Robogals Science Challenge

Minor Challenge Set #1
STEM Field: Biomedical Engineering
Level: Senior
Challenge Name: Making a Unique DNA Fingerprint
Project Cost: 0-20 USD
Materials Required:

- Laptop and internet access
- To record results: (recommended) Microsoft Word, Google Docs, or pen and paper
- Calculator

Duration:

- This challenge takes approximately an afternoon to a day to finish, however, the time guideline is an estimation only, and students and mentors can complete the tasks around their schedules.

Introduction

Each cell has DNA inside of it. DNA, or deoxyribonucleic acid, carries the genetic instructions needed for organisms to grow, function and reproduce. This set of instructions is referred to as the *genome* and genetic information is stored in a code of *nucleotides* or *bases* (A, T, C and G). These *bases* form specific pairs, or *base pairs*, in which A pairs with T, and C with G.

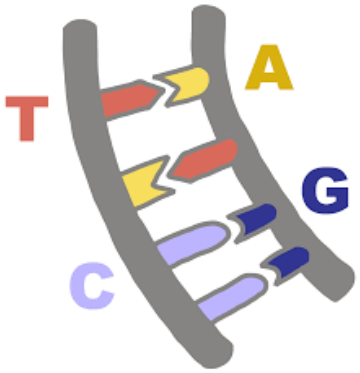

Figure 1: A diagram depicting how the nucleotides A, T, C and G can be connected together in base pairs. Nucleotides A and T are connected at the top of the DNA fragment, and C and G are connected in a pair at the bottom of the fragment.

Every individual has their own unique genetic code. However, with only 4 letters, how can we make so many different combinations of DNA? The answer is in the length of the genetic code.

For example, we define the DNA length as 2 base pairs. In mathematics, we refer to the possible ways to arrange these base pairs as permutations. That means, with 4 bases — A, T, C and G, and length being 2 base pairs, we can have 4^2 or 4 x 4 = 16 possible unique sequences! We can have: AA, AT, AC, AG, TA, TT, TC, TG, CA, CT, CC, CG, GA, GT, GC, or GG. The human genome contains 6.4 billion base pairs, and so, the number of possible DNA combinations is much much bigger than what we have calculated here!

In reality, about 99.9% of the DNA between two people is the same. The remaining percentage is what makes us unique. It sounds small, but this means there are around three million base pairs that are different. DNA fingerprinting is a method used to identify an individual from a DNA sample by looking at unique patterns in their DNA. So, how does DNA fingerprinting work?

The goal of this Minor Challenge is to generate some unique DNA fingerprints, then analyse these fingerprints using online programs.

Before attempting this project, you may want to explore more on how DNA fingerprinting works here: https://www.yourgenome.org/facts/what-is-a-dna-fingerprint.

Instruction

1. Using the example given in the Introduction section, can you calculate the total unique DNA sequences for these base pair lengths?

DNA Length	How many unique DNA sequences are possible?
2 base pairs	$4^2 = 4 \times 4 = 16$
3 base pairs	$4^3 = 4 \times 4 \times 4 = 64$
4 base pairs	
5 base pairs	
10 base pairs	

The human genome contains 6.4 billion base pairs, you can see the number of possible DNA combinations is much much bigger than what we have calculated here!

2. First, we will generate a DNA sequence with 1000 base pairs to analyse later.
   Navigate to the website
   http://www.faculty.ucr.edu/~mmaduro/random.htm
   on your computer. It is recommended that you use the Google Chrome browser. This is a free website and does not require registration.

3. Ensure the settings are set to aDNA size of 1000 base pairs (bp) and GC content of 0.5 (which will give you half G+C and half A+T base pair combinations).

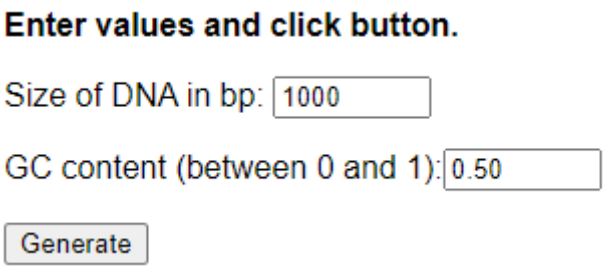

Figure 2: A screenshot of the random DNA generator, showing size of DNA in bp as 1000 and GC content as 0.50.

