Name:_____

BACKGROUND

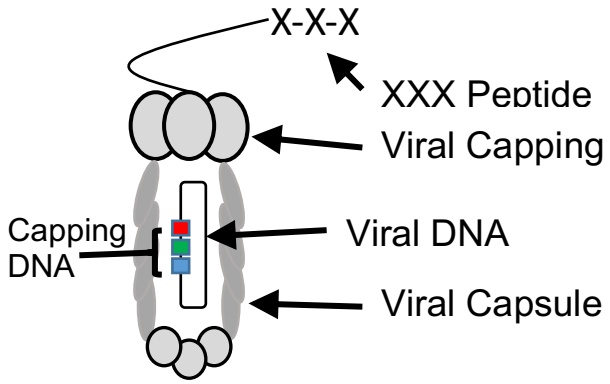
Creating a pharmaceutical drug is a difficult and expensive process. DNA biotechnology is an emerging field of genetics that vastly increases the potential of modern medicines and has provided new avenues for drug development that never existed before. Often, one of the major challenges of drug creation is delivery – targeting a new drug to attack only a specific cell type is a very difficult endeavour. Identifying particular cell receptors and then isolating chemicals that have strong affinity for those receptors is a critical step in pharmaceutical chemistry.

A biological delivery vector (like a bacteriophage virus) can allow scientists to deliver smaller quantities of a medicine that are more effective and efficient due to their proximity to their target site. Doing this, however, requires alterations to the core DNA of that bacteriophage.

Using restriction enzymes, we can add DNA sequences to existing bacteriophage DNA in order to alter the proteins that bacteriophage produces. This process involves cutting phage DNA with restriction enzymes, inserting synthetic DNA fragments, and then using DNA ligase to defragment the resulting product. If successfully done, the bacteriophage we produce has the potential to continue propagating, while at the same time producing our medicine for us.

CASE STUDY

The Derda Lab at the University of Alberta works with a virus called the **M13 Bacteriophage**. But the lab's interest isn't in the M13 phage itself – it's in using the M13 phage as a tool to mass produce proteins.



The M13 phage looks like many other viruses- it has a capsule surrounding it, a length of circular DNA inside, and a capping protein on the outside. As identified on this diagram, the Derda lab has also identified the region of the viral DNA that maps to the viral capping protein.

Using restriction enzymes, the Derda lab is able to splice in DNA that codes for specific peptide additions ('XXX' in the diagram) that they would like to be displayed on the outside of their phage. The Derda group can repeat this process to produce up to 500 million peptide possibilities they can then test at the same time. After testing peptides for their effectiveness at bonding to a target receptor, the Derda lab can isolate successful phages and sequence the DNA that remains safely inside the M13 capsule. This allows them to identify which peptide additions were the most useful.

YOUR ACTIVITY

Today you will be exploring how to alter a bacteriophage plasmid like the one from the M13 phage. First, you will be constructing your own bacteriophage plasmid. Next, you will be identifying regions of DNA on that plasmid that are essential for transcription and translation along with possible restriction sites for DNA alteration. Finally, you will be creating your own peptide addition and deciding where and how that addition will be added onto the bacteriophage plasmid. At the end of this activity, you should have a working understanding of how restriction enzymes can be used to alter a DNA sequence. You will be encouraged to explore how this might be applicable in a medical or industrial setting.

MATERIALS

- **Scissors**
- **Highlighter (Multiple colors if possible)**
- **Plasmid (Appendix 1) + Synthetic DNA (Appendix 2) Add-ons**

PROCEDURE

Part 1 - Produce your Plasmid

1. Cut out each of the 4 sections of the bacterial plasmid along the dotted lines
2. Use clear tape to join the common letters together on the ends of the plasmid sections (A → A, etc.). When you are finished, all of the BOTTOM labels should be on the same side of the plasmid.
3. Using a highlighter, locate the critical regions of the Bacteriophages' DNA plasmid. You're looking for the sequences as follows:
    a. TATA Box Promoter Region → **'TATAAA'**
    b. EcoR1 Restriction Site → **'CTTAAG'**
    c. HindIII Restriction Site → **'AAGCTT'**
    d. Kpn1 Restriction Site → **'GGTACC'**

**Note that since the restriction enzymes are almost always palindromic, these restriction sites will always occur on both the coding and noncoding strands*

Part 2 - Transcription and Translation

Though Bacteriophages normally contain 4-100 genes, there should only be 2 on your plasmid. These are indicated by a TATAAA box followed shortly by a "start codon".

