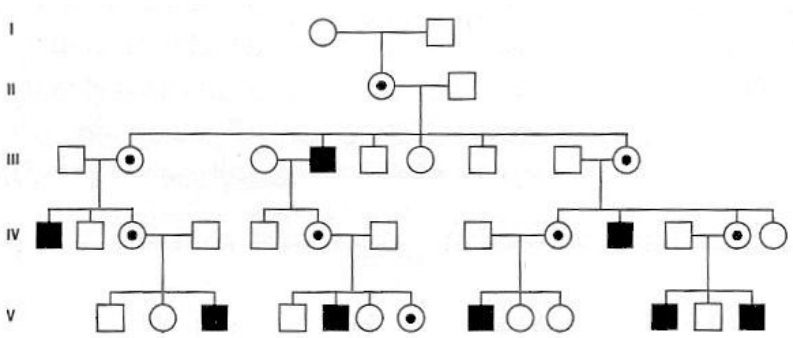
Bio 135PB Genetics Final (100 points) Name_____

Congratulations on making it to the end of this genetics course! One theme that has come up over and over throughout the course has been the study of inherited human diseases, something which you studied on your own for your research papers. In this final, all of the questions will relate to a disease called muscular dystrophy (MD), which is characterized by progressive muscular weakness. Everything in this test is based on real research, so you can learn something while you take the exam.

Although the questions build on each other, each question can be done independently, so don't worry if you cannot answer one of the earlier questions. There are easy questions scattered throughout, so read all the questions. You can do it!

1) (2 pts) Muscular dystrophy is actually a group of diseases. In other words, similar symptoms can be caused by mutation in one of several different genes. What is the term that we use to describe a disease that could be caused by mutations in different genes?

2) The most common form of muscular dystrophy is Duchenne muscular dystrophy, which is caused by deletion of exons in the *dystrophin (DMD)* gene. This is a pedigree for Duchenne MD and the females with the dot inside the circle are known carriers.

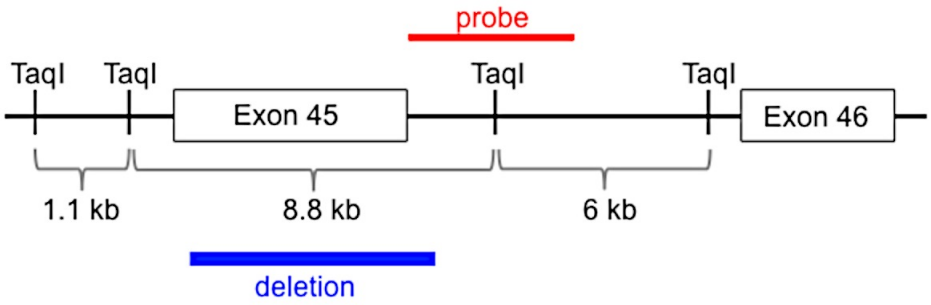


a) (1 pt) How is this disease inherited?

b) (3 pts) Although individual IV-3 (count across from left to right) does not have full-blown Duchenne MD, she does have weakness in some of her muscles, while other muscles are totally normal. Explain how that is possible (use a genetic explanation).

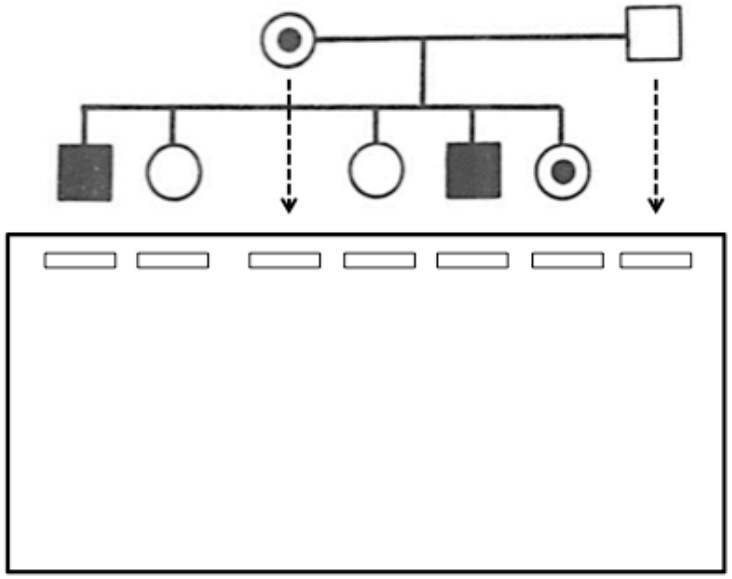
3) (7 pts) You can use Southern blot to detect the exon deletions in Duchenne muscular dystrophy.