4. Click on the generate button and you will get a random DNA sequence.

5. Copy and paste the DNA sequence into a Word document or Google Doc. Give this piece of DNA sequence a name.

6. We will now analyse the DNA sample we generated from steps 2-5.
   Navigate to this website:
   https://molbiotools.com/restrictionanalyzer.php
   on your computer. It is recommended you use Google Chrome browser.

7. Copy the DNA sequence you generated and paste it in this box.

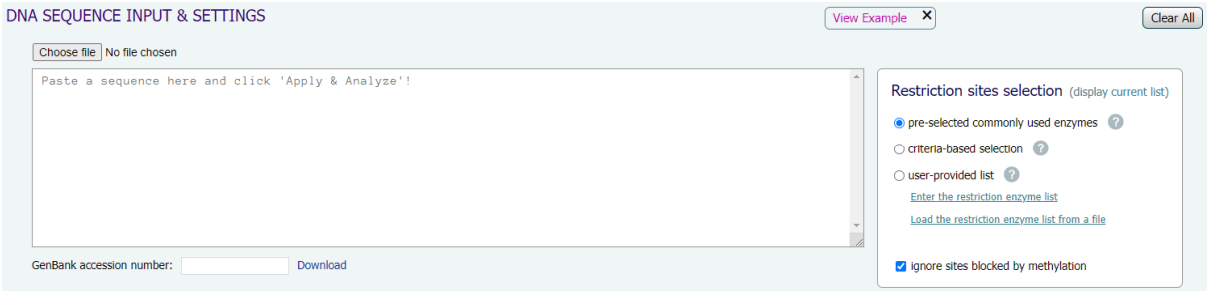

Figure 3. A screenshot of the DNA sequence section in the Restriction Analyzer tool. The blank space with the text "Paste a sequence here and click 'Apply & Analyze'" is where the DNA sequence will be pasted.

8. Leave all of the settings as the default settings. Click on the "Apply & Analyze" button.

9. Scroll down to the section called “RESTRICTION FRAGMENTS”. We will now cut the DNA sample into restriction fragments for further analysis. A restriction fragment is a short DNA fragment derived from the cutting of a longer DNA strand. To explain this process: scientists isolated restriction enzymes from bacteria. They then use these restriction enzymes to cut DNA into fragments in ways that make it easier to study, identify and characterise genes.

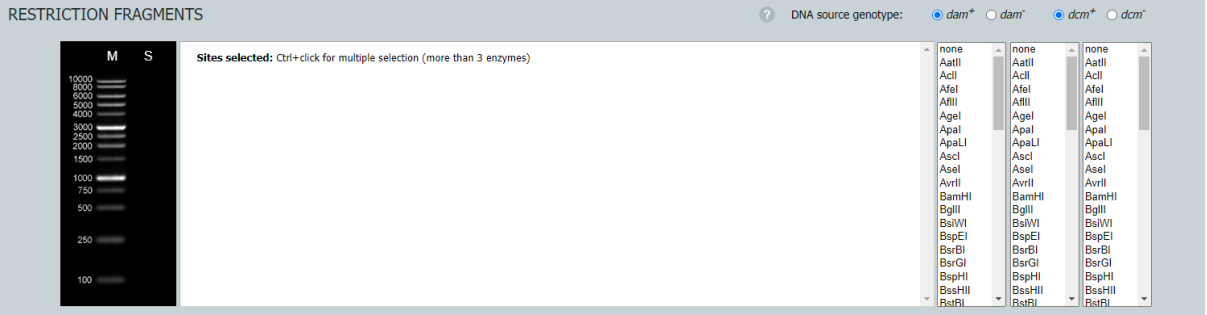

Figure 4: A screenshot of the Restriction Fragments section in the Restriction Analyzer Tool. On the left hand side is the picture of the DNA fingerprint. The blank space in the middle is where the fragment will be shown after DNA cutting. The 3 columns on the right hand side are restriction enzymes you can select to cut the DNA sequence.

