Grade 9-12 STEM Challenge

Paper Plasmids

Inspired by Dathan, a Biomedical Manufacturing Operator in the Indiana Uplands.

Published by Regional Opportunity Initiatives, Inc.

GRADE 9-12 STEM CHALLENGE

Paper Plasmids

Inspired by Dathan, a Biomedical Manufacturing Operator in the Indiana Uplands.

Students will use a paper model to understand how DNA is moved into new cells to produce needed proteins.

LESSON TIMELINE

DAY 1

- Show the inspiration video, "Dathan - Drug Substance Operator" (10 minutes)
- If necessary, introduce plasmids and transgenic organisms (15 minutes)
- Begin paper plasmid activity (20 minutes)

DAY 2

- Finish activity (20 minutes)
- Class discussion and debrief (10 minutes)
- Complete lab report

Recommended Supplies

For each group of two students:

- Paper Plasmids (Printed in color, if possible)
- Colored Pencilsor Pens
- Scissors
- Tape

CAREER CONNECTION AND LESSON OVERVIEW

Dathan is a biomedical manufacturing operator at Catalent in Bloomington, Indiana. He works in a lab where he grows cells that are modified to produce vaccines and medications in large cultures. Once the cells have grown and multiplied, Dathan isolates the transgenic proteins, performs testing, and collects data to make sure that the substance will be pure enough to be approved for further testing and use.

Biotechnology and molecular engineering allow humans to move DNA instructions from one organism into another cell (like bacteria or eukaryotic cell culture) to make large amounts of a required protein. One example of this is the manufacture of human insulin. Using restriction enzymes, the gene for insulin was isolated and “pasted” into a bacterial plasmid. When this plasmid was put back into cells, the bacteria were able to make insulin protein molecules that were identical to the insulin made by the human body. Once the cells have produced enough insulin, the lab can purify it and prepare it for use in patients.

In today’s activity, students will be doing paper cloning to understand how scientists move genes from one organism to another.

IN THIS CHALLENGE, STUDENTS WILL:

- Create a paper model of how DNA is moved from one organism to another.
- Understand how transgenic organisms are used to make important molecules for medicine.
- Explore the differences between molecular cloning and organismal cloning.

Standards

Science & Engineering Process Standards

SEPS.2 Developing and using models and tools

Preparing for College and Careers

PCC-2.1 Determine roles, functions, education, and training requirements of various career options within one or more career clusters and pathways

PCC-2.2 Analyze career trends, options and opportunities for employment and entrepreneurial endeavors for selected career clusters and pathways

PCC-2.3 Evaluate selected careers and pathways for education requirements, working conditions, benefits, and opportunities for growth and change

PCC-2.4 Use appropriate technology and resources to research and organize information about careers

Biology Standards – 9th Grade

B.1.1 Compare and contrast the shape and function of the essential biological macromolecules (i.e. carbohydrates, lipids, proteins, and nucleic acids), as well as, how chemical elements (i.e. carbon, hydrogen, oxygen, nitrogen, phosphorus, and sulfur) can combine to form these biomolecules

B.4.3 Construct a model to explain that the unique shape and function of each protein is determined by the sequence of its amino acids, and thus is determined by the sequence of the DNA that codes for this protein

Employability Skills, Grade 9-10

9-10.M.2 Able to view feedback as data that helps the learning process

9-10.WE.2 Complete tasks or activities with minimal prompting and guidance

9-10.LS.7 Predict outcomes to problems based on data and evidence.

9-10.LS.11 Able to combine concepts in different ways to create new ideas and innovative solutions

Planning and Implementation

PAPER PLASMIDS

Essential Vocabulary

- DNA: Deoxyribonucleic acid, a self-replicating molecule present in nearly all living organisms and makes up chromosomes. It is the carrier of genetic information.
- GENE: A distinct sequence of DNA, the order of which determines which proteins are made in the cell.
- DNA CLONING: A molecular biology technique that makes many identical copies of a gene or its product.
- PLASMID: A circle of DNA in a bacterial cell, separate from its chromosome.
- ENZYME: A protein that facilitates a specific chemical reaction, usually a catalyst.
- RESTRICTION ENZYMES: Proteins that recognize and cut specific DNA sequences.
- STICKY ENDS: The uneven overhang left over after a restriction enzyme has cut DNA.
- TRANSFORMATION: The transfer of new DNA into a cell.
- RECOMBINANT DNA: DNA that has been formed artificially by combining constituents from different organisms.
- TRANSGENIC ORGANISM: An organism that contains foreign DNA that has been introduced using biotechnology.
- GENOME: An organism's entire DNA complement, including all chromosomes and the genes they contain.
- VECTOR: A DNA molecule used as a vehicle to artificially carry foreign genetic material into another cell where it can be expressed.
- EUKARYOTE: Cells that have internal, membrane-bound compartments, such as a nucleus.
- PROKARYOTE: Cells that do not possess internal membrane-bound structures.