In one family (pedigree below), exon 45 is deleted, causing Duchenne MD. This region of the *DMD* gene is shown below. TaqI is a restriction enzyme. The probe used for Southern blot is shown above the DNA and you can assume it will hybridize to the DNA even if only half of it is binding. The deletion found in this family is shown below the DNA and it is 6.2 kb long.

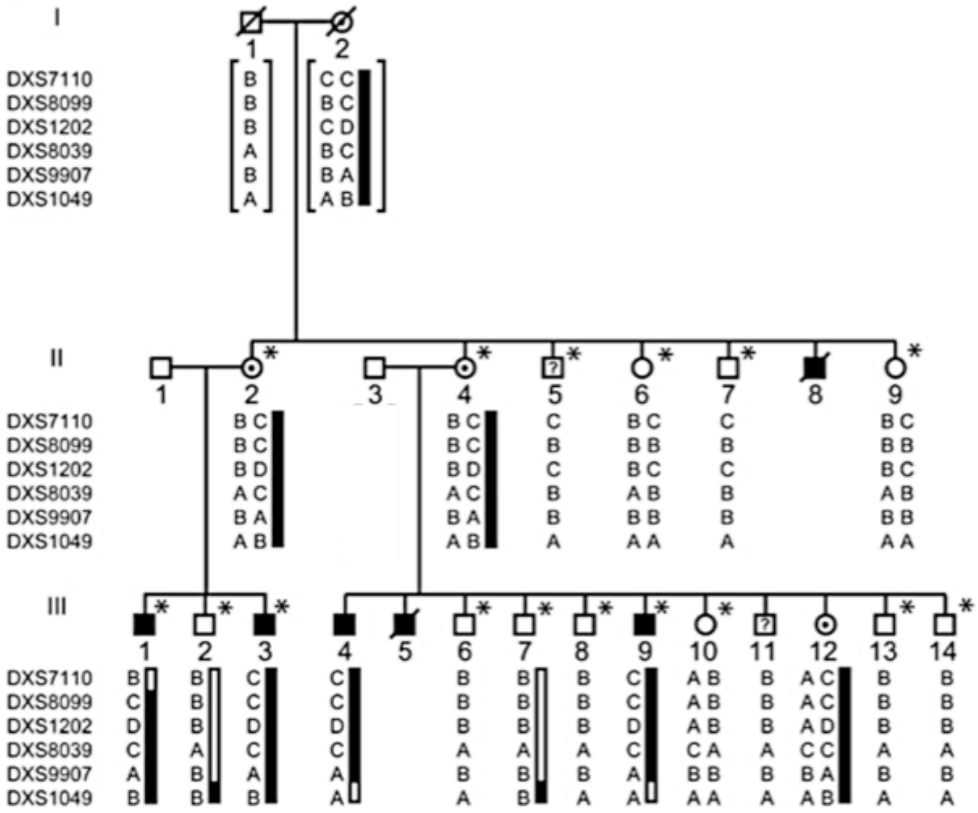


You collect genetic material from each individual in this family, digest it with TaqI and then do a Southern blot using the probe shown above.

Draw the results you would expect in the gel below. Note that each lane corresponds to the individual directly above that lane. Label the sizes of the fragments (you can do that on the side of the gel). Hint: Remember that we are diploid, and think about how this form of the disease is inherited.



4) As we learned in question 2, mutations in the gene *DMD* cause Duchenne muscular dystrophy. In the family shown here, the affected individuals have intellectual disabilities, but show no signs of muscular dystrophy. The authors trace the disease to a three base pair deletion in *DMD* that leads to deletion of one amino acid in the protein. This pedigree shows the haplotypes for a region near the mutation. You can ignore the asterisks and question marks in the pedigree. Black haplotypes are associated with the disease.



a) (2 pts) What is the haplotype of individual II-3?

b) (3 pts) Is the mutation in the first (DXS7110) or last marker (DXS1049) or somewhere in between? Explain your answer using evidence in the pedigree.

c) (2 pts) Why do you think this mutation in *DMD* does not cause muscular dystrophy? Think about the nature of the mutation (compare to the mutations in question #2 and 3).

