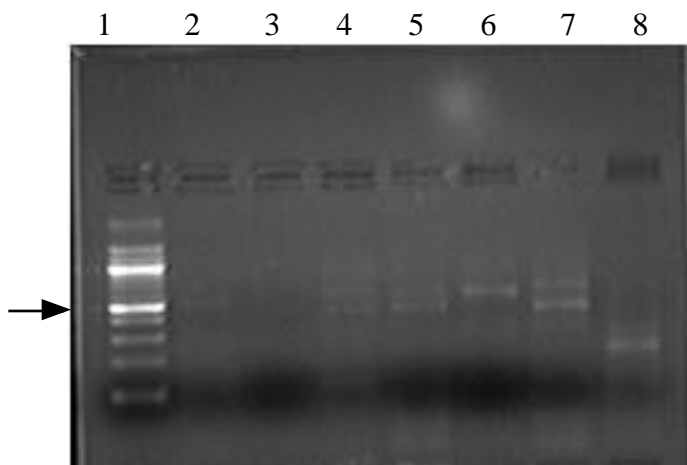


Figure 1. The results of a VNTR PCR experiment run in the BIO220 classroom laboratory. Lane 1 is the 100 bp ladder. The first band from the bottom is 100 bp, the second is 200 bp and so forth. The first bright band is 500 bp (at the arrow) and the second is 1000 bp in length. Lanes 2 is a negative control. Lanes 3-8 are the VNTR alleles of various students from that class.

Test yourself and try and answer the following questions:

In the gel above, how many students are homozygous?

How many are heterozygous?

Which student's reaction probably didn't work?

EXERCISE 4.2

Gene Mapping in Humans using DNA Markers

1. You have just started working with a research team studying a rare autosomal dominant genetic disease that causes childhood eye tumors. A pedigree of one family afflicted with this disease is shown in Figure 1, with affected individuals indicated in black.

Your job is to locate a chromosome site, or *locus*, that is located near the disease gene. Once you find a locus genetically linked to the disease gene, you can then use this information to isolate, or clone, the disease gene (see Part 2). To mark individual chromosomal sites, you decide to use DNA polymorphisms like the one introduced in Exercise 5.1, since previous researchers have already located the position of thousands of these loci on the 24 human chromosomes (for further information, see NCBI's "Human Genome Resources" web page at <http://www.ncbi.nlm.nih.gov/genome/guide/>).

To determine if a particular locus is linked to the disease gene, you isolate DNA from each family member in the pedigree and set up two polymerase chain reactions for each person. In the first reaction, you add the primer pair for the D1S80 locus on chromosome 1 (as in Exercise 5.1). In the second reaction you add a different primer pair, for the D13S1253 locus on chromosome 13. When the reactions are completed, you load a fraction of each on to separate wells of a polyacrylamide gel, electrophorese, and then stain the gel with ethidium bromide to detect the DNA. The results of this analysis are shown in Figure 2.

Do the data indicate that the "disease" gene (the gene that causes the disease when mutated) is linked to the D1S80 or D13S1253 locus (or both)? Make your answer quantitative and fully explain your reasoning.

Generation:

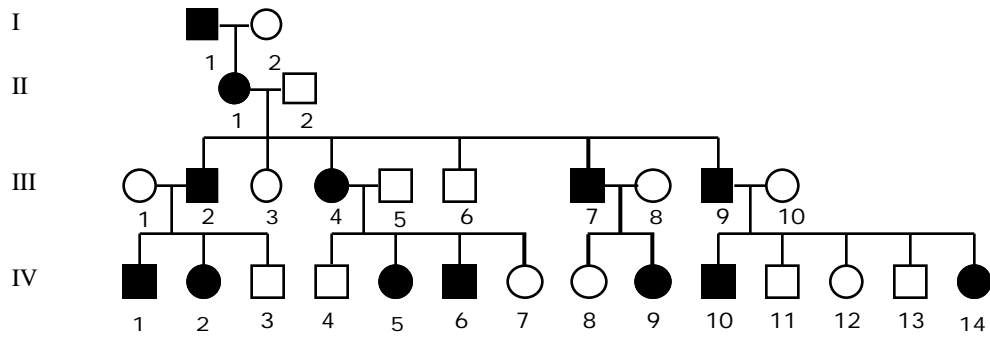


Figure 1. Pedigree Analysis. Males are indicated by squares; females by circles. Filled symbols indicate individuals affected with multiple eye tumors as a child.

