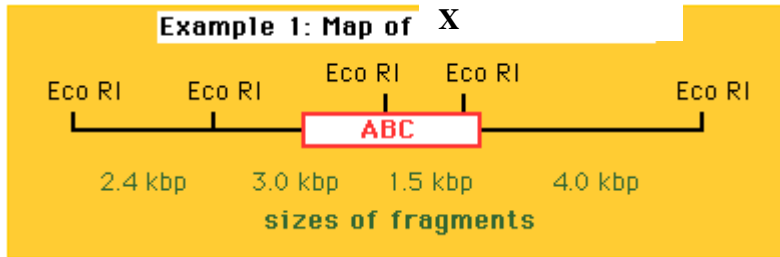


Bio 181 Internet-based exercise: Week 7

DUE: ~~Monday, April 23rd~~

Southern Blotting

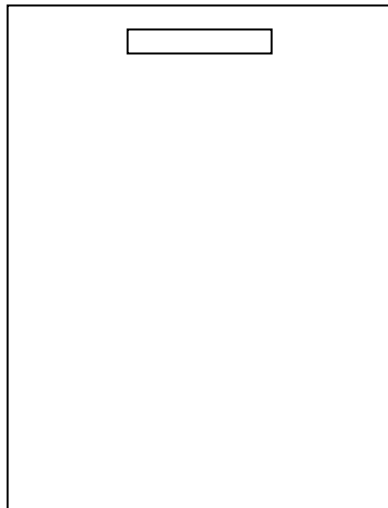
#1. Suppose the map below represents linear DNA “X”.



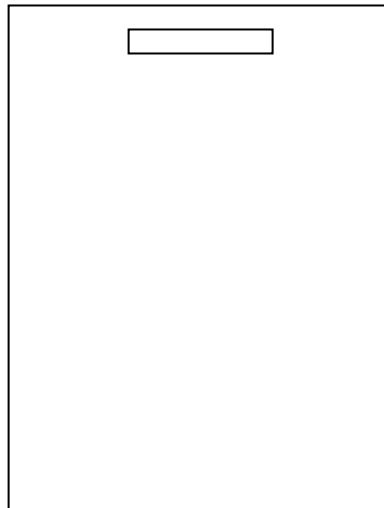
You perform a Southern blot, using the probe sequence marked ABC.

{X digested with EcoRI; run slowly on a gel for excellent resolution; transferred, probed, detected}

1. Draw appearance of gel
2. Draw appearance of Southern blot {Hint: Probe will bind to all DNA fragments containing complementary sequence}



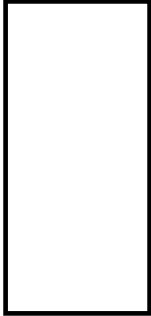
Agarose gel
(stained with EtBr)



Southern blot membrane
(after denaturation, transfer, etc.)

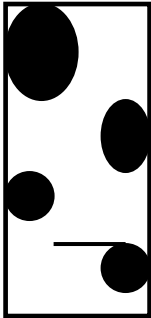
#2. You perform a Southern blot in which your probe should hybridize to a single DNA band.

Blot I: Name **THREE** possible problems that could cause this (blank blot, no bands).



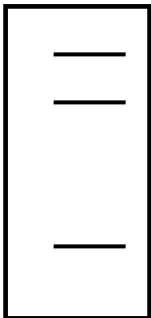
ANOTHER ATTEMPT:

Blot II: Name **ONE** problem that could cause this kind of experimental failure.



ANOTHER ATTEMPT:

Blot III: What could you do to correct this experimental failure?



#3. The following is really easy. First, visit this website and view the animation (simple description of Southern blotting for DNA fingerprinting):

<http://www.dnalc.org/ddnalc/resources/shockwave/southan.html>

Then, read below (bear in mind it is intended audience is high school students):

