7.014 Solution Set 6

Question 1

You are a UROP studying inheritance patterns in Alligators. Some of your alligators have angry dispositions while some are friendly. You determine that the gene for attitude is encoded by a single locus.

You cross true-breeding friendly male alligators by true-breeding angry female alligators and notice that all of the off-spring (male or female) are angry.

a. What are the possible modes of inheritance of angry dispositions in your alligators?

Autosomal dominant
Autosomal recessive
Sex-linked dominant
Sex-linked recessive

b. For each of the inheritance patterns you selected in (a.), cross an F1 generation angry, female alligator with an F1 generation angry, male alligator. What will be the phenotypic AND genotypic ratios of the resulting offspring?
Use A to refer to the allele conferring dominant attitude phenotype, and a to refer to the allele conferring recessive attitude phenotype.

Autosomal dominant:
F1: Aa ♂ x Aa ♀
F2: (same for both genders) genotypes: 1 AA, 2 Aa, 1 aa
(same for both genders) phenotypes: 3 angry, 1 friendly

Sex-linked dominant:
F1: X^AY ♂ x X^AX^a ♀
F2: Genotypes: 1 X^AX^A ♀, 1 X^AX^a ♀, 1 X^AY ♂, 1 X^aY ♂
Phenotypes: 2 angry ♀, 1 angry ♂, 1 friendly ♂

Assume anger is not a sex-linked trait. You notice that oftentimes the angry alligators have long tails while the friendly alligators have short tails. You determine that the short-tailed phenotype is dominant while the long-tailed phenotype is recessive.

You want to determine if tail length and attitude are linked. You cross a true-breeding, angry, long-tailed male alligator with a true-breeding, friendly, short-tailed female alligator to get an F1 progeny. You then cross your F1 generation animals and look at the results in the F2 generation.

c. What are the genotypes of the parental generation?
Use T to refer to the allele conferring dominant tail length phenotype and t to refer the allele conferring recessive tail length phenotype.
Male: AAtt *Female: aaTT*

Question 1, continued

d. What is(are) the genotype(s) of the F1 generation animals?
AaTt

e. What is(are) the phenotype(s) of the F1 generation animals?
Angry, short-tailed

f. What ratio of phenotypes do you expect to see in F2 generation if the traits are not linked?
9 Angry, short-tailed; 3 angry, long-tailed; 3 friendly, short-tailed; 1 friendly, long-tailed

g. What ratios of genotypes do you expect to see in the F2 generation if the traits are not linked?
1 AATT, 4 AaTt, 2 AATt, 2 AaTT, 1AAtt, 2 Aatt, 1 aaTT, 2 aaTt, 1 aatt

h. The F2 generation yields the following results:
Angry, short-tailed (93); Angry, long-tailed (30); Friendly, short-tailed (30)

Explain these results in genetics terms.
Because you never see the friendly, long-tailed class, it is probably a lethal phenotype. In other words, aatt alligators die sometime before they are born.

i. Based on the results observed in (h), are tail length and attitude linked traits? Explain your answer.
The traits are not linked because the cross conserves the 9:3:3:1 ratio of phenotypes.

Question 2

As it turns out, studying alligators is too dangerous for you, and you decide to turn your attention to a much less hostile organism, the fruit fly. You begin another UROP in a *Drosophila* lab. Your advisor tells you that you have to count thousands of flies. You are committed to the new UROP, but you secretly wonder if you shouldn't have stuck with alligators.

You are studying two genes known to be linked, wing shape and body color.

a. If two genes are linked, where are they located in relation to one another in the genome?
The two genes must be located on the same chromosome, and they must be found near one another.
b. Do two genes showing linked inheritance have to show a related function? Why or why not?
No. The chromosomal location of genes in the genome is generally random and not dependent on function. In bacteria, genes with related functions are often organized into operons, but these coregulation sequences do not exist in higher organisms.

Rounded wings (R) are dominant over pointed wings (r) and black bodies (B) are dominant over brown bodies (b). You perform the following cross:

Male:	$\frac{R \quad B}{r \quad b}$	X	Female:	$\frac{r \quad b}{R \quad B}$	

c. What are the expected genotypes and genotype ratios of the offspring if there is no recombination between these two genes?

1 $\frac{R\ B}{R\ B}$, *2* $\frac{R\ B}{r\ b}$, *1* $\frac{r\ b}{r\ b}$

d. What are the expected phenotypes and phenotype ratios of the offspring if there is no recombination between these two genes?

3 Round wing, black body, 1 Pointed wing, brown body

After you perform your cross, you get the following result:

725 Round wing, black body flies
255 Pointed wing, brown body flies
12 Round wing, brown body flies
8 Pointed wing, black body flies

e. What is the recombination frequency between wing shape and body color? Show your work.

