TEACHING OUTREACH RESOURCE

STR Analysis

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ŌTĀKOU WHAKAIHU WAKA

GO BEYOND WITH GENETICS

__Explore__ the origins and mechanics of life. __Discover__ causes of disease and their cures. __Solve__ the problems facing our agriculture and natural heritage. __Understand__ the past. __Create__ a better future. __Master__ the world of genetics...

WELCOME...

…to this Genetics teaching resource, created by Genetics Otago and the Genetics Teaching Programme at the University of Otago.

Our aim is to engage young minds with Genetics and to do this we have developed a range of resources that include information, worksheets and activities or experiments that will help you to plan exciting Genetics classes for your students.

Where possible we have endeavoured to align and link the content of the resources to the New Zealand Curriculum.

If you have any questions relating to the content of the resources or would like to organise an onsite teaching session on one of our topics please contact us at go@otago.ac.nz.

Contents

Overview

This resource gives a basic introduction to the concept of Short Tandem Repeats (STRs) and specifically their application to forensic investigation. A brief description of PCR (polymerase chain reaction) and gel electrophoresis are given as a basis. You may wish to introduce these two techniques to the students before completing this module.

OBJECTIVES

- To understand that there is variation in human DNA
- To learn what a Short Tandem Repeat (STR) is
- To understand that there are a number of different alleles for each STR
- To understand that the alleles at a set of STRs act as a DNA fingerprint for an individual
- To assess evidence and make appropriate conclusions

SECTIONS

Part A: Understanding the Techniques

- Polymerase Chain Reaction
    - Activity One – **PCR Cycles (no worksheet)**
- Gel Electrophoresis
    - Activity Two – **Understanding Electrophoresis**
- Short Tandem Repeats
    - DNA Conservation Handout
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Part B: Case Study

- Setting the Scene
- Evidence
- Fingerprints
    - Optional Fingerprint activities
    - Activity Four - **Fingerprint Analysis**

- DNA Analysis
    - Activity Five – **Identifying the Killer**
- Questioning Suspects
- Who is the Killer?
    - Activity Six – **Write a Case Report (no worksheet)**

CIRRICULUM LINKS

This module is designed to feed into the following curriculum areas:

- **Nature of Science**
    - *Understanding about science* – working together and interpreting evidence to support ideas
    - *Investigating in science* – carry out investigations, multiple variables, evaluations of methods
    - *Communicating in science* – Vocabulary and conventions, application of science to popular culture
    - *Participating and contributing* – drawing evidence-based conclusions

- **Living World**
    - *Life processes, ecology and evolution* – Explore the effects of genetic variation and appreciate their impact biologically, socially and ethically.

ACHIEVEMENT STANDARDS

This resource is relevant to the following standards:

- 2.2/91153 - Carry out a practical investigation in a biology context, with supervision
- 2.4/91157 - Demonstrate understanding of genetic variation and change
- 2.6/91159 - Demonstrate understanding of gene expression
- 3.6/ 90718 - Describe applications of biotechnological techniques

Background

Forensic science is a field of molecular biology that is popular among young people with a number of references to pop culture, especially TV shows, engaging students in the topic. This provides a great background to introduce students to a number of techniques fundamental to the understanding and study of genetics. Study of a forensic scenario can incorporate basic understanding of DNA, genes and heredity as well as the technical aspects of the methods used to solve such a case.

This resource focuses on the technical aspects including Polymerase Chain Reaction, Gel Electrophoresis and Short Tandem Repeat Analysis. These topics are described and there is a paper based exercise for each.

As an extension to this activity there is an optional experiment where students can run a set of STR samples on an electrophoresis gel to determine the offender of a crime from a set of suspects. If you would like to borrow the resources required for this experiment please contact go@otago.ac.nz to make a booking.

PART A

Understanding the Techniques

Polymerase Chain Reaction

Polymerase Chain Reaction (PCR) is one of the most commonly used methods in molecular biology. It is a reaction that allows the amplification of a specific piece of DNA from an entire genome. The reaction requires four key reagents to be mixed and then cycles through several steps of heating and cooling. Once DNA has been amplified it can be used in a great number of downstream applications.

