Grade: 12	**Subject:** Biology 30	**Unit:** Genetics

Rationale: Students will draw from an understanding of transcription, translation, and restriction enzymes to actively alter a crafted bacteriophage plasmid and to produce a peptide addition of their own design.

Background Information:

The Derda Research Group at the University of Alberta is interested in creating a library for ligand binders. To do this, Dr. Derda and his team use bacteriophages as a vector to mass produce genetically different peptides. Using restriction enzymes, the lab splices multiple mutated DNA variants onto the circular DNA of a bacteriophage. The targeted restriction site adds the resulting peptide onto the end of the bacteriophage capping protein. By using a third party vector (the bacteriophage) to produce the protein, researchers ensure that the DNA responsible for the resulting peptide is protected within the capsule of the bacteriophage.

The traditional approach to finding a ligand would involve cooking up individual chemicals, isolating and purifying the targeted isomer, and then testing that isomer's effectiveness at binding a specific receptor. The Derda Group has turned to an interesting and unique chemical "fishing" approach. Instead of testing chemicals one at a time, they have employed a broad spectrum approach.

Instead of purifying one individual test chemical, the lab instead uses a pure receptor. The target receptor is "dipped" into the soup of bacteriophages and the bacteriophage-peptide combinations that hold on when the receptor is removed will be those with the highest bonding strength. Though this doesn't eliminate the need for more specific testing, it does limit the number of candidate molecules for further study.

Students will be forced to transcribe and translate DNA segments multiple times. They will have to identify a suitable restriction site from one of a number of genuine restriction enzymes. And they will have to use transcription and translation backwards to produce their own peptide addition that they will then integrate into the phage plasmid. Overall, students will replicate a complex lab procedure that is relevant and done locally at the University of Alberta.

Prerequisite Learning:

- Transcription and Translation
- DNA Structures and Variations (linear, plasmid, single and double stranded)

Curriculum Connections:

- **30-C3.3k** describe, in general, how genetic information is transcribed into

sequences of bases in RNA molecules and is finally translated into sequences of amino acids in proteins
- **30-C3.4k** explain, in general, how restriction enzymes cut DNA molecules into smaller fragments and how ligases reassemble them
- **30-C3.5k** explain, in general, how cells may be transformed by inserting new DNA sequences into their genomes
- **30-C3.2sts** explain that scientific research and technological development help achieve a sustainable society, economy and environment
- **30-C3.2s** conduct investigations into relationships between and among observable variables and use a broad range of tools and techniques to gather and record data and information
    - perform simulations to demonstrate the use of restriction enzymes and ligases

Lesson Objectives/Concepts:
- Students will explore and practice the processes of transcription and translation
- Students will identify critical regions of DNA including promoter regions, start codons, stop codons, and restriction enzyme sites
- Students will create a physical manifestation of an bacteriophage plasmid and perform genetic alterations on that plasmid through restriction enzyme and ligase action

Materials:
- Student Handouts and Appendices
- Scissors (1 pair per group)
- Scotch Tape or other clear tape (Multiple Rolls)
- Highlighters (Multiple colors if possible)

Time: 1 Class Period

Introductions:
1. Read the student handout introduction and background as a class. This should help students to identify the problem and some of the potential applications of this type of technology.
2. Follow the procedure outlined in the student handout. A basic outline of the procedure is outlined below

Activities/Procedure:
1. **Create the bacteriophage plasmid (from Appendix 1)**
2. **Identify critical regions of the bacteriophage**
    a. **Initiator region**
    b. **Restriction sites of 3 potential restriction enzyme candidates**
3. **Transcribe and translate the 2 genes students should find on the phage plasmid. These sites are indicated by a TATA box promoter region closely followed by a 'TAC' sequence on the coding strand that indicates a start codon**

4. **Identify a good restriction site to use**
5. **Determine a peptide addition (8 aa long + start codon)**
   ***This can be anything students want it to be**
6. **Reverse the transcription/translation process to determine the coding sequence of the DNA required to produce that peptide addition. This sequence is added to an appropriate piece of synthetic DNA (Appendix 2)**
7. **Splice in your reverse-engineered DNA segment using scissors and tape**
8. **Answer the given extension questions**

Summary:
Overall students will be producing a bacteriophage plasmid. They will be using the template provided to create a basic plasmid. Most of the lab will be done identifying and creating a piece of synthetic DNA that will then be spliced into an appropriate DNA restriction site, given the restriction enzymes available.