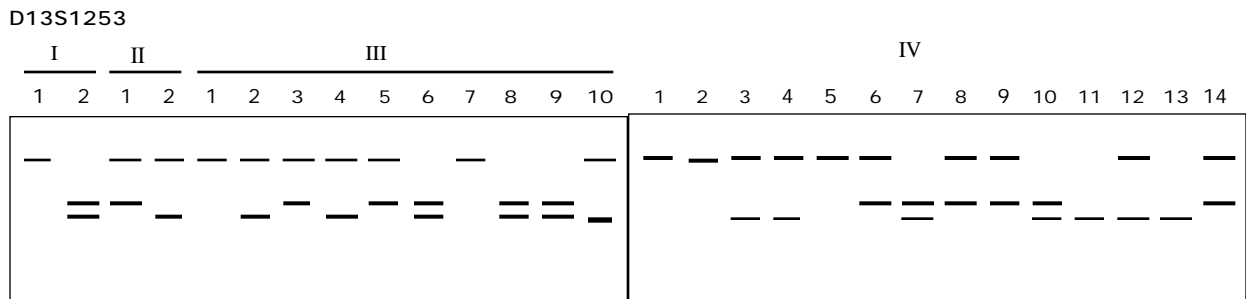
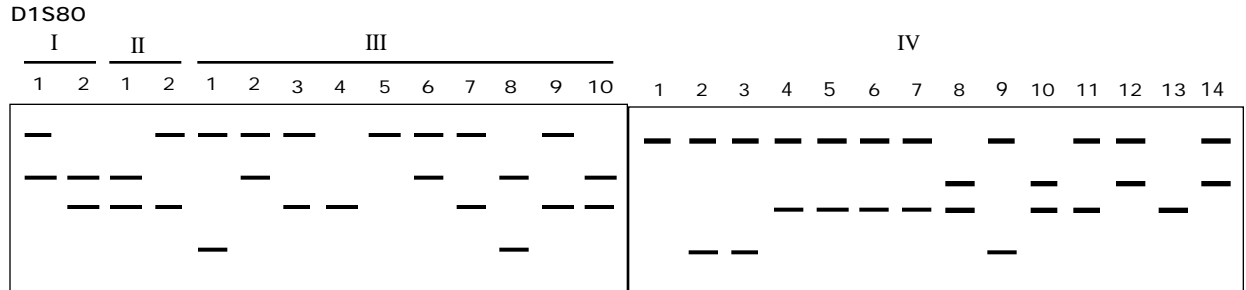


Figure 2. PCR Results. The PCR products using the D1S80 primer pair (top) or the D13S1253 primer pair (bottom) are shown for each family member in the pedigree. The numbering above each line corresponds with the pedigree labels in Figure 1.

Unpacking the Problem

(To help you through this complex problem, answer each of the following simpler questions.)

- a. Does the pedigree support the claim that this disease is inherited as an autosomal dominant trait? Explain.
- b. What does the term “disease gene” mean? All humans essentially have the same set of genes, so why doesn’t everyone exhibit this disease?
- c. Given that this genetic disease is caused by a rare autosomal dominant allele, what is the most likely genotype of affected individuals, such as I-1? What is the likely genotype of unaffected individuals, such as I-2?
- d. How can a chromosomal site (locus) be marked so that you can follow its inheritance?
- e. Why are different band patterns seen in each lane of the PCR gel? Why do some lanes have just one band? Why do some have two bands?
- f. What is genetic linkage?
- g. Perhaps the disease gene is on chromosome 1 near the D1S80 locus. Assuming so, draw a diagram of this chromosome pair in individual I-1. Indicate which alleles of the disease gene and the polymorphic locus are on each chromosome. Do this again for individuals I-2, II-1, and II-2.
- h. Assuming linkage, if a crossover occurred during meiosis in individual II-1, would a new combination of alleles be produced on a chromosome? If a crossover occurred in individual II-2?
- i. As in part (g), diagram the chromosome pairs in the offspring of II-1 and II-2.
- j. Which of these offspring are *recombinant*, having inherited a new combination of alleles from a parent? If the genes really are linked, how was this new combination produced? If the genes are not linked on the same chromosome, how was this new combination produced?
- k. Repeating steps (g) through (j), which of the offspring in the fourth generation are recombinant? Which are not? Which are uncertain?
- l. For the entire pedigree, how many of the offspring are recombinant for the D1S80 locus and the disease gene? How many of them are clearly not? Based on this data, what is the frequency of recombinants?
- m. Do these data indicate that the D1S80 locus is linked to the disease gene? That is, is the frequency of recombinant individuals significantly different from what you would expect if the D1S80 locus is not linked to the disease gene?
- n. Repeat steps (g) though (m) for the D13S1253 locus.

2. *Assuming that one of the two polymorphic loci is linked to the human disease gene, how could you use this to identify DNA clones in a genomic library that might contain the disease gene?*

Unpacking the Problem

(To help you through this complex problem, answer each of the following simpler questions.)

- a. The human genome consists of approximately 3 billion (3×10^9) base pairs of DNA. The total distance of the human genetic map is approximately 3700 centiMorgans (cM; or map units). If genetic mapping indicates that two genes are separated on a chromosome by 1 cM, approximately how many base pairs of DNA lie between them?
- b. Based on your calculated recombinant frequency, approximately how many base pairs lie between the disease gene and the linked polymorphic locus?
- c. You are given a DNA clone, isolated from a human gene library, that contains the chromosome segment for the linked polymorphic locus. The gene library was constructed using a bacteriophage vector that holds DNA fragments of approximately 15,000 basepairs. Do you expect this clone to also contain the eye tumor disease gene?
- d. How could you find a DNA clone that you expect to contain the disease gene? (Hint: See Q10-16 in *Essential Cell Biology*.)