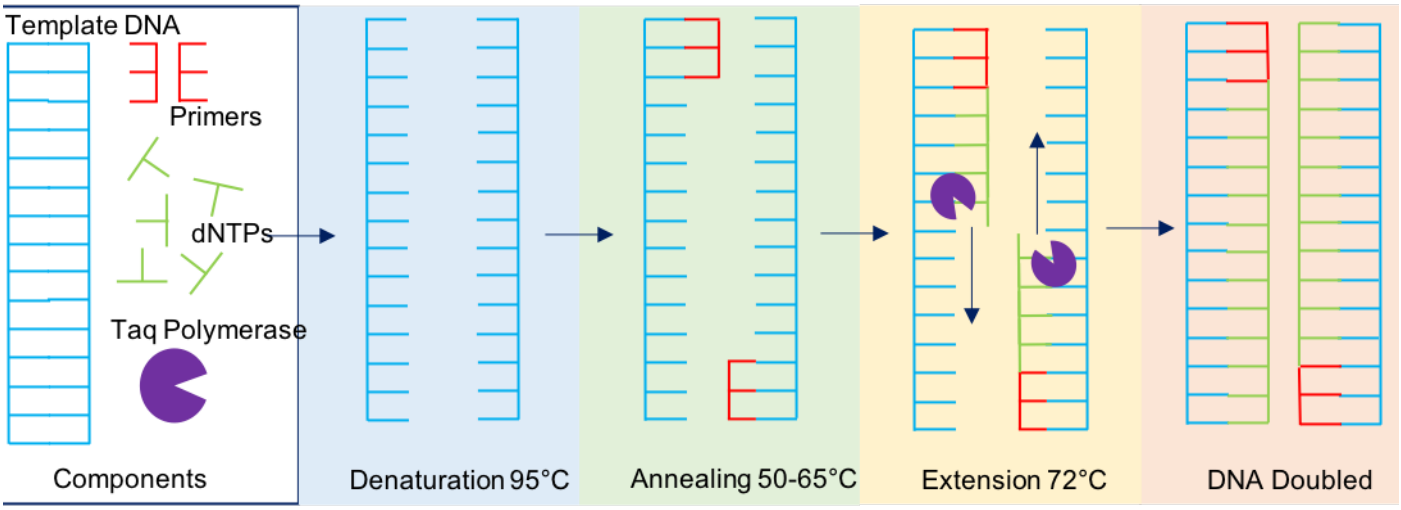
PCR Reagents

1. Template DNA – this can be from anything, a plant, a person, a blood or saliva sample etc. The DNA must be extracted and purified from the raw sample.
2. Base Pairs (called dNTPs) – these are the A, C, G and T that are the building blocks of DNA.
3. Primers – a set of short DNA sequence that perfectly match the start and end of the piece of DNA you want to amplify.
4. Taq Polymerase – a special enzyme that is heat stable and adds bases together to create a new DNA strand.

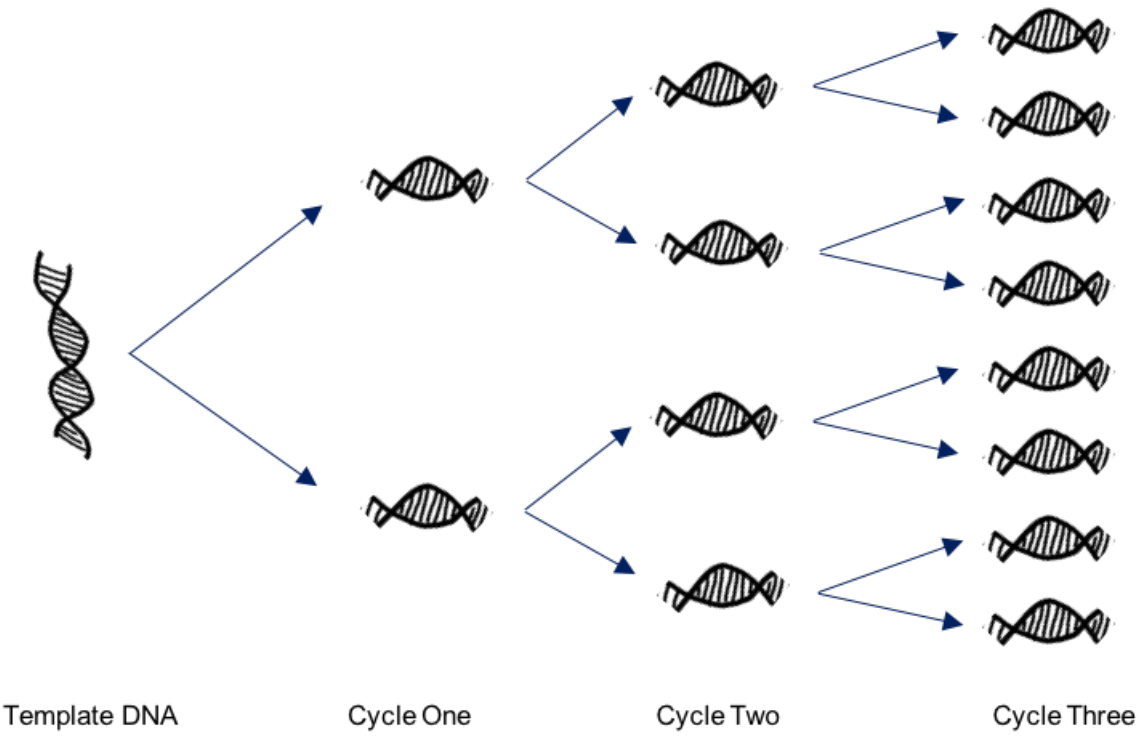
PCR Steps

1. Denature – in this step the mixture is heated to around 95°C to break apart the two strands of DNA
2. Annealing – the temperature is reduced to 50-65°C which allows the primers to bind to the start and end of the sequence you are trying to amplify
3. Extension – the temperature is raised slightly to 72°C which is the optimum temperature for the taq polymerase to synthesise the new DNA strand using the dNTP building blocks.

Multiple cycles are required to amplify the DNA to the required amount. However, due to the nature of this reaction creating two DNA fragments from a single DNA fragment, the amplification is exponential and as such the number of cycles required rarely exceeds 30.



The Figure above shows each of the steps of the PCR cycle.



The Figure above shows the doubling of the DNA at each cycle.

Activity One: Have the students work out how many copies of the DNA there will be after 2 cycles, 5 cycles, 10 cycles etc. Have them think about why the numbers they have calculated may be an under representation of the actual number of copies.

ANSWERS

The number of copies of the DNA can be calculated using 2n, or by multiplying the number of copies present by 2.

1 cycle = 2^1= 2 or 1x2 = 2
2 cycles = 2^2= 4 or 2x2 = 4
3 cycles = 2^3= 8 or 4x2 = 8
4 cycles = 2^4= 16 or 8x2 = 16
5 cycles = 2^5 = 32 or 16x2 = 32 etc…

10 cycles = 2^{10}= 1024
20 cycles = 2^{20}= 1,048,576
30 cycles = 2^{30}= 1,073,741,824
40 cycles = 2^{40}= 1,099,511,627,776

In reality the numbers are actually much higher than this because there will almost certainly be multiple copies of the DNA sequence in the sample before you begin. In the calculations above we are assuming a single starting copy.

Gel Electrophoresis

Gel Electrophoresis is another common tool used in molecular biology and is usually the next step after running a PCR reaction. Sometimes a gel electrophoresis is used simply to check that the PCR has been successful before the DNA is used in another experiment but often, it is the way a scientist will analyse the of results of their experiment.