In this challenge, students will:

- Create a paper model of how DNA is moved from one organism to another
- Explore the differences between molecular cloning and organismal cloning

Before Class:

- Read the activity outline sheet and leader notes to become familiar with the activity.
- Gather necessary materials. Be sure that you have enough materials and space for students to work in pairs.
- Before starting this activity, students should be familiar with the central dogma of biology. Specifically, they should know that:
  - DNA is transcribed into RNA, which is then translated into amino acid chains that are folded into functional proteins.
- They should also be familiar with base pairing and Chargaff's Rule:
  - A pairs with T, and C pairs with G. Because of this, the number of A bases in a molecule should equal the number of T bases, and the number of G's should equal C's.
- Copies should be made in color, if possible, so that students can see the difference between the sequences.

Guiding Questions

1. Why might humans want to move DNA from one organism to another?
2. What is DNA cloning? How is it different from organismal cloning? What is it used for?
3. What are plasmids? How are they used in biotechnology?

Introduction

When people hear the word "cloning" they often envision making identical copies of an entire organism, like Dolly the Sheep. However, in a lab setting, cloning is simply making a genetically identical copy of something. In fact, most cloning is the cloning of genes: creating a way for many identical copies of an important gene (and its resulting protein) to be made at one time.

For pharmaceutical manufacturers like Dathan, these means obtaining a culture of cells that have important genes already in them, growing them up in huge vats, and then purifying out the proteins they have been programmed to make. But how do we get those important genes into those new cells in the first place?

Scientists take the DNA sequences for genes of interest and place them into an expression vector. Sometimes these are plasmids for bacteria and other times they're entire artificial chromosomes, depending on the cell they're using to grow the product. Regardless of what they use, the outcome is the same: the cell is transformed with new DNA and the now transgenic cell starts producing the new gene and the protein it codes for.

The easiest and earliest version of this cloning was using plasmids and bacteria. Plasmids are loops of DNA separate from the bacterial genome. Bacterial cells use these loops to pass information from cell to cell. Humans can exploit this approach by using restriction enzymes to cut the loops in specific places and insert whatever genes they want made. The bacteria don't care what is on the plasmid—their cellular machinery will copy it into RNA and translate that RNA into a protein. Each of these restriction enzymes cuts leaving an overhang on one side of the DNA ladder, or a "sticky end" that will easily re-connect with other DNA that has the same overhang.

The Activity

In this activity, students will be given a DNA message for a protein to cut out and put in a plasmid. Students are given a (made up) sequence and will need to identify a restriction enzyme that:

- Cuts the plasmid sequence only ONCE.
- Cuts the target sequence twice, once on each side of the gene of interest.

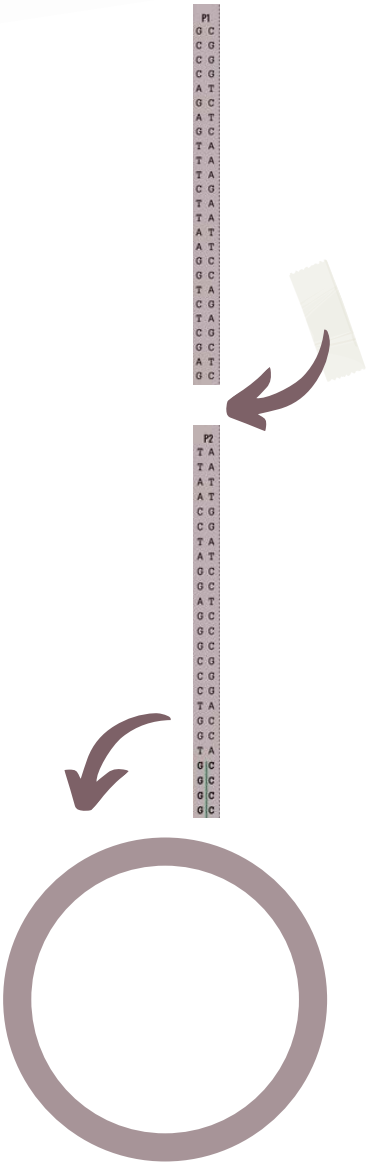
Ask students:

- What do we know about DNA? Why might we want to move DNA from one organism to another?
- Why is it important to know how the restrictions cut?