In every case of possible recombination (occurring in one parent only – female or male, or occurring in both at the same time), we only see one of the two recombinant progeny because the other one appears to have parental phenotype, even though it carries a recombinant genotype. For example, if recombination occurs in the male parent, we would see

RB/rb x RB/rb P ➔
Rb/rB x RB/rb P after recombination in first parent
Rb/RB or Rb/rb or rB/RB or rB/rb F1

In the F1 above, we can't detect recombination in the first or third possible progeny because the phenotype is obscured by the parental B and R alleles respectively. Similarly, half of the recombinants are obscured in the case where recombination occurs in both parents.

Thus, RF=recombinants/total=2x(12+8)/(725+255+12+8)=4%.

Question 3

Hemophilia is a disorder characterized by the inability to properly form blood clots. Perhaps the most famous hemophiliac in history was the heir to the Russian throne, Alexei, the son of the last Tsar of Russia, Nicholas II and Tsarina Alexandra, granddaughter of Queen Victoria. Hemophilia is sometimes called "the royal disease" because it entered the royal lines of Germany, Russia, and Spain through the descendants of Queen Victoria. Alexei, together with his parents and four sisters, was executed in 1918.

Until recently, hemophilia was untreatable, and only a few hemophiliacs survived to reproductive age because any small cut or internal hemorrhaging after even a minor bruise was fatal. Now hemophilia is treated with blood transfusions and infusions of a blood derived substance known as anti-hemophilic factor.

Here is the pedigree that includes Alexei (Alexis in the figure), starting with his grandmother Alice, second daughter of Queen Victoria. Known carriers are depicted with half-filled circles in the figure.

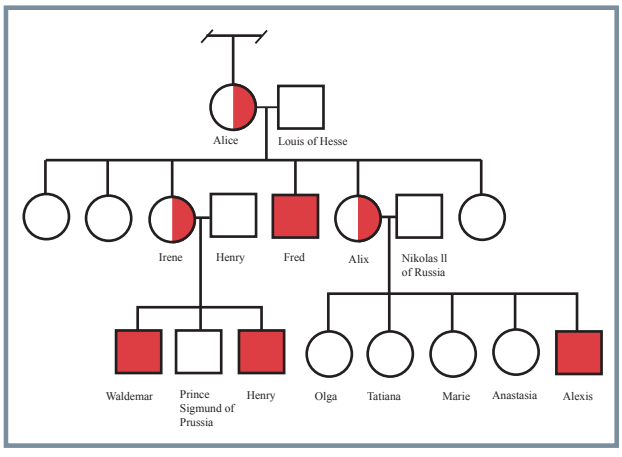


Figure by MIT OCW.

Question 3, continued

a) What is the mode of inheritance of hemophilia?
Hemophilia is an X-linked recessive disease.

b) Is it possible for females to get hemophilia? If yes, explain how. If no, explain why not.
For a female to exhibit hemophilia, she must be a daughter of a carrier mother and an affected father. It used to be rare for an affected individual to survive to reproductive age, but some, like Queen Victoria's son did. Now, due to advance treatments, it is much more common.

All four of Alexei's sisters are depicted as non-carriers. However, their status was never actually ascertained due to their untimely demise.

c) What is the probability that none of the sisters was a carrier of hemophilia? Justify your answer.
All sisters got a wild-type X from their father, and had a .5 probability of getting a wild-type X from their mother. Thus, the probability that none of the sisters were carriers is $(.5)^4=6.25\%$

Question 4

C. elegans cDNA library is commercially available.

a) What is the difference between a *C. elegans* cDNA library and a *C. elegans* genomic library?

A genomic library contains all the DNA, coding or not. A cDNA library contains onlt the DNA that is transcribed and processed into mature mRNA. No introns or non-coding DNA is represented.

You want to clone a *C. elegans* gene into the pUC19vector, shown below. The Ap region is the ampicillin resistance gene.

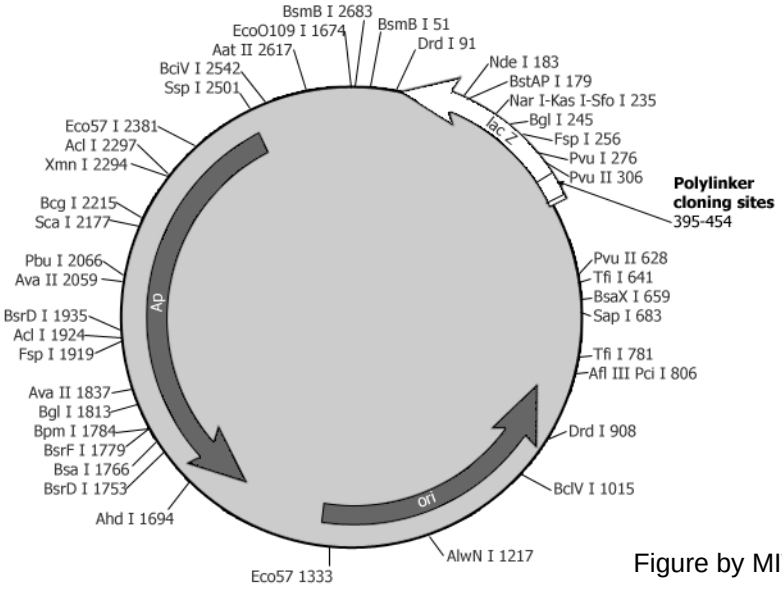


Figure by MIT OCW.