DNA Structure

Deoxyribose Nucleic Acid (DNA) is the code of life. It is the set of instructions that is used to build an organism and keep it going. DNA is made up of millions of nucleotides all bound together into a double helix structure, which looks like a twisted ladder. Each nucleotide is made up of one of four nitrogenous bases (Adenine [A], Cytosine [C], Guanine [G] or Thymine [T]), a sugar called deoxyribose and a phosphate group. The nucleotides bind via a covalent bond between the sugar of one nucleotide and the phosphate group of the next – this forms the backbone of the DNA (or the sides of the ladder). The nucleotides are also bound via their nitrogenous bases, which always pair A with T and G with C, via a different kind of bond called a hydrogen bond. The pairing of the bases creates the 'rungs' of the ladder. The phosphate group of the structure makes the DNA negatively charged which is the basis of how gel electrophoresis works.

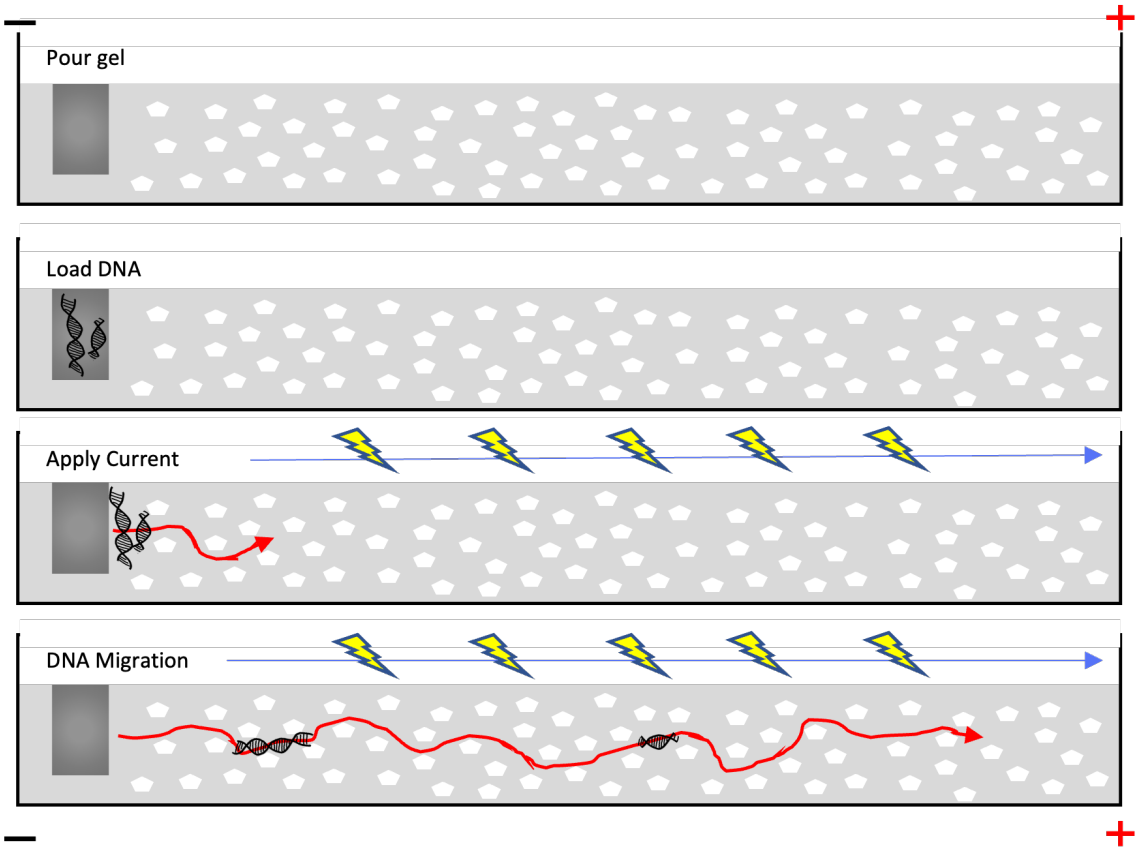
The Gel

An electrophoresis gel is made using agarose and buffer. Agarose is a substance that is extracted from red seaweed and comes in a powdered form. The agarose powder is added to a buffer, which provides ions to carry the current of electricity, and is heated to dissolve the agarose. This mixture is then poured into a special cast with a comb inserted (to create loading wells) and left to set. The gel sets as a cloudy, jelly-like substance. Once set the the gel it takes on a matrix like structure with tiny pores that cant be seen, this matrix structure is also very important to how electrophoresis works.

Electrophoresis - Running the Gel

The gel is placed into a tank (or electrophoresis chamber) and fully submerged in buffer. The DNA samples, controls and size markers (ladder) are then loaded into the wells created by the comb. An electric current is the passed through the buffer and gel using a power pack.

The negative charge of the DNA means that it is attracted towards the positive terminal of the gel tank, this is what makes the DNA move through the gel. The matrix structure of the gel determines how far the DNA moves. The DNA has to move around the pores of the matrix and the smaller the piece of DNA the faster it can do this. The biggest fragments take the longest to move and so stay closer to the wells while the smallest fragments move quickly towards the positive end.



In the figure above, the pores of the matrix are represented by the white spots and the blue arrow shows the direction of the current.

Activity Two: Have the students complete the worksheet '**Understanding Electrophoresis'**. Using a ruler or a piece of paper is helpful to determine the size, line up your ruler with the band and draw a real (or imaginary) line back to the ladder.

ANSWERS

1.	*Lane*	*2*	*Size*	*~2800bp*
2.	*Lane*	*8*	*Size*	*~2200bp*
3.	*Lane*	*5*	*Size*	*~2000bp*
4.	*Lane*	*3*	*Size*	*~1190bp*
5.	*Lane*	*7*	*Size*	*~500bp*
6.	*Lane*	*4*	*Size*	*~320bp*
7	*Lane*	*6*	*Size*	*~100bp*

Short Tandem Repeats

Although we all look different, our DNA is actually very similar to each other. In fact, we share 98% of our DNA with Chimpanzees (see '**DNA Conservation**' handout), so imagine how similar two humans DNA is!