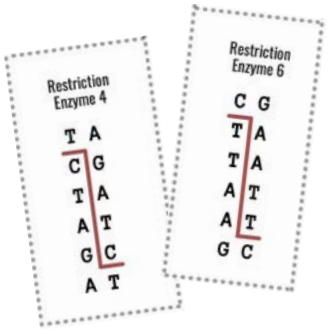
The Protocol

Each pair of students should have a copy of the DNA sequence handouts, scissors, and markers and/or colored pens.

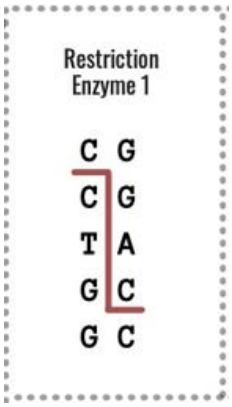
1. Carefully cut out each column.
2. Tape together the strips labeled P1, P2, P3, and P4 (they are shaded). Tape each strip together in order end to end, with P2 taped to the bottom of P1, P3 to the bottom of P2, etc. This plasmid has a sequence, marked in bold and with a green line, that must be intact for the gene of interest to be transcribed. Without this promoter sequence, nothing in the plasmid will be made.
3. Tape the end of P4 to the beginning of P1 to form one continuous circle of DNA. Congratulations! You have made a PLASMID!
4. Cut out the strips representing your target DNA and tape the strips labeled #1, #2, #3, #4, and #5 together end to end in numerical order just like in step 2. This time do NOT tape this DNA sequence into a closed circle. This is the DNA that contains your GENE OF INTEREST. Unlike a plasmid, this DNA is linear. The sequence for the gene of interest is in bold and has a blue line.

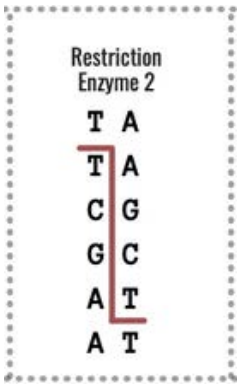


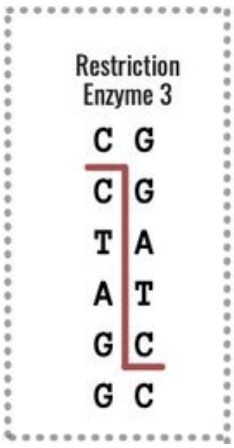
5. Cut out the restriction enzyme cards. These are labeled Restriction Enzyme 1, Restriction Enzyme 2, etc. Each of these represents real sequences that restriction enzymes recognize! Cut out all 6 cards and set these aside.
6. Your mission: determine which restriction enzyme will cut both the sequence around the gene of interest (the GoI) AND the plasmid so that the gene of interest can be spliced into the plasmid's DNA. To insert the gene into the plasmid you will want a restriction enzyme that cuts the target DNA twice but the plasmid DNA only once. Ask students:
    - How many times should the restriction enzyme cut the plasmid? How many times should it cut the target DNA sequence we're cloning from?
    - Where should the restriction enzyme cut the target DNA sequence? Should it cut in the gene itself? Why or why not?
    - What do we want to avoid cutting on the plasmid? The promoter sequence.
7. Compare the restriction enzyme cut sequences to the PLASMID DNA sequence loop and mark on each where the enzymes would cut with a marker or colored pencil. These enzymes always recognize the same sequence and will always cut there. Remind students not to cut the sequences yet.
    - Educator note: to simplify this activity, you may omit restriction enzymes 3 and 6.
        - Make sure students note the "Sticky ends" when they mark the sites on the plasmid DNA. Colored pencils make this part MUCH easier.
        - Some restriction enzymes may not cut the plasmid at all. Any enzyme that doesn't cut the plasmid won't help us so we can set those cards aside.
        - Remind them: we want an enzyme that cuts ONCE and doesn't disrupt the promoter.
          This will already eliminate:
            - RE4: no cut site
            - RE6: two cut sites

