Name _____ Date _____ Pd _____

Outbreak!! Fingerprinting Viral DNA

It is the year 2015. You are a molecular biologist working for the Centers for Disease Control and Prevention (CDC). Your job is to help track epidemics and to monitor emerging diseases.

Five years ago, a cluster of cases of hemorrhagic fever occurred in an isolated town in northeastern Alabama. The disease killed approximately 30% of those that caught it. The most alarming aspect of the new disease was that it was highly contagious from human to human, thus posing the threat of an epidemic. Medical authorities, including your office, believe the only reason the outbreak did not erupt into a major epidemic was that the Alabama town was so small and isolated. A CDC team traced the disease to a virus carried by the numerous local squirrels. An extensive trapping campaign was carried out in an attempt to eliminate the virus by eliminating the squirrels infected with it. No further cases have been reported in Alabama.

Three years ago suspicious outbreak occurred in Pennsylvania. Several people fell ill with a hemorrhagic fever. The symptoms of the disease were the same as those of the Alabama fever, but no one died. The Pennsylvania fever was apparently less contagious than the Alabama fever since the Pennsylvania patients were exposed to the many people who did not come down with the disease. However, the Pennsylvania virus was also traced to the local squirrel population.

You were asked to compare the viruses that caused the two outbreaks. You found that the virus particles looked similar. Both viruses had a DNA genome, but the base sequences of the genomes were different in many places.

Now, three people in Missouri have fallen ill with a hemorrhagic fever. Their symptoms are similar to the symptoms of the Alabama and Pennsylvania fevers. Local medical personnel were alarmed and called the CDC to determine if the dangerous Alabama virus had reappeared. The patients have been placed in quarantine. You are flown to the scene to research the situation.

You must immediately determine whether the Missouri patients are infected with the highly contagious and deadly Alabama virus, the Pennsylvania virus, or some other agent.

One of the techniques you decide to use is examination of viral DNA by restriction analysis. Wearing protective clothing, you enter the patients' isolation rooms in the Missouri hospital, carefully draw samples, place them on ice and rush back to the biological containment laboratory at the CDC for a variety of tests. For the restriction analysis, you isolate virus particles, extract DNA, and subject the samples to restriction digestion and agarose gel electrophoresis. In your gel you should include samples of DNA from the Pennsylvania and Alabama viruses for comparison.

Procedure:

____1. Put on gloves and safety glasses. For this lab, the gel is already made and in the box. If it were not, you would be given specific instructions on making the gel.

____2. Obtain approximately 250 ml of TBE buffer in the 400 ml beaker and pour into the box **(Not over the gel itself).** The box should be filled completely and the top of the gel should be covered with about 2 mm of the solution. After filling the box, pour any remaining buffer back into the carboy.

____3. *****Those groups that are not working in a gel box you will wait to pour your buffer solution until after you have loaded the wells***

____4. Each group should obtain vials containing samples of the Pennsylvania, Alabama, and Missouri strains.

____5. Vials should be microcentrifuged so that all the contents are found at the bottom of the vial.

____6. Use a micropipette set to _____ uL to draw the sample out of the vial.

____7. Carefully load the virus DNA into a well in the gel and label it accordingly on the following diagram.

____8. **Be sure not to release the button until you have removed the pipette from the well!!!!**

____9. When your group has completed loading your wells, prepare your gel box with the other group sharing it with you and close the lid.

____10. CAREFULLY carry the gel boxes to the power source and plug them in (RED TO RED & BLACK TO BLACK)

____11. Set the voltage to 70 and turn the machine on after all groups are plugged in.

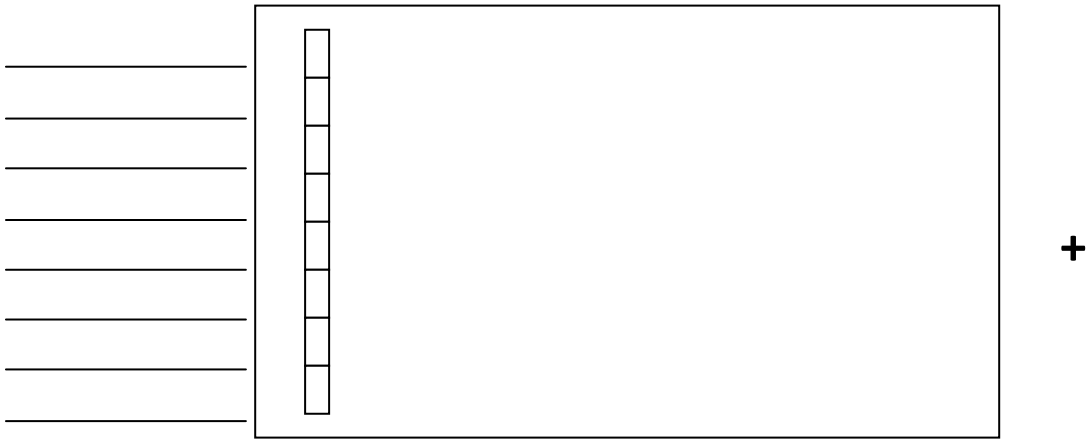
____12. The DNA will separate into two bands of color. The faster moving purple band represents a DNA sample that is roughly 300 base pairs long. The slower moving aqua band represents a DNA fragment that is 2000 base pairs.

____13. Allow the DNA to electrophorese until the purplish band is about 2 cm away from the end of the gel.

____14. After the electrophoresis is complete turn off the power source and disconnect all cords.

____15. Carefully removing gel from the casting tray and slide gel into staining tray. Take your tray to your instructor for staining.

Diagram



After the staining procedure is complete use colored pencils to show where each viral DNA strand has ended up in the gel.

Questions

1. Compare the fingerprints of the Pennsylvania, Alabama, and Missouri virus strains. What can you conclude about the virus infecting the Missouri patients?

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2. Why would the fragment patterns from two different viruses be look different after running an electrophoresis?

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