Some of this variation is because there are locations in our DNA where a short sequence of bases are repeated many times. The example below shows six repeats for the sequence 'CAG'

```
CAG CAG CAG CAG CAG CAG
GTC GTC GTC GTC GTC GTC
```

Individuals differ in the number of repeats they have at these locations. In a particular area of DNA, one individual might have 100 repeats and a second 200 repeats. These regions are called Short Tandem Repeats (STRs) or microsatellites, and the different number of repeats at each site are called alleles.

There are several different alleles for each STR and each of these will be shared by between 5-20% of individuals. The alleles at each STR are inherited from an individuals' parents. If both of your parents pass on an allele of the same size you will be homozygous; if each parent passes on an allele of different size you will be heterozygous.

Due to the fact that STRs are inherited people who are related are more likely to have the same STR lengths, however if several different STR loci are used the chances of two people having the same sizes across all of them are very low. For example, the FBI uses 13 different STRs when doing a forensic analysis, with this many sites the chance of identifying the wrong person is about 1 in 10 billion (there are only about 7.5 billion people in the world).

So the pattern of several different STRs is unique to each individual much like a fingerprint is. We can look at this 'DNA Fingerprint' of an individual using PCR and Gel Electrophoresis.

Activity Three: Have the students complete the worksheet '**Find the Odd One Out**'. Remember that if an individual is homozygous they will appear to only have one band though it may be bolder than a single allele band because there are actually two bands in the same place. A heterozygous individual will have two bands at each STR.

ANSWERS

Babies 2 and 5 are not the offspring of parents 1 and 2.

Parent 1 has alleles 1 (~1000bp) and allele 2 (~600bp)
Parent 2 has alleles 2 (~600bp) and allele 3 (~200bp)

Child 1 has alleles 2 and 2
Child 2 has alleles 1 and 4 (~2500bp)
Child 3 has alleles 1 and 3
Child 4 has alleles 2 and 3
Child 5 has alleles 1 and 1

PART B

Case Study

Setting the Scene

Computers have been going missing from schools in you area for over a week now. The school board is worried that your school will soon be the target of these thieves and employs a security guard to monitor the school overnight, without anyone's knowledge.

This morning the school grounds keeper has arrived to discover the body of the security guard in a pool of blood in the computer suite. The room is in chaos, with tables upended, chairs tipped over and several computers lying broken on the floor. One of the screens has shattered leaving shards of glass on the carpet, upon closer inspection there is blood on some of these shards. A keyboard is found near to the victim with blood splattered on it.

The grounds keeper alerts the police and the Principal of the situation. When the computer teacher becomes aware of the situation she is visibly distressed.

Police arrive on the scene to collect evidence.

Setting the Scene

Police have found evidence in several forms at the crime scene. Fingerprints have been lifted from the keyboard, assumed to be the murder weapon as well as on the door handle and on several surfaces around the room.

Blood samples were taken from the keyboard and from the shards of glass of the broken computer screen.

The cause of death has been determined to be blunt force trauma to the head but the victim also shows signs of a struggle with bruises on the face and DNA found under the fingernails. The time of death was determined to be some time between 3:00pm and 8:00pm the previous evening.

The Principal, Grounds Keeper and Computer Teacher have all had their fingerprints and DNA samples taken to eliminate their contamination of the crime scene. The officer collecting the evidence also noticed a hair that appeared to match the victim on the scarf of the Computer Teacher – this was also collected and DNA was extracted.

All DNA samples have been taken to a lab for STR analysis.

Fingerprints

Fingerprint analysis has been used as a tool in forensic analysis for over 100 year and is still important in forensic situations today because no two people ever have the same fingerprints, even identical twins who have identical DNA. Additionally, they remain essentially unchanged over the course of a persons life, the exception to this is extremely deep damage to the finger which will result in a permanent change to the fingerprint that is still unique.

Fingerprints are made up of unique sets of patterns made by the friction ridges and furrows, it is the friction ridges that are often analysed as these are what are captured by an ink print for example. These patterns are classified into three types – loops, whorls and arches (these are described in the info sheet 'Fingerprint Types').

In reality, the prints lifted from crime scenes are often incomplete or partial prints. In this case study we use complete prints that are easier to recognise and match without the use of specialist equipment.

Optional Activity: Using inkpads have the students try taking their own or a partners fingerprints, identify the three types of pattern and see how partial prints are likely.

Physically there are two different kinds of prints that can be left at a crime scene Patent prints are impressions left in a soft surface and can be captured using photography, sometimes with dyes and alternate lighting sources. Latent fingerprints are more common and are formed by oils and sweat from the skin leaving a residue on a surface. Latent prints can be collected in a number of ways including using special lights, fingerprint 'dust' or chemicals that react with the proteins left behind to change the colour of the prints.

Optional Activity: Have the students press a finger onto a solid non-porous surface (glass is good). Sprinkle a small amount of dark powder (carbon powder or dark eye shadow works well) over the area and gently remove excess using a soft paint brush. Apply clear tape over the print and then peel the tape off and stick it to a a piece of white paper. Are the prints all complete or are some only partial?

Activity Four: Have the students complete the worksheet **'Fingerprint Analysis'.**

ANSWERS

Door Handle *– Security Guard, Computer Teacher and Grounds Keeper and Principal*
Keyboard *– Computer Teacher, Principal and Security Guard*
Surfaces *– Computer Teacher, Grounds Keeper and Security Guard*

The Grounds Keeper's fingerprints are present in the room and on the door handle but not on the murder weapon (the keyboard). This suggests that he is less likely to be the killer, however he can not be excluded on these grounds alone as he may have been wearing gloves at the time of the murder and the other fingerprints may have been from earlier interactions with the room.