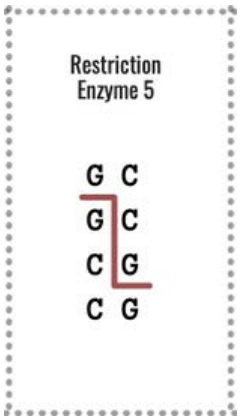


8. Compare the restriction enzymes to the source DNA and mark on the sequences where each enzyme would cut the sequence. DO NOT cut yet!
    - *Remind students to show the "sticky ends" of each restriction enzyme's cut site when they mark their DNA*
    - *The students want an enzyme that will cut the sequence on either side of the gene of interest. They also want to pick the restriction enzyme that will cut closet to the gene without disrupting it. You don't want to clone in a bunch of extra DNA. They should already have eliminated RE4 and RE6 because they aren't appropriate for the plasmid.*
        - *RE1: Eliminate. Does not cut the target DNA (no restriction sites)*
        - *RE2: Best choice. Cuts twice and cuts closest to the gene of interest.*
        - *RE3: Possible choice. Cuts twice but further away from the gene of interest than RE2.*
        - *RE5: Eliminate. Cuts too many times (three.) This is a good time to point out that this is a problem with restriction enzymes that recognize very short sequences. A sequence of only four bases is going to much more common than one with six or more.*
9. Once students have done this comparison, they should be down to one enzyme (RE2, which a. cuts the plasmid once and b. cuts the target DNA twice and closest to the ends of the gene of interest). Cut both the plasmid and the target DNA along the restriction site leaving sticky ends.
10. Line up the sticky ends on the gene of interest and the plasmid DNA. Use tape to stick these together. Congratulations! You've inserted a gene into a plasmid!









Discuss and Report

Once students have completed their plasmids, discuss the following:

- Great! You have a plasmid. Now what would you do with this?
  - *Answers will vary, but students should be able to explain that the plasmid would be transformed into a bacterial cell. These cells would then be grown up into a large culture and all of these cells would be producing the protein encoded by the gene they inserted.*
- What kind of products could we use this technique to produce?
  - *Answers will vary. Some students may be familiar with Humalin, the human insulin produced by bacteria and used by thousands of people with diabetes every day. Some students may offer ideas about agriculture and transgenic foods, like RoundUP Ready Corn.*

Career Exploration and Extension

Prompt students to think about and research what a career as a biomedical manufacturer or researcher might entail.

- What does a biomedical manufacturing operator do all day? What does Dathan do?
- What kind of training would a student need to manufacture biomedical products? What kind of training would they need to become the scientist who designs these systems?
- Are jobs like Dathan's in high demand? Will more people be hired to develop and manufacture new medicines in the future?
- What kind of education is needed to be a biomedical manufacturing operator? Where could a student be trained locally for this career? What types of classes are important?

Encourage students to research how transgenic organisms are used in medicine. Human insulin is an excellent topic to explore here.

Name: _____

PAPER PLASMIDS

STUDENT PROTOCOL

Pharmaceutical manufacturers, like Dathan at Catalent, use other cells to make proteins and other gene products for medical use. Sometimes these cells are eukaryotic cell cultures, but the first way scientists learned to do this was by using plasmids to take genes from one organism and have the bacteria's machinery make the protein in large quantities.

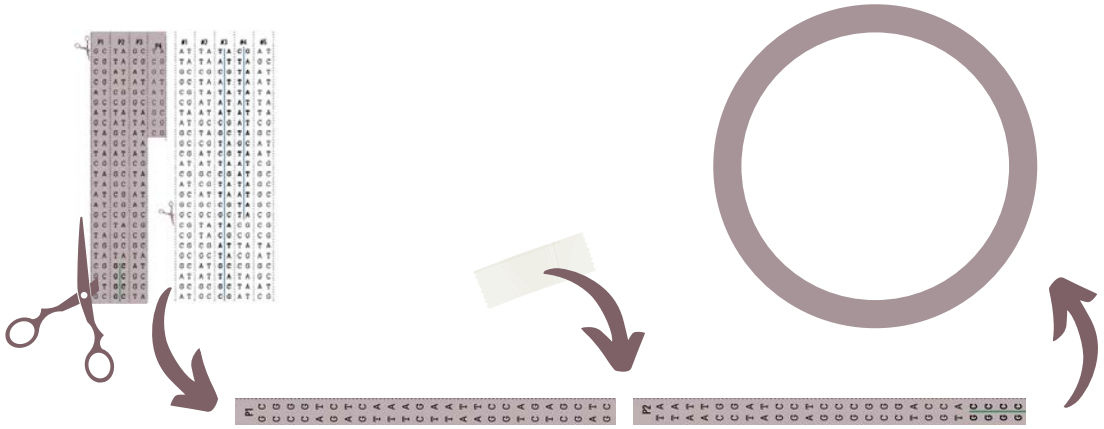
Remember that generally, information for how to build the molecules a cell needs flows in one direction:

DNA → RNA → PROTEIN

That is, the information stored in DNA as genes is transcribed into an RNA message, which is then sent out into the cell to be translated by the ribosome into a chain of amino acids. Once these amino acids are folded correctly, they become a functional protein. Some of these proteins are structural but most of them are enzymes that make other molecules or catalyze chemical reactions. It goes without saying that proteins are very important!

YOUR MISSION

You will be making a paper model of a target DNA sequence (a gene of interest, or GOI) and a bacterial plasmid. To cut the gene of interest out and open up the plasmid, you'll be looking at restriction enzyme sites. Restriction enzymes are proteins that cut DNA a specific sequence, leaving over hangs, or "sticky ends." These ends will easily re-connect with another DNA molecule, as long as it has the same overhang sequence. Once cut, you can mix DNA samples with these sticky ends together and the gene of interest will integrate into the plasmid.



Name: _____

STUDENT PROTOCOL

Your mission: determine which restriction enzyme will cut both the sequence around the gene of interest (the GoI) AND cut the plasmid so that the gene of interest can be spliced into the plasmid's DNA. To insert the gene into the plasmid you will want a restriction enzyme that cuts the target DNA twice but the plasmid DNA only once.

1. Carefully cut out the strips of your DNA sequences. The shaded sequences will be your plasmid DNA and the white sequence contains your gene of interest.
2. Tape together the plasmid sequence strips labeled P1, P2, P3, and P4 (they are shaded). Tape each strip together in order end to end, with P2 taped to the bottom of P1, P3 to the bottom of P2, etc. This plasmid has a sequence, marked in bold and with a green line, that must be intact for the gene of interest to be transcribed. Without this promoter sequence, nothing in the plasmid will be made.
3. Tape the end of P4 to the beginning of P1 to form one continuous circle of DNA. Congratulations! You have made a PLASMID!
4. Cut out the strips representing your target DNA and tape the strips labeled #1, #2, #3, #4, and #5 together end to end in numerical order just like in step 2. This time do NOT tape this DNA sequence into a closed circle. This is the DNA that contains your GENE OF INTEREST. Unlike a plasmid, this DNA is linear. The sequence for the gene of interest is in bold and has a blue line.
5. Cut out the restriction enzyme cards. These are labelled Restriction Enzyme 1, Restriction Enzyme 2, etc. Each of these represents real sequences that restriction enzymes recognize! Cut out all 6 cards and set these aside.
6. To insert the gene into the plasmid you will want a restriction enzyme that cuts the target DNA twice but the plasmid DNA only once.

    - How many times should the restriction enzyme cut the plasmid? How many times should it cut the target DNA sequence you're cloning from? Why?

    - Where should the restriction enzyme cut the target DNA sequence?

Name: _____

7. Compare the restriction enzyme cut sequences to the plasmid DNA (shaded) and mark on the sequence with colored pencils where each enzyme would cut. These enzymes always recognize the same sequence and will always cut there. Don't cut your target DNA or plasmid just yet!
    - Make sure you note the “Sticky ends” when you mark the sites on the plasmid DNA.
    - Some restriction enzymes may not cut the plasmid at all. Anything that doesn’t cut the plasmid won’t help us so we can set it aside.
    - You want an enzyme that cuts ONCE and doesn’t disrupt the promoter sequence.
8. Compare the restriction enzymes to the source DNA (unshaded) and mark on the sequence where each enzyme would cut the sequence. DO NOT cut your DNA sequence yet!
    - You want an enzyme that will cut the sequence on either side of the gene of interest. You also want to pick the restriction enzyme that will cut closest to the gene without disrupting or damaging it. You don’t want to clone in a bunch of extra, unnecessary DNA.
    - Don't forget to mark your sticky ends!
9. Once you’ve identified the optimal restriction enzyme, cut the both the plasmid and the target DNA along the restriction site leaving sticky ends.
10. Line up the sticky ends on the gene of interest and the plasmid DNA. Use tape to stick these together. Congratulations! You’ve inserted a gene into a plasmid!