4. The start codon has the mRNA "**AUG"** meaning the **coding strand** of DNA that produces that mRNA will appear as... _____ . Locate the first iteration of this sequence that occurs **after each promoter region** and **highlight it**. This is where to begin your transcription for that gene.

5. Using the transcription and translation template provided, determine the length and amino acid sequence of each peptide this bacteriophage will produce.

Peptide # 1		**Length: ____ Amino Acids**
mRNA sequence (from start codon to stop codon):		
_ _		
Amino Acid sequence:		
______ ______ ______ ______ ______ _______ ______ ______ ______ ______ ______ ______ ______		

Peptide # 2		**Length: ____ Amino Acids**
mRNA sequence (from start codon to stop codon):		
_ _		
Amino Acid sequence:		
______ ______ ______ ______ ______ _______ ______ ______ ______ ______ ______ ______ ______		

6. **Label** each of the plasmid DNA sequences that code for these peptides (labels should be peptide #1 and peptide #2. This will be important later.

Part 3 - Choosing a Restriction Site

7. **Evaluate** the potential restriction sites you located in step 3. You will be using restriction enzymes and synthetic DNA to insert additional DNA segments at one of these locations. To guide your decision of which restriction site to use, answer the following questions:

A. Which transcription sites might be most appropriate if you wanted to insert a gene into this bacteriophage without disrupting or altering any normal viral processes? Explain.

Student answers may vary

B. Why might it be disastrous to use a restriction enzyme with multiple restriction sites? Give an example of a restriction enzyme that may have this issue in your explanation.

C. Are the bonds formed by restriction enzyme "sticky ends" considered permanent? How is this normally addressed with restriction fragments?

For this assignment, it is essential that the peptide you will be designing is added to the bacteriophage capping protein. This will allow your protein to be produced and then displayed on the exterior of the bacteriophage. The phage capping protein is 10 amino acids in length. You should notice that there are 2 restriction sites in the direct proximity of the capping protein DNA sequence. You may assume for the purpose of this activity that producing a few extra amino acids in addition to capping protein is forgivable- but altering the capping protein itself in any way will ruin its function and cause your phage to fail.

D. Which transcription site would most likely allow your protein to be produced along with the bacteriophage capping protein, but without affecting the capping protein structure? Explain.

E. Complete the following statement:

We will be using synthetic DNA strand number _____ to complete our restriction site addition, because it matches the sticky ends created by the restriction enzyme _____.

Creating Synthetic DNA

8. You will now be creating the DNA sequence that codes for the peptide you want to add to the bacteriophage capping protein. Decide on a peptide that is 8 amino acids long (not including the start codon that must be at the beginning of the sequence). Be creative!

Our Amino Acid sequence:

-START- _____ _____ _____ _____ _____ _____ _____ _____

9. Using your knowledge of transcription and translation, work backwards to **determine the coding strand and non-coding strand** sequences (including the start codon) for your chosen peptide. You are encouraged to work on a scrap piece of paper first – it is easy to make transcription/translation mistakes.
10. When you are certain of your coding/non-coding strands, **transfer** your DNA sequence to the synthetic DNA strand of your choosing.
11. **Cut out** your completed synthetic DNA strand.

Using a Restriction Site

12. Using scissors, **cut** your bacteriophage plasmid at its restriction site to open up the sticky ends. Sticky ends should be 4 base pairs long as shown in this example using the BamHI restriction enzyme:

13. **Insert** your completed synthetic DNA strand into the restriction site. Be sure that the coding strand for the peptide you have created matches up with the coding strand side of the existing plasmid. In addition, the sticky ends up your synthetic fragment should form complementary base pairs with the sticky ends of your phage plasmid restriction site.
14. Using clear tape, **join** your fragmented bacteriophage plasmid together.
15. **Transcript** and **translate** your bacteriophage gene again to ensure your peptide will be produced as intended. When you are confident in your completed plasmid, complete the analysis questions below on a separate sheet of paper and then hand in your phage product!