5) Facioscapulohumeral muscular dystrophy (FSHD) in another form of muscular dystrophy. It is linked to a series of microsatellite repeats on the end of chromosome 4, known as the D4Z4 repeats. When the repeat number decreases below 10 repeats, then this can lead to muscular dystrophy.

a) (2 pts) A similar set of repeats is found at the end of chromosome 10. The ends of chromosome 4 and 10 often participate in balanced translocation. Why do you think these two chromosomes often do translocation? Explain.

b) (2 pts) When the D4Z4 repeats on chromosome 4 decrease below a certain critical number, this area of the chromosome (and areas nearby) gets hypomethylated (less methylated). What would be the likely effect of hypomethylation on gene expression? Briefly explain.

c) (2 pts) The nearest gene to the D4Z4 repeats is called *DUX4*, which encodes for a transcription activator. Patients with FSHD show increased expression of *DUX4* in their muscles compared to controls. What is one way you could measure expression of *DUX4* (not including microarrays)? Briefly explain.

d) (3 pts) Given all this information, how do you think FSHD is inherited? Explain.

6) Researchers have found a connection between Facioscapulohumeral muscular dystrophy (FSHD) and telomere length.

a) (3 pts) What are telomeres? How do they help solve the "end replication problem"?

b) (3 pts) Remember from question #5 that this form of the disease involves overexpression of the gene *DUX4* found near the end of chromosome 4. Researchers noticed that patients with FSHD have shorter telomeres on this chromosome compared to normal control subjects, and in fact the disease only occurs when telomeres are shortened. This disease is unusual in that it has a later age of onset compared to the other muscular dystrophies. Given this information, why do you think there is a late age of onset for FSHD?

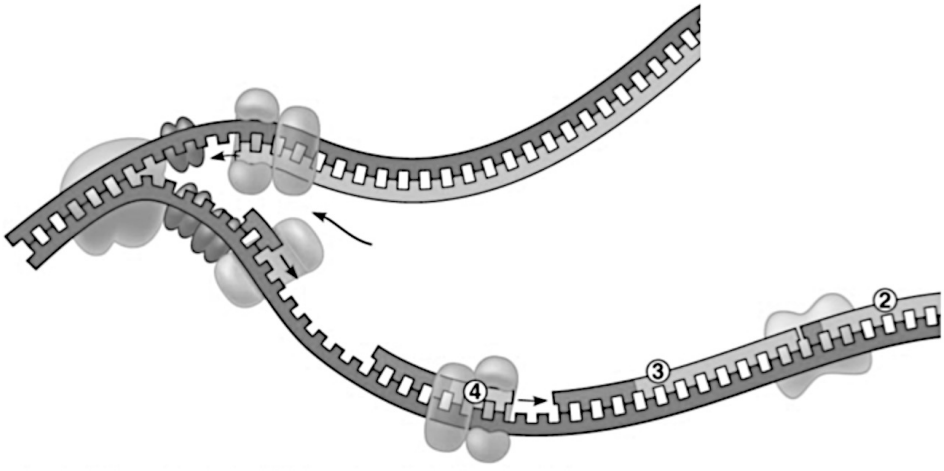
c) (4 pts) Since we are discussing DNA synthesis, let's label this diagram of replication.

- Label the 5' and 3' ends of the darker template strands (i.e. the very top and very bottom strands)

- Label DNA ligase

- Label helicase

- Label an Okazaki fragment



7) Myotonic muscular dystrophy is an autosomal dominant form of the disease that is caused by expansion of CTG repeats in the 3' UTR for the gene *DMPK*.

