TEACHING OUTREACH RESOURCE

Who Attacked the Kiwi?

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SAPERE AUDE
Te Whare Wānanga o Otāgo
NEW ZEALAND

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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, would like to organise an onsite teaching session on one of our topics or would like to book the use of one of our electrophoresis kits please contact us at go@otago.ac.nz.

Contents

Overview

Scientists, such as Dianne Gleeson at Landcare research, use forensic science techniques, including DNA profiling, to help catch wildlife criminals. In this resource we provide background information, instructions and worksheets that follow one such case. Students will be given the opportunity to work through a series of paper exercises to identify the attacker of a kiwi. This paper exercise can be followed up with a practical experiment using gel electrophoresis to confirm the results (electrophoresis kits are available on request).

OBJECTIVES

- Successfully follow a scenario through a logical set of steps to reach an informed conclusion.
- Understand that more than one piece of evidence is often necessary to identify a culprit of a crime.
- Understand that different methods of identification can be used in forensic conservation.
- Understand that DNA is unique to a species (and an individual in the extension exercise).

SECTIONS

Part A: Who has been there?

- Exercise One – Tracking Large Animal Footprints (**Exhibits A and B**)
- Exercise Two – Tracking Small Animal Footprints (**Exhibit C**)
- Exercise Three – Hair Samples (**Exhibit D**)
- Exercise Four – Post Mortem Results **(Vetschool Post-mortem Results)**

Part B: Who attacked the kiwi?

- Exercise Five – DNA Extraction
- Exercise Six – DNA Sequence Analysis **(Exhibit E)**
- Extension Exercise **(Extension Exercise)**

CURRICULUM LINKS

This module is designed for year 10 students, to introduce science concepts in a fun and engaging manner, however it may still be a fun activity for older students. In particular it is designed to feed into the following curriculum areas at level 5/6:

- **Nature of science**
  - *Understanding about science* – working in groups, working with current scientific theories and collecting evidence and developing a logical argument.
  - *Investigating in science* – carrying out investigations, working scientifically with multiple variables and evaluating the suitability of investigative methods.
  - *Communicating in science* – Using science vocabulary and relating science understanding to scientific texts.
  - *Participating and contributing* – develop an understanding of socio-scientific issues and draw evidence based conclusions.
- **Living World**
  - *Life processes* – Investigate environmental factors that affect key life processes in animals.
  - *Evolution* – Explore the importance and implications of genetic variation,
  - *Ecology* – investigate the impact of natural events and human actions on a NZ ecosystem

Background

A kiwi has been found harmed in a Northland bush. The Department of Conservation rangers have found the kiwi close to it's den and estimate that it was injured not more than 12 hours ago. The kiwi has been sent for a vet examination at Massey University.

The DoC ranger has been able to gather the following information from nearby:

From the surrounding area:
1. Animal tracks in muddy soil (exhibits A and B)
2. Animal tracks through the Black Trakka system (exhibit C)
3. Hair samples (exhibits D)

From the dead kiwi:
4. Saliva samples from the wounds on the kiwi (exhibit E)

PART A

Who Has Been There?

Exercise One

TRACKING LARGE ANIMAL FOOTPRINTS

The DoC workers have been lucky; it rained heavily in the area three days ago but has been fine ever since. Therefore, the ground has been muddy for three days but any footprints have not been washed away.

Activity: Students are to complete the worksheet, **Exhibit A**. Using their skills of observation they are to match up the muddy prints with the likely object that formed them.

When questioned, the DoC ranger said that before discovering the kiwi they had been walking around the area. Their boots are provided for comparison as **Exhibit B**.

Activity: What can the students deduce from the muddy prints found around the area? Who has been walking in the area and in what time frame?

ANSWERS

A – Dog, Labrador, B – Gumboot, C – Hiking boot, D – Dog, Fox Terrier

Tracks from two people and two dogs have been found. It is likely that the hiking boot prints found in the mud are from the rangers boots as the sole pattern matches. However, due to the lack of definition in the print this cannot be proved definitively, it is possible that someone else wearing a similar pair of boots has also been in the area. The Prints are all likely to have been made in the last three days after the heavy rain.

Exercise Two

TRACKING SMALL ANIMAL FOOTPRINTS

The Black Trakka is a NZ invention (full credit to Warren Agnew) that consists of a small tunnel that contains a sheet of black inked card, followed by a sheet of white card and some bait (usually peanut butter). Small animals such as rats, mice and stoats walk into the tunnel attracted by the bait, they walk over the dye then leave tracks on the white card. This information can be used by DoC workers to determine what species are present in an area of bush.