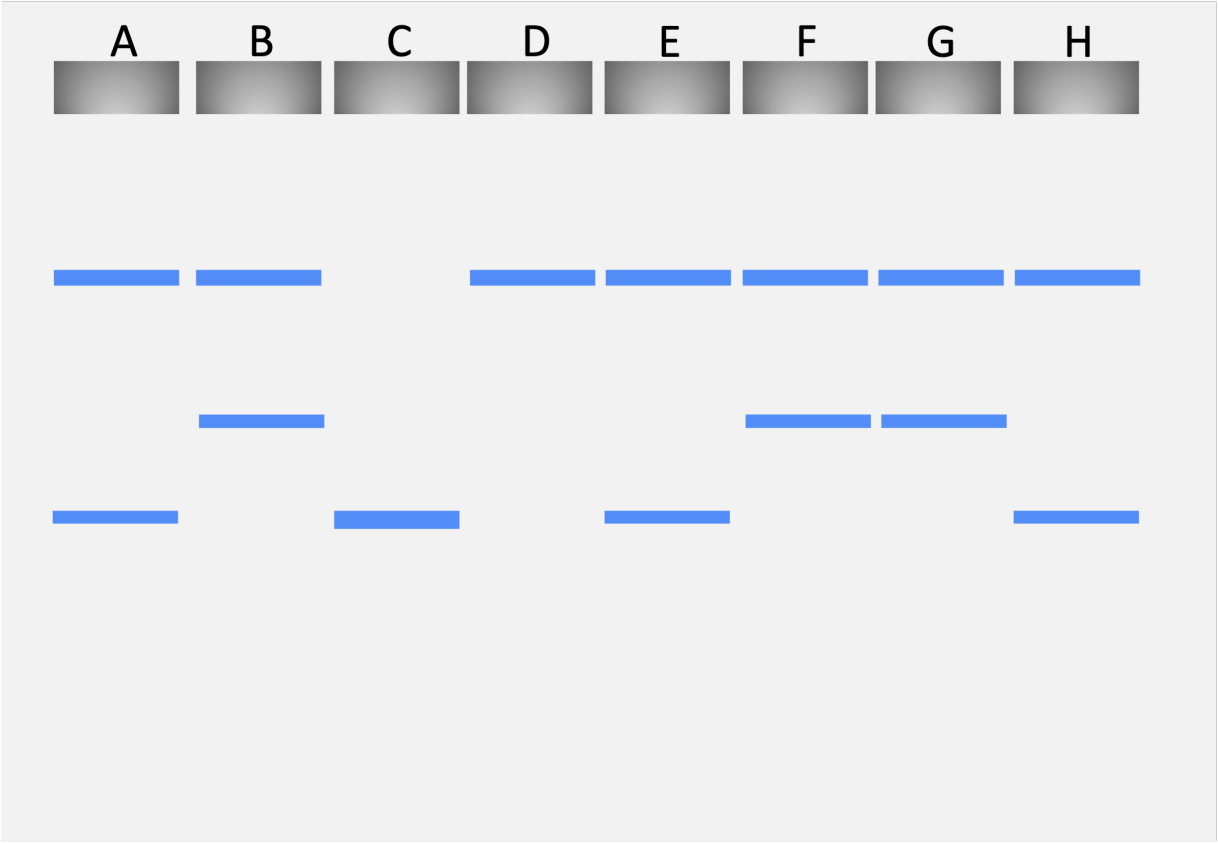
DNA Analysis

Activity Four: Draw the following gel diagram on the board before having the students answer the '**Identifying the Killer**' worksheet.

DO NOT draw this if you plan to run the practical experiment, the students will use the results from the experiment to complete the worksheet.

ELECTROPHORESIS KITS

This final identification step can be done as a practical exercise using the Genetics on the GO kits available from us. In this case the students will make and pour an agarose gel that they will then load 'DNA' samples into and analyse the results to decide which individuals are affected by the disease and which are carriers. For this exercise we supply dye samples as the DNA to eliminate the need for staining of DNA meaning that the exercise can be done in a single lesson. If you would like to borrow one of these kits please contact us at go@otago.ac.nz.



A – Security Guard DNA
B – Principal DNA
C – Grounds Keeper DNA
D – Computer Teacher DNA
E – Blood from keyboard
F – Skin under nails
G – Blood on glass shards
H – Hair DNA

ANSWERS

1. The Security Guard (victim)
2. The Principal
3. The Principal
4. The Security Guard (victim)

5. The Grounds Keeper has no DNA present at the crime scene and his fingerprints are not on the murder weapon so he can be excluded.
6. The Principal has fingerprints on the murder weapon and his DNA is on the shards of glass and under the fingernails of the victim.
7. The computer Teacher can not be ruled out as her fingerprints are also on the murder weapon and the victims hair was found on her clothing.

Questioning Suspects

The police have questioned the Principal, Computer Teacher and Grounds Keeper and the following was discovered:

- The night before the murder was discovered the Grounds Keeper was at home with his wife from 5:30pm. His wife confirmed this.
- The Grounds keeper arrived at school at 7:50am as usual and noticed that there were several cars in the carpark which is not unusual, these included the Computer teachers and the Principals cars.
- The Grounds keeper saw that the principal was alone in his office and the Computer teacher was in the staff room chatting with two other teachers as he walked through the administration block at 8:00am.
- The Grounds keeper arrived at the computer room at 8:20am and immediately alerted the police and the Principal of the situation.

- The night before the murder was discovered the Computer Teacher went to the supermarket after work and then headed home where she was alone until her children arrived at 5:00pm.
- The Computer teacher arrived at school at 7:55am and went straight to the staff room to grab a coffee and start some marking. She was planning to head to her classroom just prior to the morning bell at 8:45am.
- The Security Guard was the ex-partner of the Computer Teacher, they were currently seeking a divorce and share two children.