Question 4, continued

b) You successfully clone a gene into the Alwn I site at 1217, but then realize that the resulting plasmid will not help you generate more copies of the cloned gene. Why?
Cutting at the AlwN I site at 1217 will cut within the origin of replication and render the vector unable to be propagated by the bacteria.

The region of this vector where you want to insert the gene is called the polylinker site. Each of the enzymes shown in that site cut the vector once and only once.

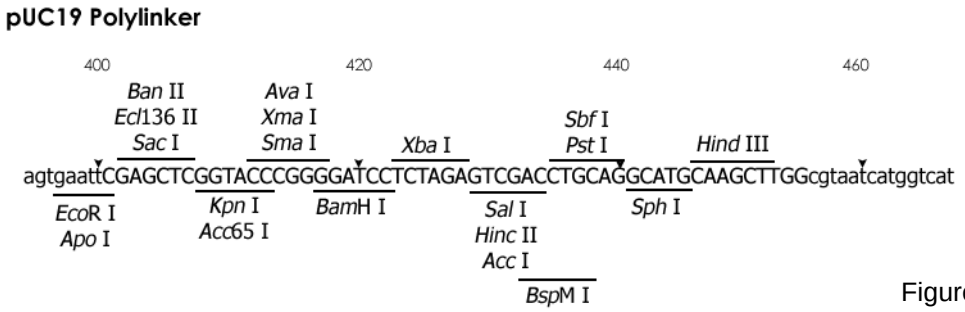
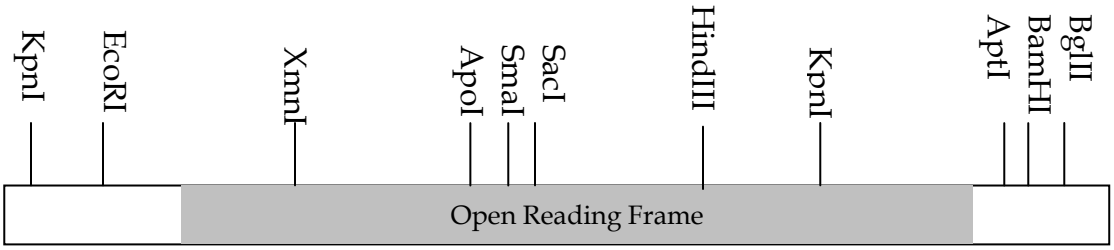


Figure by MIT OCW.

Below is the restriction map for the *C. elegans* gene of interest.



c) Given the above restriction map for the gene, what restriction enzyme(s) would you use to cut the gene and the polylinker region of the vector?

EcoRI and BamHI

You successfully ligate your cDNA and pUC19, and are now ready to proceed to the transformation. You want to be able to distinguish transformed from untransformed cells.

d) Therefore, the *E. coli* cells you choose for transformation have the following property (circle one):

ampicillin resistant *ampicillin sensitive*

Explain your choice
The E. coli cells that you are going to transform should be ampicillin sensitive so that after transformation you can screen for those cells that have acquired a plasmid by plating on ampicillin containing media.

e) To select for bacteria that now carry a plasmid, you then plate the bacteria from the transformation on medium containing __*ampicilin*_____.
Why?
Only the cells that have acquired the plasmid will be able to survive in the presence of ampicilin. This is a selection.

Question 4, continued

A day later, many colonies appear on your plates.

f) Not all the colonies represent cells that contain the *C. elegans* gene? Why?
Some colonies may represent a vector that has reacquired its original insert – the portion of the vector between the EcoRI and BamHI sites. However, if we gel-purified the vector away from that fragment, we could ensure that vast majority of the colonies represent a vector with a gene insert. This is because a vector cut with two different incompatible (different sticky ends) restriction enzymes can not close without an insert.

g) How do you now find the colonies that contain the *C.elegans* gene?
Cloning by hybridization, sequencing, or PCR to determine the length of the fragment of the new vector located between two points on different sides of the cut sites are all applicable.

Suppose that when successfully cloned, the protein of interest is expressed in the cell.

h) What promoter is used to drive the expression of the cloned *C. elegans* gene?
The C. elegans promoter can not be recognized by the E. coli machinery, so it is not the native promoter. The map of the plasmid indicates that the poly-cloning site, where the cut sites are located (the point of insertion of the gene) is located at the beginning of the lac Z gene. Therefore, if there is expression of the C. elegans protein, it must be driven by the lac promoter.