Activity: Use the poster (**Guide to Animal Tracks**) provided to learn more about different animal tracks. Students should draw up a table that summarises the following features for each animal:

- Approximate footprint size
- Description of footprint shape
- Description of footprint components
- Description of footprint patterns (where applicable)

Activity: Then, students use the information they have prepared and the posters provided to complete the tracking card exercise (**Exhibit C**). Students need to try and identify each of the tracks found on the sample tracking cards.

The final card in **Exhibit C** was the card recovered from the area around the kiwi den. The card was last changed 48 hours ago. What species does this suggest were present and what does this information tell us?

ANSWERS

Card A: Ferret, Card B: Rat, Card C: Stoat.

The final card shows overlaid prints from Possum and Stoat (students could list the possibility of a rat as well). This tells us that both possums and stoats were definitely in the area in the past 48 hours and rats may have been. Whether they were present during the past 12 hours is not known.

Exercise Three

HAIR SAMPLES

Activity: Using the poster provided (**Guide to Mammal Hair**) students should compare the hair samples provided as **Exhibit D**. This worksheet is best printed or displayed in colour.

What species do they think the hair might have come from and what other details can they provide?

If you have access to a microscope, slides of these hair samples are available from Genetics Otago on request (go@otago.ac.nz).

ANSWERS

From top to bottom:

This hair is likely to be from a stoat. The unmagnified sample is from a stoat tail and the medulla is clearly a lattice formation, consistent with animals such as stoats, rats and possum. It is possible that this could be a rat or possum sample, this level of detail is not conclusive but the students will likely match it to the magnified image at the bottom of the hair identification poster which shows a stoat hair.

This hair is likely to be human hair. There is no clear medulla and the unmagnified sample is consistent with the texture and form of human hair.

This hair is likely to be dog hair, from a dog with a light coloured coat. The medulla closely matches the example given in the poster and the unmagnified image supports this conclusion.

This hair is likely to be dog hair, from a dog with a dark coloured coat. The medulla is not obviously matched to the samples in the poster but the medulla is too wide to be from human hair. This combined with the unmagnified view suggests dog hair, although further evidence could disprove this theory.

Exercise Four

VET EXAMINATION RESULTS

At the end of this analysis it is possible to confirm that the following species have been in the area where the dead kiwi was found:

- Two different dogs, one with dark hair and one with light hair
- Two different people, one perhaps is the DoC ranger
- At least one possum
- At least one stoat

Activity: Compare these findings to the details from the **Vet Examination Results**. What can you conclude so far?

ANSWERS

This information, in combination with the post-mortem results, suggest that the dogs and the stoat are the most likely candidate for attacking the kiwi.

PART B

Who Attacked the Kiwi?

Exercise Five

We now have a list of main suspects, two breeds of dog and a stoat. However, it is still possible that a possum or rat is responsible as there is no firm evidence to rule them out.

DNA EXTRACTION

Sample swabs have been taken from the area around the wounds. These samples will contain both kiwi DNA and the DNA from whoever attacked the bird. Before analysis, the DNA must be isolated from the rest of the material.

Activity: For the current module this step has been done for you but students can practice this technique using the **'DNA Extraction'** resource which demonstrates the process in fruit. This is a very crude representation of a DNA extraction but will allow the students to see the DNA and understand the steps required to extract DNA from cells.

There are a number of methods available for extracting DNA but they all rely on the following basic steps:

1. **Break open the cell membranes to expose the DNA (cell lysis).** This is generally a mechanical step, involving breaking or grinding the sample. If the sample is a liquid (such as saliva) this may not be necessary.
2. **Remove the cellular membranes from the sample.** This is achieved with a detergent of some kind that destroys the fatty cellular membranes.
3. **Remove the cellular proteins from the sample.** This is achieved with a protease, an enzyme that targets and destroys proteins. Some extraction protocols miss this step, which means the final product isn't as clean.
4. **Remove the RNA from the sample.** Because DNA and RNA are closely related it is best to remove all the RNA from the sample using an RNase enzyme, again some protocols skip this step, which means that the DNA is not well suited for other downstream applications such as PCR or sequencing.
5. **Precipitate the DNA with alcohol.** DNA is not soluble in alcohol and will precipitate to form white strands. In this step the alcohol is often layered on carefully to form a separate layer to the water-based cell mixture. This allows for easy identification of the DNA.

Exercise Six

DNA SEQUENCING

Once the DNA from the saliva samples has been extracted the sample will be amplified using PCR (PCR is beyond the scope of this lesson plan but easy to use experimental kits are available for those that would like to explore this technique). The amplified sample is then sequenced and compared to known marker sequences for different species, breeds or individual animals depending on what the investigators need to know.

