

~~Question 3, continued~~

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~~b) Is it possible for females to get hemophilia? If yes, explain how. If no, explain why not.~~

~~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.~~

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?

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

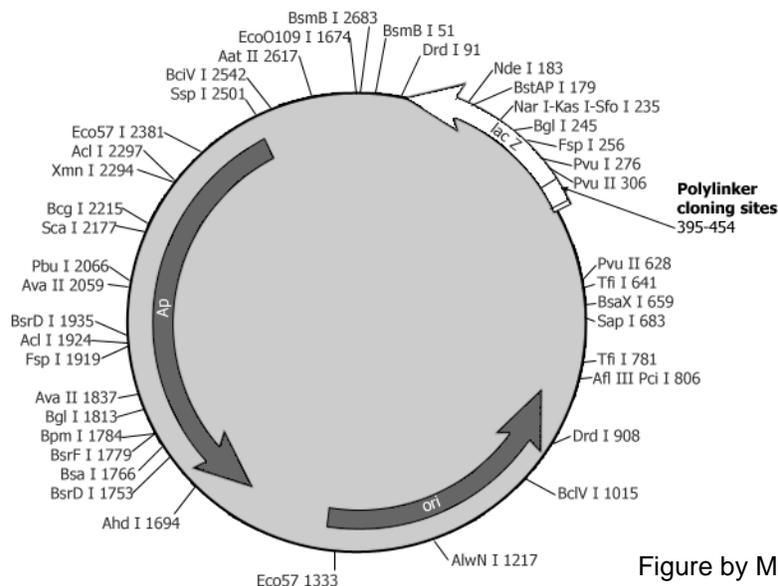


Figure by MIT OCW.

Question 4, continued

A day later, many colonies appear on your plates.

f) Not all the colonies represent cells that contain the *C. elegans* gene? Why?

g) How do you now find the colonies that contain the *C.elegans* gene?

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?

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 only 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 pUC19 vector, shown below. The Ap region is the ampicillin resistance gene.

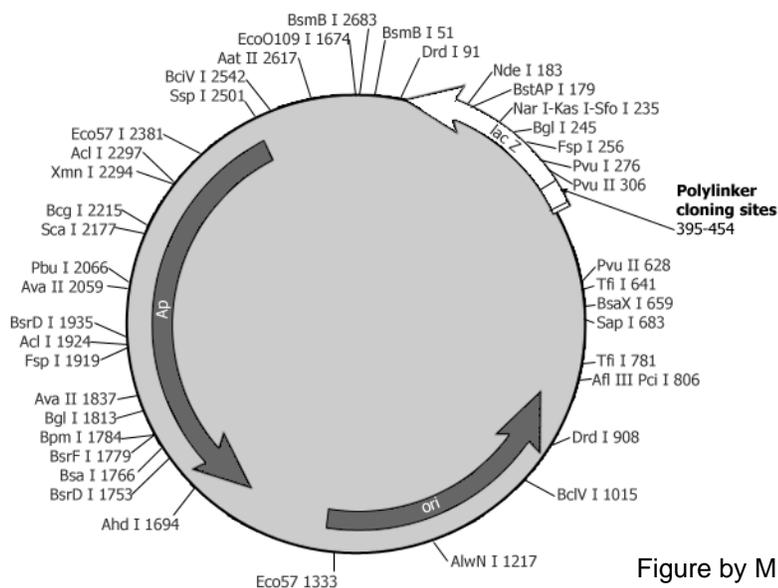


Figure by MIT OCW.

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.

pUC19 Polylinker

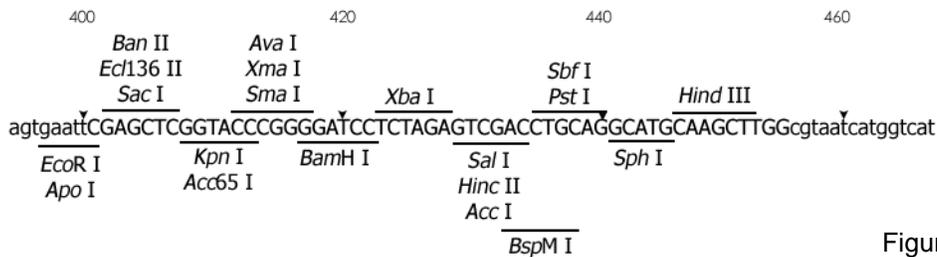
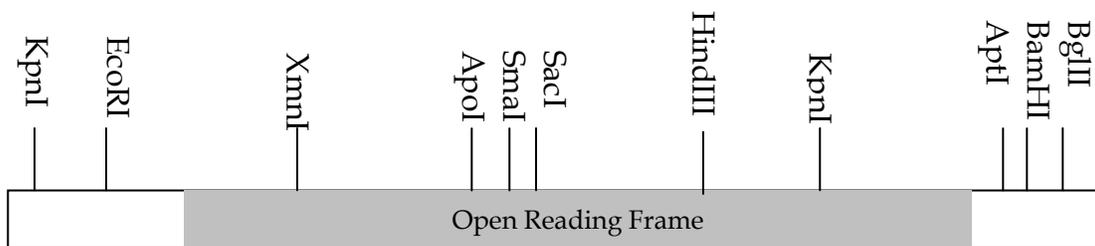


Figure by MIT OCW.

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 ampicillin.

Why?

Only the cells that have acquired the plasmid will be able to survive in the presence of ampicillin. 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.