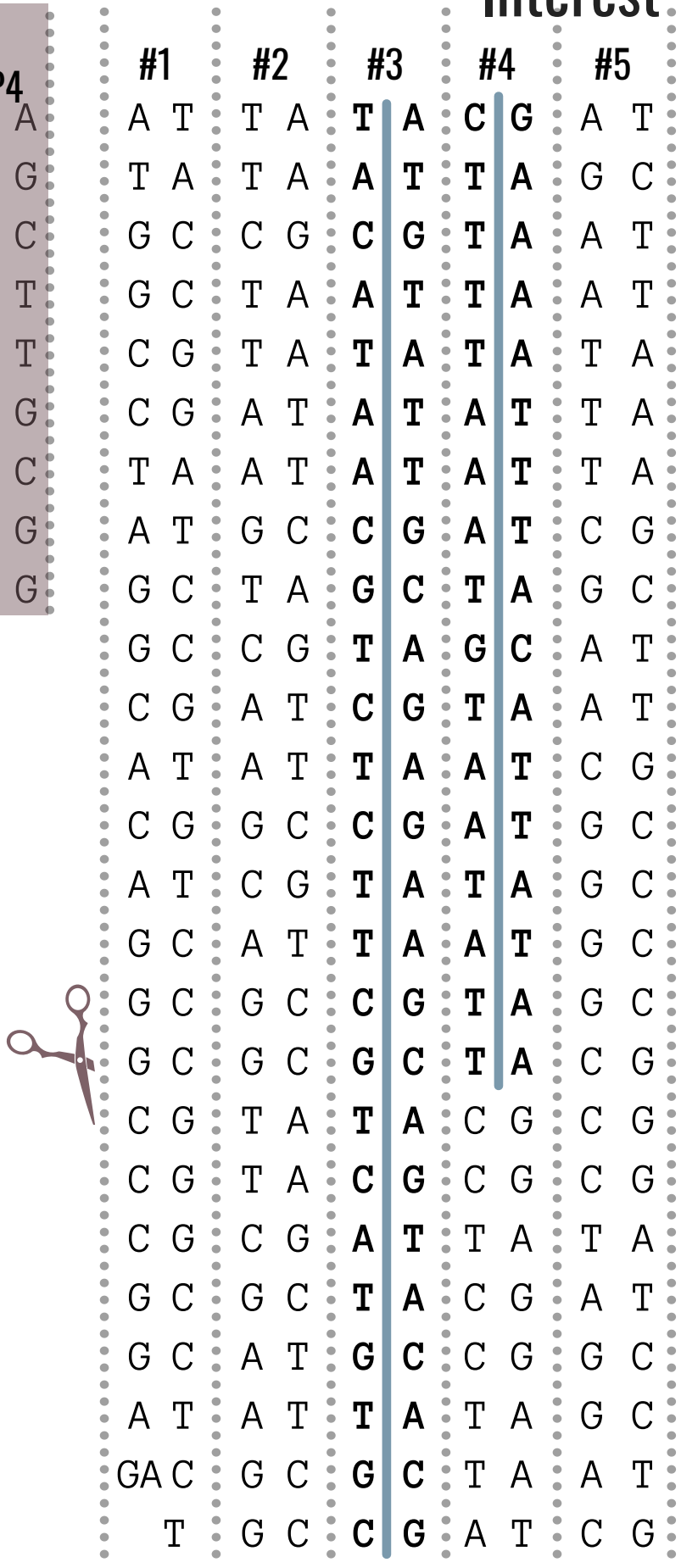
DISCUSSION AND REPORT

- How did each restriction enzyme cut?

- Which was the best restriction enzyme for cloning this gene in to this plasmid? Why?

- What could you do with this plasmid now that it contains your gene of interest?

Plasmid DNA and Gene of Interest



Restriction Enzyme Cards

Restriction Enzyme 1 C G C G T A G C G C	Restriction Enzyme 2 T A T A C G G C A T A T	Restriction Enzyme 3 C G C G T A A T G C G C
Restriction Enzyme 4 T A C G T A A T G C A T	Restriction Enzyme 5 G C G C C G C G	Restriction Enzyme 6 C G T A T A A T A T G C

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IMAGE AND CONTENT CREDITS

Images

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Content

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