“This lesson gives students a more detailed idea of how DNA fingerprinting works and how it can be useful. There is a large amount of “junk DNA” — DNA that does not code for protein — in the human genome. Junk DNA is made up of repeated sequences that are called repeats. Although individuals may have identical genes, there may be different numbers of repeats between these genes. For example, one person may have 7 repeats while another has 12. The more repeats, the longer the junk DNA between genes. One method of DNA fingerprinting — which produces a Southern Blot — begins by taking a DNA sample from something such as skin, saliva, blood, or hair. The DNA is cut into pieces using restriction enzymes. The resulting collection of DNA pieces will consist of some pieces of junk DNA and some genes. The sample DNA pieces are placed into a clear gelatin, where an electric current pushes the DNA pieces through the gel. Short pieces move farther than long ones, so a piece of DNA that had 7 repeats would move faster than a piece of DNA with 12 repeats. Since DNA has no color more steps must be completed so scientists can “see” particular DNA pieces. The sequences are denatured so only a single strand remains. They are transferred onto a nylon sheet where the strands are permanently fixed. A radioactive probe with a known sequence is then added. After a radioactive probe of single stranded DNA has been allowed to bond by basepairing with the denatured DNA on the paper, X-ray film reveals only the areas where the radioactive probe sits. This allows researchers to identify, in a particular person’s DNA, the occurrence and frequency of the particular genetic pattern contained in the probe.”

Problem:

Within a span of three months, Haywood and Clark counties suffered eight bank robberies. The banks were mostly small and rural, with minimal security. The robber wore a ski mask, carried a large gun, and got in and out within minutes. However, the robber got more bold in his last robbery. He chose a larger bank with new surveillance cameras and a security guard. The robber still escaped, but not before he was chased by the guard. When the guard caught the robber, the robber shot and killed him, but not before the guard managed to pull off the robber’s ski mask, revealing his face to the surveillance cameras. Meanwhile, a woman leaving a hair salon across the street witnessed it all.

Police Chief Harry Gilford felt pressure from the state to make a quick arrest. Using the pictures from the tape, he brought in two possible suspects, both with prior arrests. The woman identified Suspect 1 who could not account for his whereabouts at the time of the robbery. Within weeks, Gerald Walker was convicted and sentenced to life in prison, despite his plea of innocence. Chief Gilford was pleased to close the case, but he couldn’t help but wonder if they really got the right man. Four years later, a new governor was elected. With advancements in genetic technologies, he soon signed a law that allows prisoners on death row or life sentences to demand a retrial that would use DNA testing on the evidence. Gerald Walker hired a lawyer and pressed for a new case. Using hair samples from the ski mask recovered by the security guard, the crime lab did a Southern Blot test. During the investigation, hair samples were also taken from both suspects. Use the suspects’ hair samples to determine if the right man was convicted.

Below, you will see the Southern blot data. Note the following:

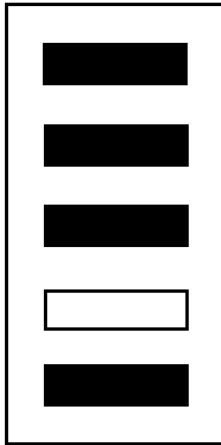
- Gels are “upside down” (direction of migration is upward)
- Southern blot had 5 DNA fragments on it; 4 hybridized to the probe (AGGT)
- Probe sequence will hybridize to *complementary* sequence in the suspects’ DNA
- Gerald Walker & Suspect 2 both produce 5 DNA fragments (see sequences below; / indicates restriction enzyme cut site). Predict what their Southern blots would look like.

Your assignment:

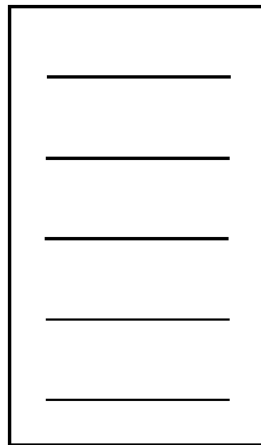
1. Tell me who the Southern blot matches: Gerald Walker, or Suspect 2?
2. How is the innocent party’s Southern blot different?

HS Lesson 8 Handout: The Case of Gerald Walker

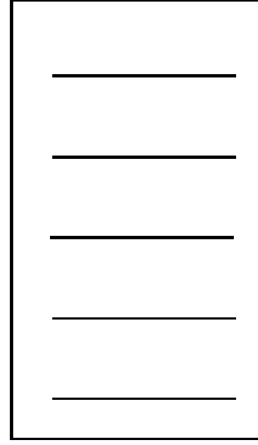
Southern Blot from blood
found at Crime Scene



Gerald Walker



Suspect 2



*DIRECTION
OF
ELECTRICITY*

Gerald Walker

TCCATCCA / TCCATCCATCCA / TCCA / TCCATCCATCCATCCA / TGGATGGATGGATGGATGGA

Suspect 2

TCCATCCA / TCCATCCATCCA / TCCA / TCCATCCATCCATCCATCCA / TGGATGGATGGATGGA

Probe

AGGT