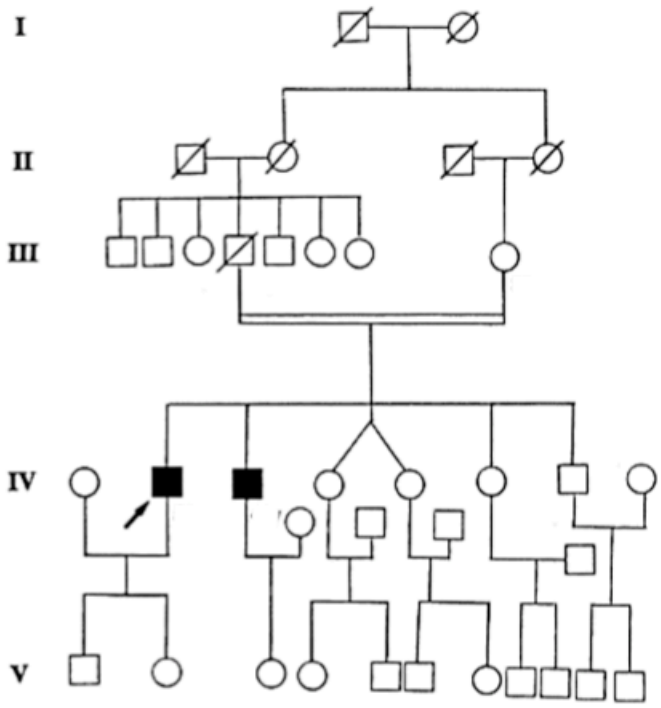
a) (2 pts) If CTG is in the template strand and is going 5' → 3', then what is the complementary mRNA sequence? Write the sequence 5' → 3'.

b) (2 pts) What is the 3' UTR? What function does it have?

c) (3 pts) Patients with myotonic muscular dystrophy are at increased risk of developing cancer. Although the mechanism is not completely clear, it appears that expansion of the CTG repeats somehow impairs mismatch repair in general. Explain why this mutation makes people more susceptible to getting cancer.

d) (2 pts) Would you consider this mutation more like an oncogene or tumor suppressor? Explain.

8) Oculopharyngeal muscular dystrophy is very rare and first affects the muscles that hold the eyelids open. The mutation for this form of MD occurs in the *poly-A binding protein (PABP)* gene, which is found on chromosome 14.



a) (2 pts) This form of muscular dystrophy is inherited differently depending on the nature of the mutation. In the family shown in this pedigree, what is the likely inheritance pattern? Briefly explain.

b) (2 pts) Find the individual in generation IV with the arrow. What is the probability that his next daughter will have the disease?

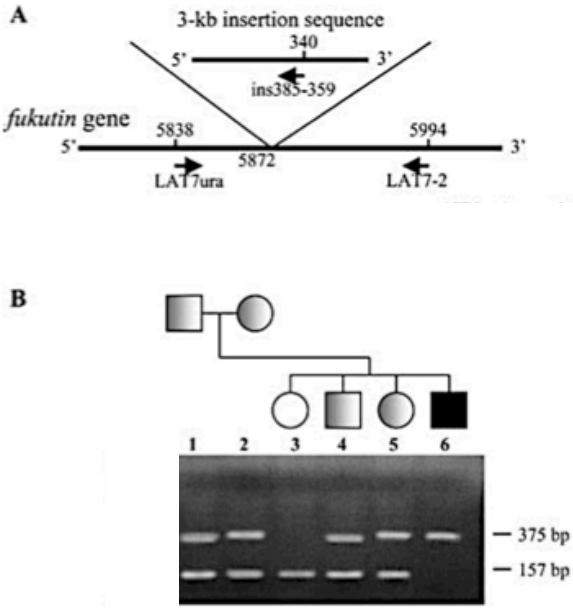
c) (3 pts) What is the probability that his unaffected brother is a carrier?

d) (3 pts) What kind of cellular problems would you expect in individuals without a properly functioning PABP? Explain. Hint: the name of the gene tells you exactly what it does.

9) Last pedigree, I promise. This form of muscular dystrophy is called Fukuyama-type congenital MD (FCMD) and is one of the most common autosomal recessive disorders in Japan.

The gene that causes FCMD is called *fukutin* and most patients with the disease in Japan have a 3 kb insert within the gene.

a) (3 pts) Explain how the insert might cause an autosomal recessive disorder. In other words, what is the insert doing to the function of the *fukutin* gene? Where did it likely insert within the gene?



b) (4 pts) In the figure shown above, the authors are describing a new method for testing for carriers of the disease. They did a PCR from genomic DNA using the three primers shown in figure A as arrows (LAT7ura, etc). Note that one primer hybridizes in the insert 340 bp from the 5' end of the insert (and remember the insert is 3 kb total). The results of the PCR for a family with FCMD is shown in figure B and the grey symbols in the pedigree indicate heterozygous carriers. Explain the method and the results in the gel. How can three primers be used to do PCR? Why do some people have two bands and others have one?