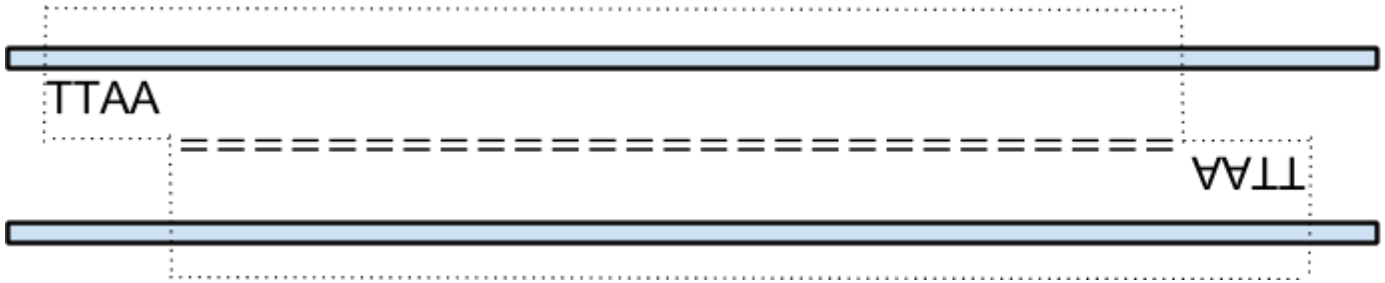
Analysis Questions:

1. What were the scissors and tape metaphorically representing in this activity?
2. Even without the requirement of being attached to the bacteriophage capping protein, why is the restriction site you have chosen still valuable? Which important DNA sequence are you hijacking to encourage the phage to produce your peptide?
3. Restriction enzymes are used for a number of purposes in research. One of the first industrial uses of this technology involved isolating the human insulin gene and splicing it into bacterial genomes for mass production. Describes the conditions you would look for on the bacterial plasmid if you were tasked with choosing a potential restriction site for this gene.

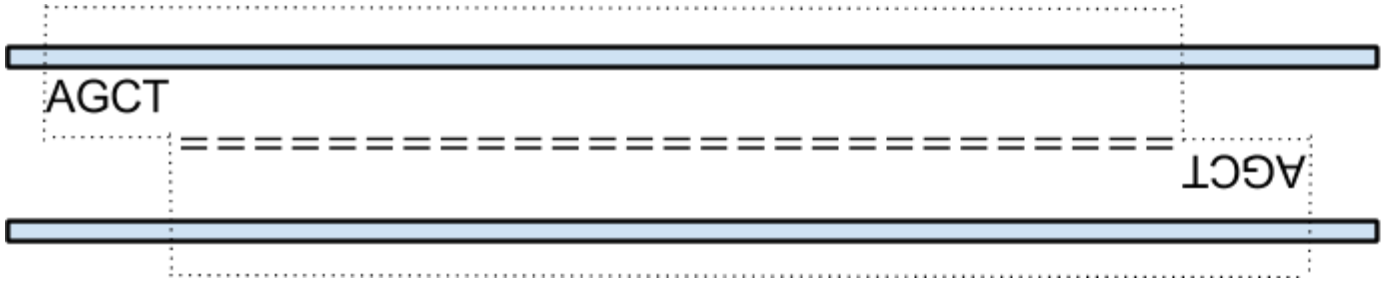
4. Using the tools you have been presented with today, theorize how you might be able to increase production of a product like insulin. Would it be possible to force a vector like a bacterium or a phage to produce multiple copies of a peptide at the same time?
5. In reality, there are far more than 3 restriction enzymes to choose from. Most restriction enzymes produce 'sticky ends', but some cut straight through the plasmid at a particular DNA sequence. Hypothesize some of the advantages and disadvantages these types of restriction enzymes may have.


A
ACTCTTAAGGACCGGTTGAGTGACCATCCAGTTGTATAAACCTTAAGGTCATAC
GTATGACCTTAAGGTTTATACAACTGGATGGTCACTCAACCGGTCCTTAAGAGT
B
BOTTOM
B
AGGGTATTTGGTACCCGGCTAGAAGAGCCGATCGATTGACACCTAGGACGATAT
ATATCGTCCTAGGTGTCAATCGATCGGCTCTTCTAGCCGGGTACCAAATACCCT
C
BOTTOM
C
AGCTGCGTAAGCTTCGATTGTATAAATCTCATACTTCTTCAGAAATGCATAG
CTATGCATTTCTGAAGAAGTATGAGATTTATACAATCGAAGCTTACGCAGCT
D
BOTTOM
D
GATACTCACGGTACCTCAGGTACGATGACCAGTAGCGTGGTAAGCTTCCCATAG
CTATGGGAAGCTTACCACGTACTGGTCATCGTACCTGAGGTACCGTGAGTATC
A
BOTTOM

Synthetic DNA Fragment #1



Synthetic DNA Fragment #2



Synthetic DNA Fragment #3