Assessment:
Students will be marked according to the rubric provided. They will largely be assessed on their identification skills, successful transcription and translation, and assembly of their final plasmid.

Extensions/Connections:
- After the process of peptide chain production is complete, the Derda lab chemically adds a small carbohydrate onto the end of their peptides. Could this be done by adding additional DNA onto the bacteriophage? How? What might you have to consider?

Video Links:
Derda Research Group:
- Research Group - Information/current research - http://derda.chem.ualberta.ca/
- Derda Lab - Current Research - https://www.youtube.com/watch?v=YvdGGvaUmIc&list=PLb6QL_MiNdwKkIkw_0QayINWp_prjwxAE

Bacteriophages:
- Khan Academy - Types of Viruses - https://www.youtube.com/watch?v=4kIKySxUYuk

Restriction Enzymes/Biotechnology:
- Khan Academy - Restriction enzyme overview - https://www.youtube.com/watch?v=U2cKywEn6KY

ng a Bacteriophage - Transcription, Translation, and Restriction Enzyme Activity

ent Name _____ **Date** _____

Altering phage plasmid DNA through use of restriction enzymes **Total: /25**

Level / a	Proficient (5)	Adequate (3-4)	Limited (1-2)	Insufficient/ Blank*
iophage Plasmid DNA sites identified /4)	All DNA sites are correctly identified and highlighted (2 TATAAA, 2 start codons, 5 restriction sites)	Most DNA sites are correctly identified (1 or 2 errors)	Some of the critical DNA sites are identified (3 or more errors)	No score is awarded becaus there is insuffici evidence of stud performance ba on the requirements of assessment task
d gene Transcription anslation is complete curate (step 5)	Transcription and Translation are done neatly and correctly on the chart.	Transcription and Translation are largely correct (1-2 amino acid errors)	Transcription and Translation are done poorly or on the wrong DNA segment	
tion Enzyme Questions mplete (1 sheet per and site correctly n (step 7)	Restriction enzyme is chosen accurately (EcoR1) and questions are answered correctly and accurately	Restriction enzyme is chosen correctly (EcoR1) and some questions are answered correctly.	Restriction enzyme is chosen incorrectly OR questions are mostly answered inaccurately	
hage plasmid is ete and correct, along ynthetic DNA strand	Final phage has Synthetic DNA strand inserted in line with the plasmid promoter and sticky ends matched correctly	Final phage has synthetic DNA inserted, but synthetic strand is inverted, does not contain a start codon, or is otherwise incorrect	Final phage doesn't have a synthetic DNA strand, or has a synthetic strand inserted into the wrong restriction site	
is Questions are ete and correct	Analysis questions are complete, correct, and	Analysis questions are complete, but may be	Analysis questions are partially completed or	

	use terminology properly	partially incorrect or use poor terminology	poorly completed	

hen work is judged to be limited or insufficient, the teacher makes decisions about appropriate intervention to help the stude
improve.

ACTCTTAAGGACCGGTTGAGTGACCATCCAGTTG**TATAAA**C**CTTAAG**GTCATAC

TATGAC**CTTAAG**GTTTATACAACTGGATGGTCACTCAACCGGTCCTTAAGAGT

AGGGTATTT**GGTACC**CGGCTAGAAGAGCCGATCGATTGACACCTAGGACGATAT

TATCGTCCTAGGTGTCAATCGATCGGCTCTTCTAGCCG**GGTACC**AAATACCCT

AGCTGCGT**AAGCTT**CGATTG**TATAAA**TCTCA**TAC**TTCTTCAGAAATGCATAG

CTATGCATTTCTGAAGAAGTATGAGATTTATACAATCG**AAGCTT**ACGCAGCT

GATACTCAC**GGTACC**TCAGGTACGATGACCAGTAGCGTGGT**AAGCTT**CCCATAG

TATGGG**AAGCTT**ACCACGTACTGGTCATCGTACCTGA**GGTACC**GTGAGTATC

ent Procedure Questions (ANSWER KEY)