The way DNA is analysed after extraction has changed dramatically over the past few years. Initially DNA samples were cut into chunks with restriction enzymes and run out on an agarose gel. The bands were then compared from one sample to another to look for matches. This is the basis of DNA fingerprinting (again if you would like to try this, get in touch we have kit that can help you).

However, technology has developed to the point now where sequencing using high-throughout machines and fluorescent dyes is becoming the standard technique. This allows much greater accuracy, it's faster and is cheaper than the older DNA fingerprinting technique.

To analyse which animal has attacked the kiwi the amplified DNA sample is tagged with markers and run through a bench-top sequencer. In less than 4 hours a DNA sequence will be generated. Only a small region of DNA is amplified and sequenced but this region is very carefully selected to ensure that it contains a DNA sequence that is unique across species or breeds. By comparing the sample sequence to a reference sequence, investigators can determine who left behind the saliva.

DNA SEQUENCE ANALYSIS

Activity: Students should complete the worksheet **Exhibit E** (please note that this worksheet needs to be provided in colour) to determine which animal left the saliva on the kiwi.

In a sequencing reaction, each base shows up as a peak in a different colour. All the students need to do is read (from left to right) the colours of the bases and record them in the table provided.

Once the sample sequence has been determined, students compare the sample sequence to the reference sequences to find the *best match*. Due to mutations the match may not be 100% but they are looking for the best match from the options.

ANSWERS

The full DNA sequence of the sample DNA should read:

A	A	G	T	C	C	T	G	G	A	C	T
Start …											
…	C	C	C	A	G	A	A	T	T	C	A
…	G	G	G	T	T	T	G	T	G	A	.
											… End

This sequence is most closely aligned with the reference sequence for the dog. From this analysis students can determine with reasonable accuracy that a dog was the culprit. This is consistent with the rest of the evidence that has been found at the scene.

Extension Exercise

WHICH DOG?

An extension exercise is provided whereby students can go on to determine which of the three possible dogs is likely to have been the attacker.

Do not give out the extension worksheet until students have completed the sequence analysis otherwise you will give away the answer! If you are running the practical experiment do this exercise after the gel electrophoresis to confirm the results.

Activity: Students should complete the worksheet **Extension Exercise** (again this worksheet needs to be provided in colour) to determine which of three dogs left the saliva on the kiwi.

ANSWERS

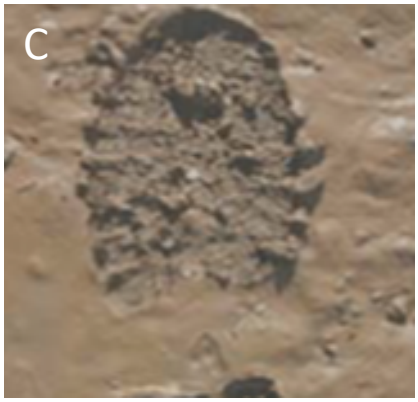
At the end of the exercise students will be able to identify dog C, the Jack Russell terrier as the likely culprit. In reality it is not common for DNA analysis to be used to uniquely identify one culprit within wildlife forensics. Often getting species or breed details is sufficient. However, this analysis is completely within the realms of possibility and can be undertaken if time and budget permit.

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 dog is the culprit. For this exercise we usually 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 go@otago.ac.nz.

Exhibit A

Match the footprints in the mud to the item that made them



Labrador

Fox Terrier

Gumboot

Hiking Boot

A _____

B _____

C _____

D _____

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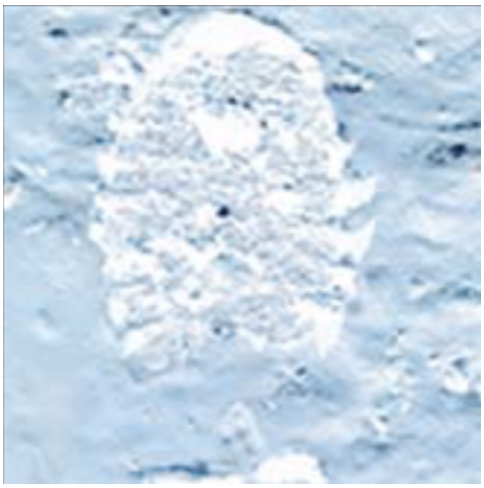
Exhibit B

Samples provided by the Department of Conservation (DoC) worker, of the boots they were wearing on the day they discovered the kiwi.

Photo of boots supplied by DoC ranger. Testified as being the shoes worn on the day the kiwi was discovered.