- The night before the murder the Principal told police that he had gone for a run after at 5:45 pm returning to the school at around 7:00pm to shower before heading home to his family at around 8:00pm.
- The Principal arrived at school at 7:30am and has been in his office all morning preparing for a meeting with the Board.
- The Principal had a scratch on his neck that was just visible above his collar. When asked about this he said that he had got the scratch a couple of days ago while playing rugby with his sons after school.
- Further examination revealed a cut on the Principals knee that he claimed was also from the rugby practice.

Who is the Killer?

Activity Six: Have the students work in small groups to write a short 'story' of what they think has happened and who the killer is. It can be fun to have them read these out to the class to see how they differ and the ideas that they have come up with.

The following is our version of events – based on the evidence the students should come to the same conclusion of who the killer is but their motives and descriptions will certainly vary.

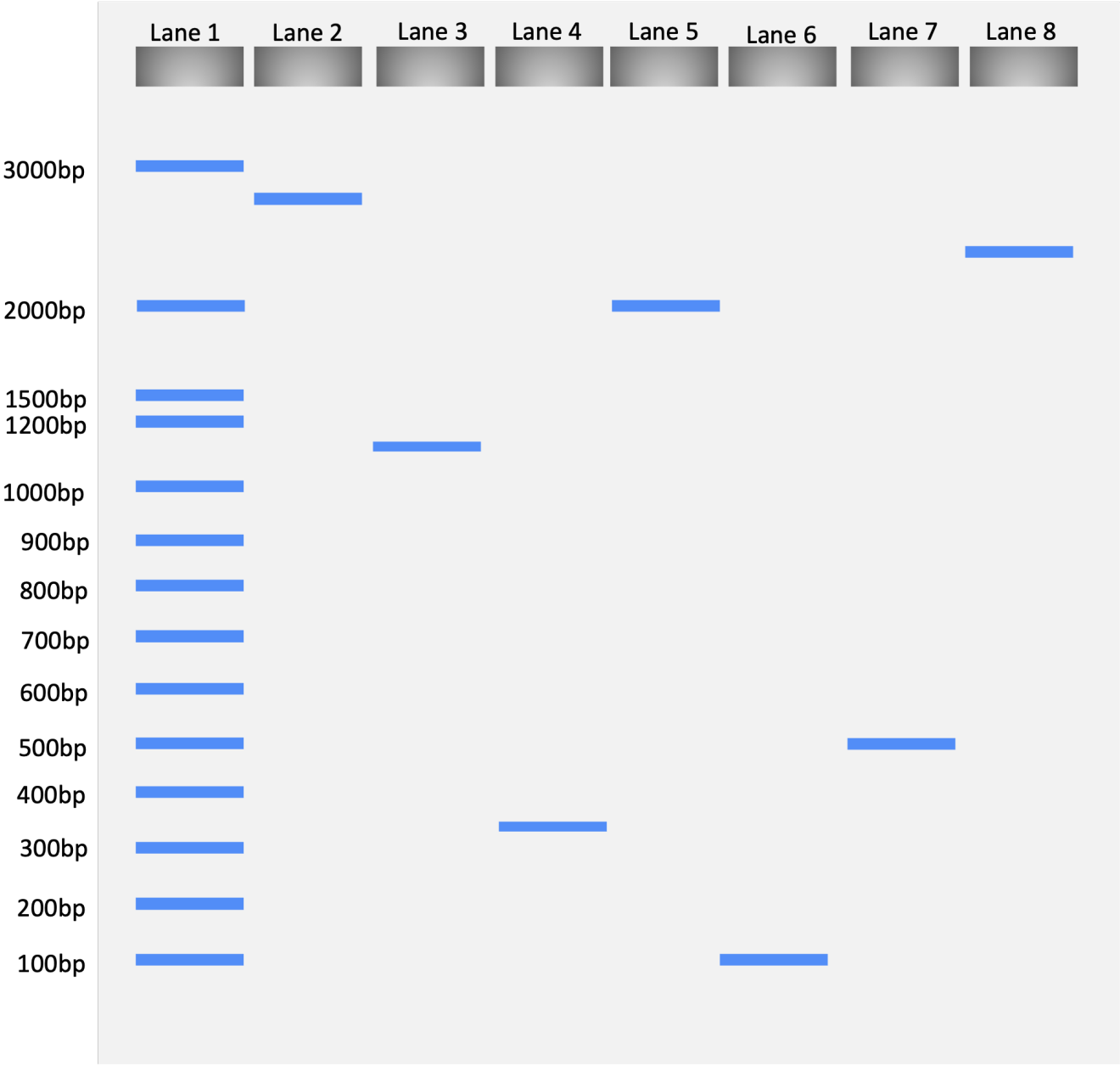
The Security Guard visited the house of his ex-partner, the computer teacher, to drop off their kids at 5:00pm before heading to his new job at the school. As he was leaving his daughter ran to give him a last hug knocking him off balance and into the coat rack, on which the Computer Teachers scarf was hanging – leaving the hair that was found by the police.

The Principal went to have a chat with the Security Guard before he went home for the night at around 6:45pm. He discovered that the Security Guard employed by the school Board was in fact the computer thief himself and he was in the act of bundling up computers to steal from the room.

An altercation ensued with the Principal attempting to stop the burglary. The Security Guard upended tables in an attempt to escape, throwing a computer to the floor and shattering the screen. The Principal dove to catch him and landed on the glass cutting his knee. A physical struggle occurred with the Security Guard resulting in him scratching the Principals neck and then pinning him down. In a desperate attempt to get free of the Security Guard the Principal managed to reach for a nearby keyboard and hit the Security Guard on the head with it. The Security Guard slumped to the ground bleeding from the head and not breathing. The Principal panicked and fled the scene.

Understanding Electrophoresis

Put the bands in order from biggest to smallest and estimate their size using the DNA Ladder in Lane 1.