10. After cutting, scientists will use restriction enzymes and a process called electrophoresis to digest and separate the DNA fragments. In the column (highlighted in red), you can choose between the following enzymes to digest DNA fragments: BamHI, EcorI, HindIII, SmaI, SpeI, XbaI and XhoI. These enzymes are chosen as they have the capacity for recognising and separating the DNA fragments.

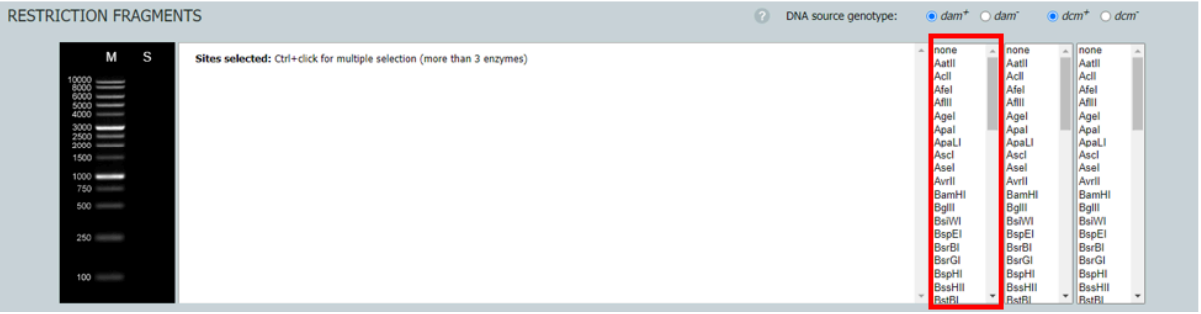

Figure 5: A screenshot of the Restriction Fragments in the Restriction Analyzer tool. The right hand side column out of 3 columns is highlighted in red. This column is where you will select the restriction enzyme to do DNA cutting.

11. Once you have clicked on an enzyme, you should see that the different fragments show up in the middle section. On the left hand side you will see an image of your piece of DNA. This piece of DNA was cut by your selected restriction enzyme.

    The following image shows an example of DNA fragment/s.

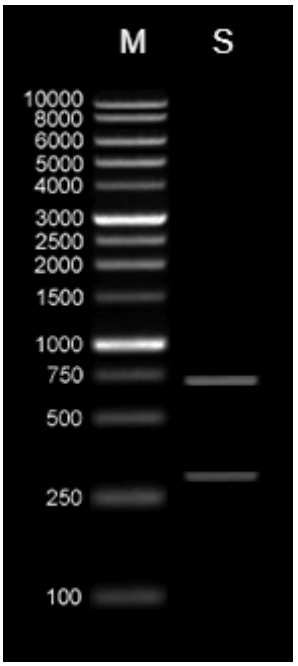

Figure 6: A screenshot of an example of a DNA fragment/s after cutting.

On the left hand side, labelled “M”, is a standard DNA ladder that contains DNA fragments with sizes from 100 to 10000 base pairs.

The right hand side, labelled “S”, shows the different DNA fragments in your cut DNA sample which you can compare to the DNA ladder to determine the size of your DNA fragment/s.

12. Click on “Save the image” to download the image of your DNA fragment/s. Insert this to your previous notes of the DNA sequence. Ensure you saved this document!

13. Go back to the Random DNA sequence generator on the website http://www.faculty.ucr.edu/~mmaduro/random.htm and repeat steps 3-12. Give this DNA sequence a new name. Save the image of your new DNA fingerprints to your notes.

14. Repeat the process three more times. Compare all of your DNA sequences and DNA fingerprints. At the end of this experiment you should have five DNA fingerprints to compare and analyse.