4: Start Codon 'UAG' mRNA becomes **'TAC'** on the coding strand

5: Transcription and Translation (either order for Peptide 1/2):

otide # 1		**Length: _10_ Amino Acids - This is the Capping Protein Sequence**

UCCCAUAAACCAUGGGCCGAUCUUCUCGGCUAG

<u>NA sequence</u> (from start codon to stop codon):
<u>ino Acid sequence</u>:

TART_ _SER_ _HIS_ _LYS_ _PRO_ _TRP_ _ALA_ _ASP_ _LEU_ _LEU_ _GLY_ _STOP_

otide # 2		**Length: _7_ Amino Acids - Not the Capping Protein**

<u>NA sequence</u> (from start codon to stop codon):
AAGAAGUCUUUACGUAUCCUA-UGA

ino Acid sequence:

ART_ _LYS_ _LYS_ _SER_ _LEU_ _ARG_ _ILE_ _LEU_ _STOP_

7: Choosing a Restriction Site

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

*est answer is **HindIII**. The EcoR1 and Kpn1 restriction sites both affect the gene for the bacteriophage capping protein. If you w*
oid the bacteriophage genome entirely, HindIII offers a few restriction sites that are outside of the active phage DNA regions.

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

riction enzyme with multiple restriction sites is more difficult to control effectively. The restriction enzyme will cut the plasmid
*ple regions and may actually splice out entire regions of DNA. Both **HindIII** and **Kpn1** have this issue on our phage.*

e the bonds formed by restriction enzyme "sticky ends" considered permanent? How is this normally addressed with restrictio
ents?

onds formed when sticky ends come together are only hydrogen bonds. To form proper covalent bonds between the base pairs
ction site, ligase enzyme is required. This enzyme will make sticky end connections permanent.

hich transcription site would most likely allow your protein to be produced along with the bacteriophage capping protein, but
ut affecting the capping proteins structure? Explain.

coR1 transcription site on the first bacteriophage plasmid segment (initially labelled "A") is the best option. This transcription s
ed after a promoter region, but before the actual TAC segment. This means that the protein we are producing will take advanta
xisting plasmid promoter and will be produced continuously with the phage capping protein (there is no stop codon or phase sh
een the addition we are adding and the existing DNA).

mplete the following statement:

vill be using synthetic DNA strand number _*1*_ to complete our restriction site addition, because it matches the sticky ends
ed by the restriction enzyme _*EcoR1*_.

8/9/10: Reversing Transcription and Translation

ent answers may vary. Answers are correct as long as the sequence students provide an 8 amino acid long peptide. When stud

*heir peptide back into mRNA and then into a coding strand of DNA, there will be multiple possibilities due to redundancies in ar coding. When students are finished creating their synthetic DNA sequence, they should have the blank spaces completely full o side of the synthetic strand (27bp per side). The coding strand should begin with the '**TAC**' sequence and all base pairs shoul hed appropriately with a complementary base pair on the non coding strand.*

ysis Questions:

What were the scissors and tape metaphorically representing in this activity?

*Scissors are representative of the chosen **restriction enzyme** (specifically EcoR1). Tape is representative of the **DNA ligase** enz*

Why is the location of a restriction site so crucial? With respect to this activity, which transcription mechanism are you capitalizing on by using the restriction site you have chosen?

*The location of a restriction site is critical because DNA is not used equally. Many DNA regions are rarely accessed. This partic restriction enzyme takes full advantage of the phage's existing **promoter region**. Promoters play a large part in identifying DN regions that should be accessed and transcribed. Using a promoter increases the likelihood of transcription occurring- especia since the bacteriophage capping protein is critical to the structure of the bacteriophage, one can assume this protein will be transcribed with a high frequency.*

Restriction enzymes are used for a number of purposes in research. One of the first industrial uses of this technology involve isolating the human insulin gene and splicing it into bacterial genomes for mass production. Describes the conditions you wo look for on the bacterial plasmid if you were tasked with choosing a potential restriction site for this gene.

To find a region of DNA that is useful for mass production of a protein, we should be looking for areas with one or more promoter regions. These promoter regions will ensure that the inserted DNA sequence will be "seen" and accessed by transcription mechanisms. A good place to look would be an area on the organism's genome where there are critical section DNA for that organism.

Another important factor would be to look for areas with appropriate restriction sites. If we have a sequenced copy of the organism's DNA, this would mean looking for restriction sites that take advantage of the promoter regions while not critically affecting the function of the organism itself.

