**Activity 1.4.2: Vaccine Development Day 3**

Bacterial plasmids can be used to generate protein products that are part of a disease antigen. These products can be used to trigger an immune response. In this activity, you will engineer a plasmid to produce a protein used as a vaccine against the virus, Hepatitis B. You will transfer a gene for a viral envelope protein for Hepatitis B into a bacterial plasmid. This protein resides on the surface of the virus and assists with identity and infection. Once copies of this protein are made in bacterial cells, the protein can be purified for use as a vaccine.

1. Recall that each restriction enzyme recognizes a specific DNA sequence called a *restriction sequence* or *restriction site*. When the enzyme encounters this sequence, it makes a distinct cut in the DNA. Refer to the chart below for the restriction sites of five common enzymes.

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| **Restriction Enzyme** | | **Restriction Site** | **Cut** |
| *Bam*HI | 5’ GGATCC  3’ CCTAGG | | 5’ ---G GATCC--- 3’  3’ ---CCTAG G--- 5’ |
| *Eco*RI | 5’ GAATTC  3’ CTTAAG | | 5’ ---G AATTC--- 3’  3’ ---CTTAA G--- 5’ |
| *Hae*III | 5’ GGCC  3’ CCGG | | 5’ ---GG CC--- 3’  3’ ---CC GG--- 5’ |
| *Hind*III | 5’ AAGCTT  3’ TTCGAA | | 5’ ---A AGCTT--- 3’  3’ ---TTCGA A--- 5’ |
| *Pst*I | 5’ CTGCAG  3’ GACGTC | | 5’ ---CTGCA G--- 3’  3’ ---G ACGTC--- 5’ |

1. Answer Conclusion question 6.

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| 1. Explain why HaeIII is unlikely to be used to create recombinant DNA. How does this enzyme differ from the others described in the table? |  |

1. Obtain a Student Resource Sheet, a paper plasmid, scissors, tape and colored pencils from your teacher.
2. Refer to the table of restriction enzymes and their restriction sequences shown above or on the Student Resource Sheet. Scan the plasmid DNA and locate the restriction sites for each enzyme. These sites have been shaded in or colored for you. Identify the enzyme that would target each designated site and create a color key in your laboratory journal.
3. Scan the viral DNA and locate restriction sites for each enzyme. Using the same color key you designated in Step 20, shade each restriction site with the appropriate color using colored pencils.
4. Scan the plasmid DNA as well as the gene from the Hepatitis B virus to find an appropriate enzyme to engineer the plasmid. Your goal is to open the plasmid ring and insert a piece of the viral gene. Follow the guidelines listed below to determine the most efficient enzyme to complete your task.

* Use an appropriate restriction enzyme to open the plasmid ring as well as cut out a piece of the viral gene. You do not want to use an enzyme that digests the plasmid into many tiny pieces.
* Do not cleave more than twenty base pairs off either side of the viral DNA. The remaining base pairs are required for production of the protein of interest. Important promoters at the beginning of the sequence are vital to transcribing the genetic code.
* Choose an enzyme that does not interrupt the antibiotic resistance gene in the plasmid or the origin of replication (ori), the sequence that initiates replication of the plasmid. The antibiotic resistance gene provides scientists with a way of determining if an engineered plasmid successfully made it into a target cell. If bacterial cells that were not antibiotic resistant now grow on medium with antibiotics present, they must contain the new plasmid.
* Make sure you create sticky ends on both the plasmid and the viral DNA.

1. When you think you have located an appropriate enzyme, present your plan to the teacher. Be prepared to defend your recommendation with evidence on your DNA.
2. Use scissors and tape to create your recombinant plasmid. Be sure to make your cuts according to the pattern shown on the restriction enzyme table.
3. Examine your completed plasmid. Compare your final product to that of a classmate’s. Discuss any differences.
4. Note that an engineered plasmid must be put back into a bacterial cell to produce the desired protein. Refer to your discussion of bacterial gene transfer in Lesson 1.2. In your laboratory journal, describe which gene transfer method can most likely be used to insert the new plasmid into a bacterial cell.

Conclusion

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| 7. What molecular tools do scissors and tape represent in this activity? Explain. |  |
| 8. . Edible vaccines, a more controversial approach to vaccine development, have been investigated by scientists. Plants can be genetically modified to produce viral proteins and when ingested, these proteins ignite the immune system of the consumer. What are the advantages to edible vaccines? What are some of the possible disadvantages? |  |
| 1. How could you compare the original plasmid and the engineered plasmid in the lab using restriction digestion and gel electrophoresis. Look at your paper plasmid and think about the number of restriction sites for the enzyme you used in the activity before and after you altered the plasmid. Describe your experiment below. |  |
| 1. Jimmy has always been told that if you have chicken pox once, you will not get the disease again. He had chicken pox when he was eight, but he only had five to ten small pox on his skin. As an adult, Jimmy is again showing symptoms of chicken pox. Using your knowledge of the human immune system, explain how this is possible. |  |
| 1. A new flu vaccine is formulated and released every year. Explain why one shot against influenza does not necessarily protect a person from year to year. Think about the genetic material of the virus. |  |