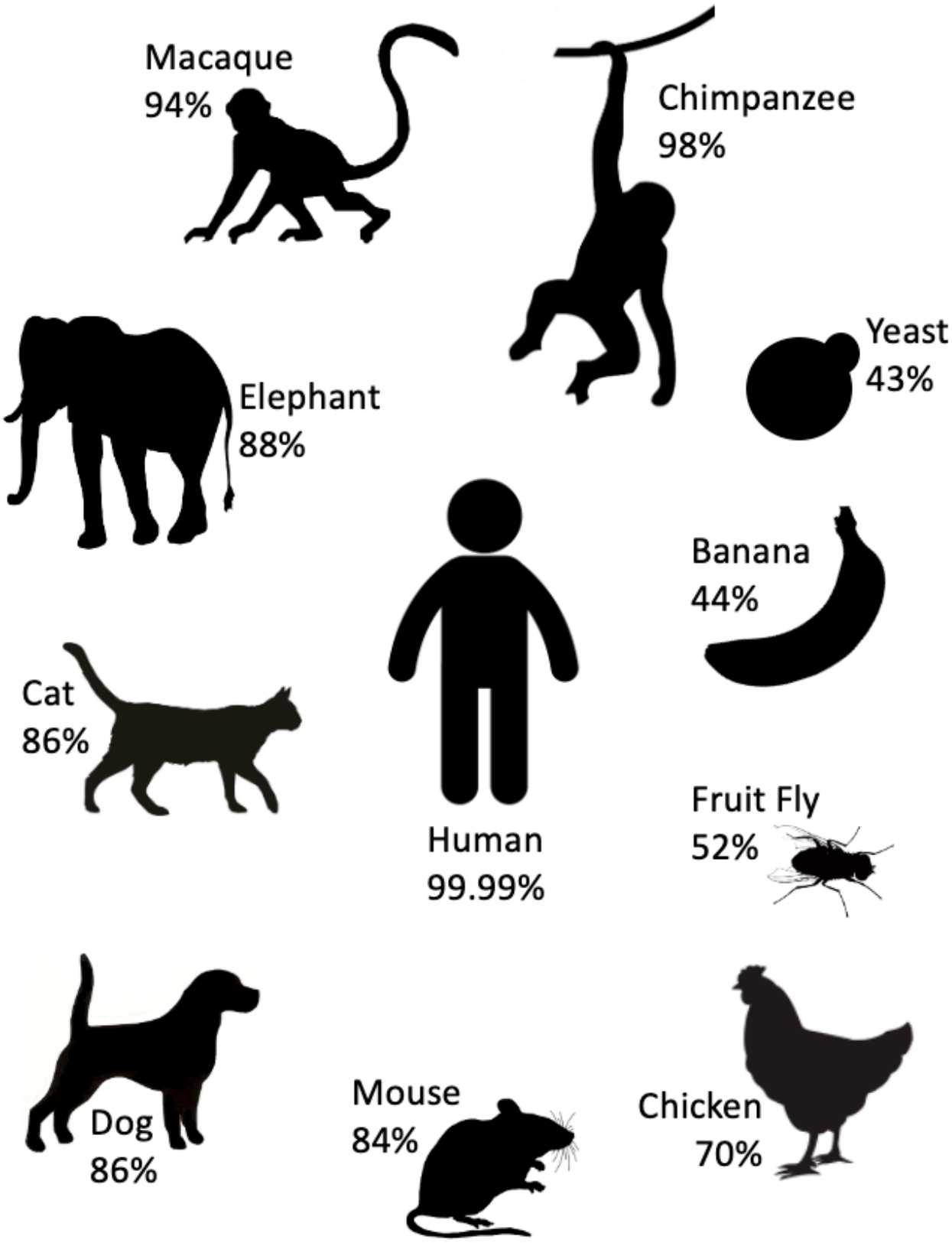


1. Lane _____ Size _____
2. Lane _____ Size _____
3. Lane _____ Size _____
4. Lane _____ Size _____
5. Lane _____ Size _____
6. Lane _____ Size _____
7. Lane _____ Size _____

Tip: Use a ruler to draw a real or imaginary line from the band to the ladder to estimate the size.