*It may be useful to note an instance where a restriction site may hurt an organism. A site that cut a gene in half that codes critical membrane receptor, enzyme, or even DNA transcription protein could negatively affect the organism, potentially eve killing it. This would prevent the organism from reproducing. Because a single organism's contribution is negligible and these

industrial processes require thousands of organisms producing insulin (for example), refraining from negatively impacting th bacteria's life cycle is critical.

Using the tools you have been presented with today, theorize how you might be able to increase production of a product lik insulin. Would it be possible to force a vector like a bacterium or a phage to produce multiple copies of a peptide at the sam time?

There are a number of possibilities for this question. Namely, we are looking for students to devise a solution using correct terminology and correct usage of the DNA technologies they have practiced. An example of a potential solution would be inserting multiple copies of the insulin gene into the same restriction site on the biological vector. Another possibility could b inserting additional promoters into an area to encourage more frequent transcription.

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 an advantage and disadvantage these type restriction enzymes may have.

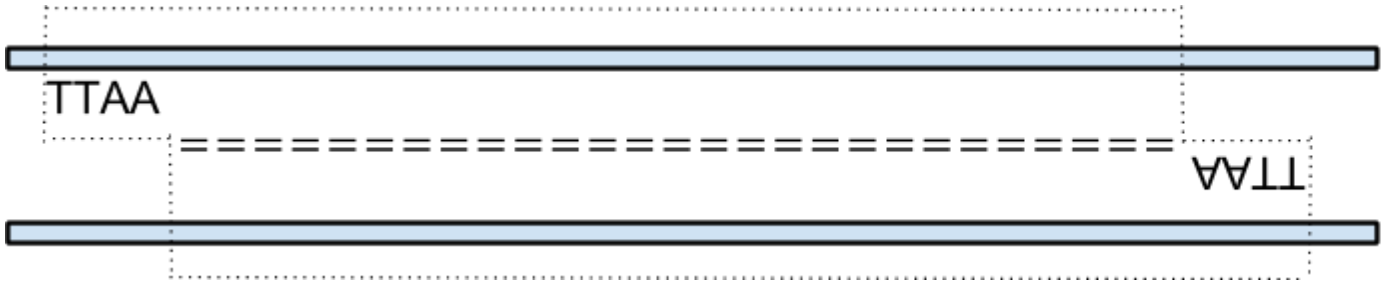
Again, student answers may vary here and do not necessarily need to be correct for full marks. We are looking for proper understanding and usage of restriction enzyme terminology.

Restriction enzymes that cut straight through a DNA strand produce a "blunt end". They are often disadvantageous because are harder to control. Because sticky ends provide a substrate for a new natural or synthetic DNA strand to stick to, it is easie target a sticky end with a DNA strand you wish to incorporate into your biological vector.

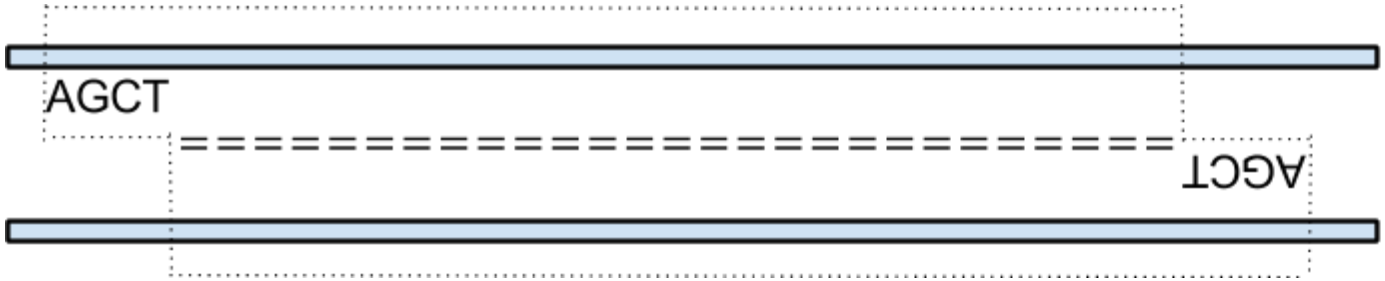
Though they are difficult to use, there are no limitations on which DNA strands can be spliced together because there are no complementary base pairs to match.


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