15. Choose one DNA sequence out of the five sequences you generated. This time, we will only change a few letters of the sequence. For example, change the first 10 letters to something new, and leave the rest of the sequence the same. Repeat steps 3-12 and see if there would be any changes to your DNA fingerprint.

16. Using what you have learned from this activity, you will now attempt to analyse a DNA fingerprint and find the culprit of a crime using this virtual activity.

    Navigate to the website:
    https://www.pbs.org/wgbh/nova/interactive/create-dna-fingerprint/
    on your laptop. Follow the instructions to analyse the fingerprint and identify the culprit!

Extension:

Task 1: Virtual Lab - Restriction Enzyme Digest

This is an optional, but highly recommended task to complete if you are interested in biotechnology. This simulation provides an opportunity to practice a restriction enzyme digest in a virtual lab setting. They have step by step instructions to follow and provide more context for the process of restriction digestion.

Navigate to the website on your computer:
https://www.labxchange.org/library/items/lb:LabXchange:1fb8b9d5:lx_simulation:1

Click on “Start simulation”, choose “Level 1” to start, then follow the instructions as shown on screen.

Task 2: Interactive - How Do Restriction Enzymes Cut Plasmids?

Navigate to the website on your computer
https://www.labxchange.org/library/items/lb:LabXchange:783397ff:lx_simulation:1

Click on “Start interactive” and read the context. This interactive demonstrates how restriction enzymes work and how they can be used as tools to analyse DNA.

Reflection Questions

- Are there any improvements you would make to this challenge?
- What real world application/s can you apply this challenge to?
- What are the key science and engineering concepts that relate to this challenge?

- What do you notice about the 5 DNA fingerprints you generated? Do unique sequences of DNA result in different DNA fingerprints?
- In step 15, what changes did you make to the DNA sequence you chose?
- Did you notice any changes to the fingerprint after making minor changes?

- If you attempt the extension task, please write a short reflection on what you learned.
- Optional question: In real life, restriction enzymes can be used as a tool to recombine or join different DNA fragments. Have you heard of DNA cloning? In biotechnology research, DNA cloning is used to create a large number of copies of a piece of DNA for research purposes. The first step is to cut and paste DNA using restriction enzymes. You can do further research on the application of restriction enzymes, then tell us some interesting points about the applications that you learnt.

  A resource that may help this research is:
  https://www.khanacademy.org/science/biology/biotech-dna-technology/dna-cloning-tutorial/v/dna-cloning-and-recombinant-dna

Submission Guidelines

- Submit your research notes, the images of your DNA fingerprints and your answers to the reflection questions.

> Note: Remember, if you want to upload pictures of your Minor Challenge that also include you, please check if it is OK with your mentor first.

- The submission form is on the Minor Challenges page: https://sciencechallenge.org.au/index.php/minor-challenges/
  Fill out the details and make sure you upload your submission.

Learn More! Resources

- If you enjoyed this task, you may want to read more on possible careers to pursue in the biomedical or biotechnology field.

  Biomedical engineer:
  https://www.sciencebuddies.org/science-engineering-careers/health/biomedical-engineer

  Biotechnologist:
  https://www.prospects.ac.uk/job-profiles/biotechnologist

  Bioinformatics Scientist
  https://www.sciencebuddies.org/science-engineering-careers/health/bioinformatics-scientist

  Forensic Technician
  https://www.sciencebuddies.org/science-engineering-careers/earth-physical-sciences/forensic-science-technician

Bibliography

- Agee, S. (2021, May 1). What Makes a DNA Fingerprint Unique? Retrieved from https://www.sciencebuddies.org/science-fair-projects/project-ideas/BioChem_p016/biotechnology-techniques/what-makes-a-dna-fingerprint-unique
- Innovative Genomics Institute (IGI). 2022. Complementary. [online] Available at: <https://innovativegenomics.org/glossary/complementary/> [Accessed 20 January 2022].
- Pray, L., 2008. Restriction Enzymes | Learn Science at Scitable. [online] Nature.com. Available at: <https://www.nature.com/scitable/topicpage/restriction-enzymes-545/> [Accessed 20 January 2022].