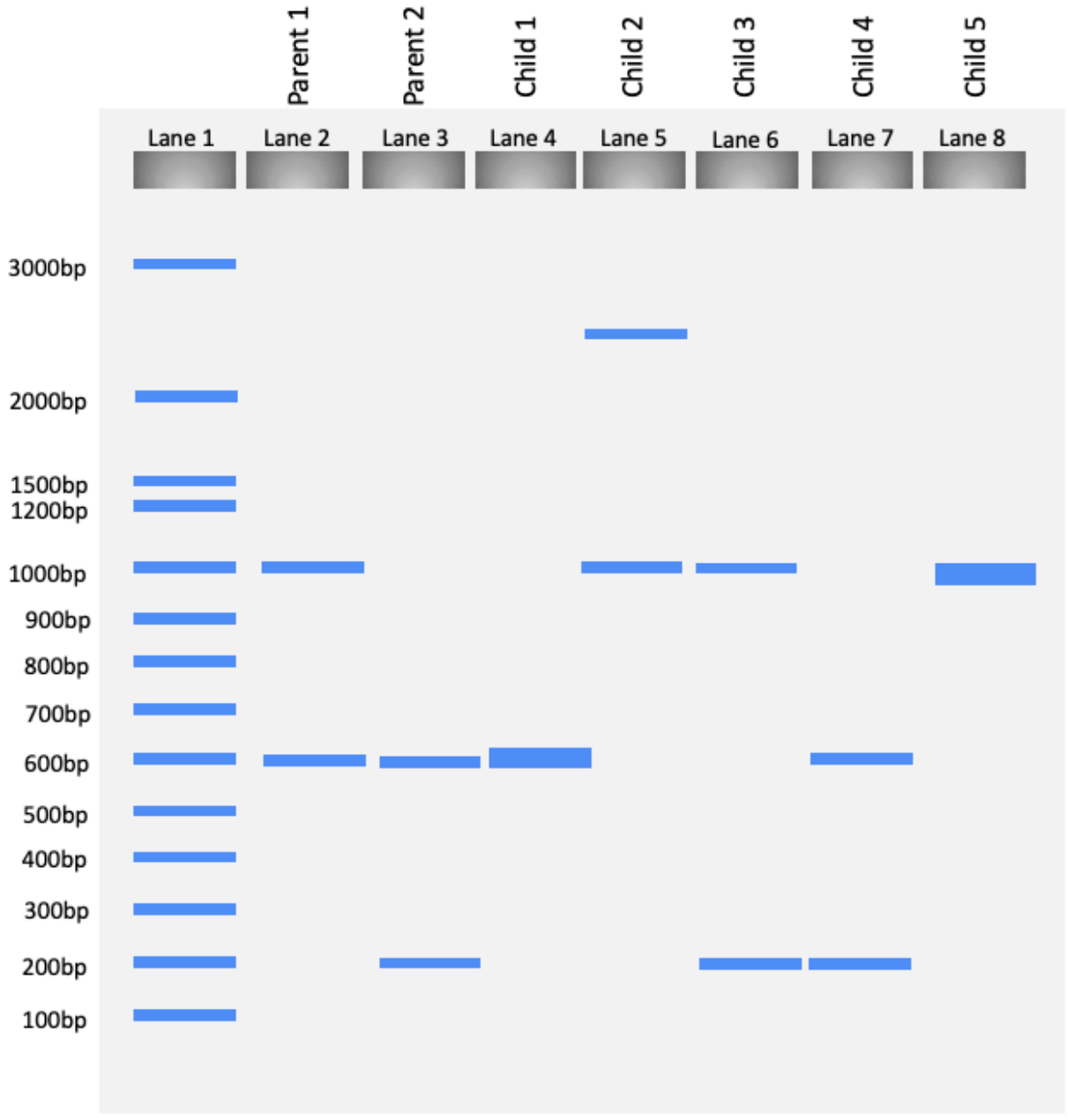
DNA Conservation

Percentage of DNA different species share with humans



Find the Odd Ones Out

A Department of Conservation worker is interested in knowing the genetic lineage of five young Kea in order to avoid inbreeding in a breeding program that is being set up. Can you identify which two Kea are not the offspring of the parents shown.



_____ and _____ are not the offspring of parents 1 and 2.

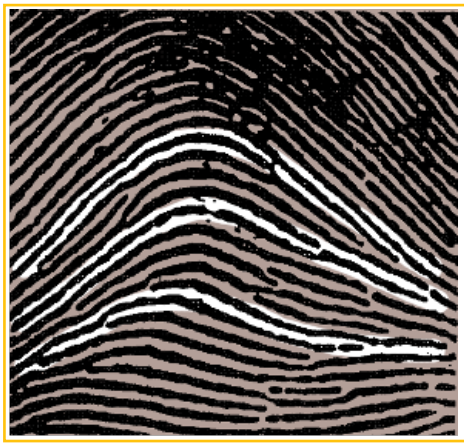
Fingerprint Types

On our fingers our fingerprints are made up of friction ridges (the raised parts) with furrows (recessed parts) in between. Most commonly when you look at a fingerprint image what you are seeing is the friction ridges in ink with the furrows as empty space.

The friction ridges have three distinct pattern types – loops, whorls and arches. These can all have unique variation in different individuals depending on the specific shape and spacing of the ridges.

Loops - prints that recurve back on themselves to form a loop shape. Divided into radial loops (pointing toward the radius bone, or thumb) and ulnar loops (pointing toward the ulna bone, or pinky), loops account for approximately 60 percent of pattern types.

Whorls - form circular or spiral patterns, like tiny whirlpools. There are four groups of whorls: plain (concentric circles), central pocket loop (a loop with a whorl at the end), double loop (two loops that create an S-like pattern) and accidental loop (irregular shaped). Whorls make up about 35 percent of pattern types.



Arches - create a wave-like pattern and include plain arches and tented arches. Tented arches rise to a sharper point than plain arches. Arches make up about five percent of all pattern types.

Images and descriptions sourced from: http://www.forensicsciencesimplified.org

Fingerprint Analysis

Compare the file fingerprints for the Principal, Computer Teacher, Grounds Keeper and Security Guard. Can you identify as the killer at this point?

Security Guard (Victim)

Thumb | Index | Middle | Ring | Little

Computer Teacher

Thumb | Index | Middle | Ring | Little

Grounds Keeper

Thumb | Index | Middle | Ring | Little

Principal

Thumb | Index | Middle | Ring | Little

Door handle

Keyboard

Surfaces

1. Whose fingerprints are on the door handle?

_____

2. Whose fingerprints are on the keyboard?

_____

3. Whose fingerprints are on the surfaces?

_____

4. Can any of these people be eliminated as suspects?

_____

Identifying the Killer

Using the DNA evidence answer the following questions:

Matching the DNA

1. Whose DNA was on the keyboard?

_____

2. Whose DNA was found under the victim's fingernails?

_____

3. Whose DNA was on the shards of glass on the floor?

_____

4. Who did the hair belong to?

_____

Interpreting the Evidence

5. Who can be excluded as a suspect based on the fingerprint and DNA analyses?

_____

6. Who is the main suspect at this point?

_____

7. Why can the third person not be completely ruled out at this point?

_____

Koha

While these kits will remain free for schools in need, we ask that you please consider a koha of whatever your school can afford to help in the continued development and upkeep of these ever-popular kits. Each kit costs us $50 - $120, depending on class size and location. Our resources have been used by more than 1500 students, from Invercargill to Whangarei, annually over the past few years.

You can donate by simply visiting:
https://alumni.otago.ac.nz/donate/genetics-otago-on-the-go

Thank you!

Feedback

We hope you have enjoyed this resource.

Feedback is very welcome to:
go@otago.ac.nz