Question 9 continued

c) (1 pt) The insert in the *fukutin* gene is made up of repeats of *Alu*. Given the other information in the problem, about how many of these repeats are in the insert? Pick one:

2 repeats 10 repeats 100 repeats 1000 repeats

d) (2 pts) Briefly describe what an *Alu* repeat is.

e) (2 pts) Besides directly sequencing the insertion or using this PCR technique, what is another way you could test if someone is a carrier? Briefly explain.

10) Break time! Only a few more pages to go! Take a moment to answer these questions about the course.

a) (2pts) What was your favorite topic? Examples: transcription regulation, cancer genetics, inheritance patterns, the Holliday model, etc

b) (2 pts) What was your favorite assignment? Circle one (or more).

Quizzes – I like answering easy fact-based questions
Exams – I like the challenge!
Homework – Fun problems without the time crunch
Research paper – Finally, I can study what interests me
Student presentations – I like sharing my knowledge with others
Extra credit questions – I like researching the nitty gritty details

11) You want to study Duchenne muscular dystrophy using an animal model system, so you find the homologous gene in fruit flies. The *dystrophin (dys)* gene in flies is located in chromosome 3 and is 14 map units away from a marker gene called *Stubble (Sb)*.

Mutations in *dystrophin* are recessive and the mutants have impaired muscle function
Mutation of *Stubble* is dominant and it causes the hairs on the fly to be short

a) (5 pts) You set up the following experiment to confirm the distance between *dys* and *Sb*:

P generation: True breeding wildtype females mated with true breeding males with impaired muscles and short hairs

F1 generation: You take the F1 females and cross them back to the males from the P generation (true breeding mutants)

What are the expected percentages for the different phenotypes in the F2 generation? Show your work.

b) (3 pts) If you had not known the map distance between these two genes, would these crosses have been helpful for determining the distance? If not, what was the problem? How would you improve this experiment to help you figure out where *dystrophin* is located on chromosome 3?

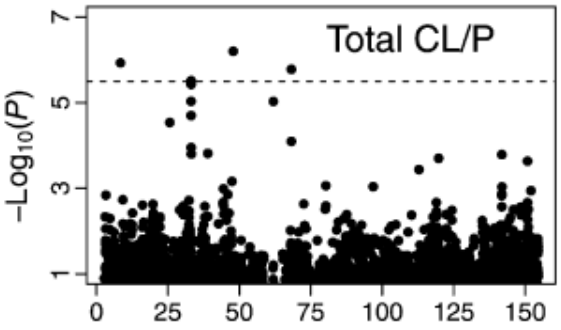
12) In a recent study, researchers were investigating the genetic basis of cleft lip and palate (CL/P) (this is when the lip and mouth palate do not close all the way during development). They decided to focus only on the X chromosome and look for SNPs associated with this birth defect.

a) (2 pts) What is a SNP? Don't just write what it stands for.

b) (3 pts) They looked at over 14,000 SNPs on the X chromosome in more than 1000 subjects. How were they able to genotype so many SNPs in so many people in a reasonable amount of time? Explain.

c) (2 pts) The results of the study are shown in this Manhattan plot for the X chromosome. The numbers on the x-axis are the position along the X chromosome (in megabases). The researchers found 5 fairly significant SNPs in the *dystrophin* gene associated with Duchenne muscular dystrophy.

Circle on the graph the likely location of the SNPs that are in the *dystrophin* gene.



d) (3 pts) Given that there are 5 SNPs *within* the *dystrophin* gene associated with cleft palate, does it seem likely that this gene plays an important role in the development of the lip and palate? Explain.

13) (8 pts) Let's finish the class the way we started – with a concept map! Use the terms in one of the word banks to create an accurate and cohesive concept map. You choose which set of words you want to use, but you must use all the words within that set. Every word must have at least one connection with the whole concept map. Every linkage must use a word or phrase to describe the connection. Only make one concept map total, using only one set of words.

Choose one

Set #1: DNA, heterochromatin, histone acetylation, mRNA, microRNA, RNA polymerase, TATA box, TFIIH, transcription,

Set #2: chromosome inversion, genetic variation, haploid, homologous chromosomes, karyotype, meiosis II, mutation, prophase I, recombination