Colour inverted image of photo taken from area around kiwi den.

Ink print test taken from the bottom of DoC rangers boots.

What does this mean for the prints that you have described in the analysis of Exhibit A?

_____

_____

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Exhibit C

Tracking cards are used to capture the footprints of small animals. They are made using a long card with an ink section. Animals are lured into a small tunnel containing the tracking card (usually using food) and they walk through the ink and then across the card to get out of the tunnel leaving high definition foot prints behind.

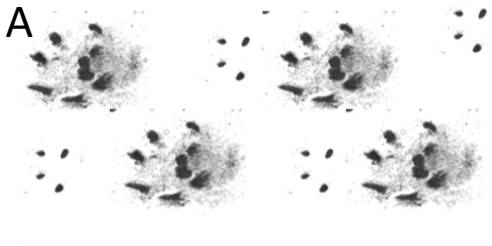
What do you think made each of these tracks?

A _____

B _____

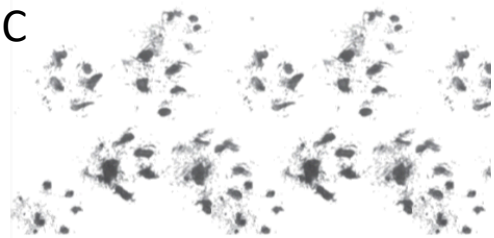
C _____

A

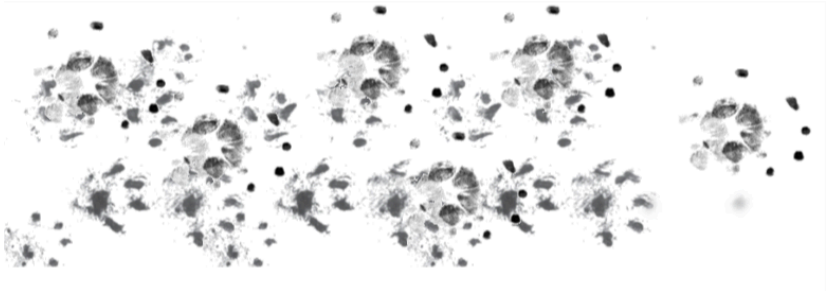


B

C



This tracking card was recovered from the area around the kiwi den



What do you think this means?

_____

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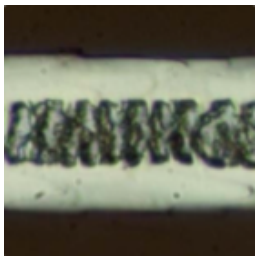
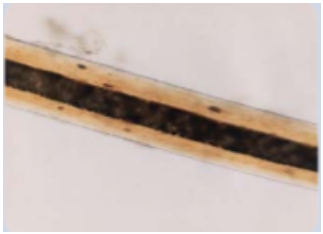
Exhibit D

Below are four hair samples that were recovered from the area around the kiwi den, can you identify the species that most closely matches each of the samples?

NO MAGNIFICATION	40X MAGNIFICATION (Light or fluorescence microscope)	PROBABLE SPECIES
		
		
		
		

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Vetschool

Sample: NZND98392X
Description: Kiwi

Details: Results of the veterinary examination a kiwi found in the Northland region 20/08/11 by Department of Conservation

Initial findings:

Height: 36cm
Weight: 2.76kg
Bill length: 134cm
These features identify the kiwi as a female bird of approximately 2 years old. Size, colouration and feather patterns are consistent with it being a Northland Brown Kiwi (*Apteryx mantelli*)

Detailed external examination:

The kiwi has been attacked and shows many wounds to its body. Several of these wounds are puncture wounds made by sharp teeth consistent with a range of species from rat through to dog. The wounds sustained were largely superficial and did not penetrate the deep tissue layers. Crushing of the main body cavity was detected.

Detailed internal examination:

Internal bleeding was obvious throughout the main body cavity, this is consistent with a crushing injury. The bird was in good health with no obvious signs of disease. Heart, lungs and liver were all found to be in good condition, although all organs had suffered crush damage. All biochemistry and toxicology screens were clear. No evidence of ingestion of a foreign body was found.

Cause of Injury

From these results it is concluded that the bird was injured in an animal attack. The attack resulted in numerous wounds, including crushing of the body leading to internal bleeding which was stopped during surgery in our clinic.

Time of Attack

Time of attack markers are consistent with the reports from the DoC ranger with ToA ranging from 10 to 14 hours before discovery.

This report was prepared independently by Dr John Swindon, BVSc. ICVM, QMP, IPMS.

Signed:

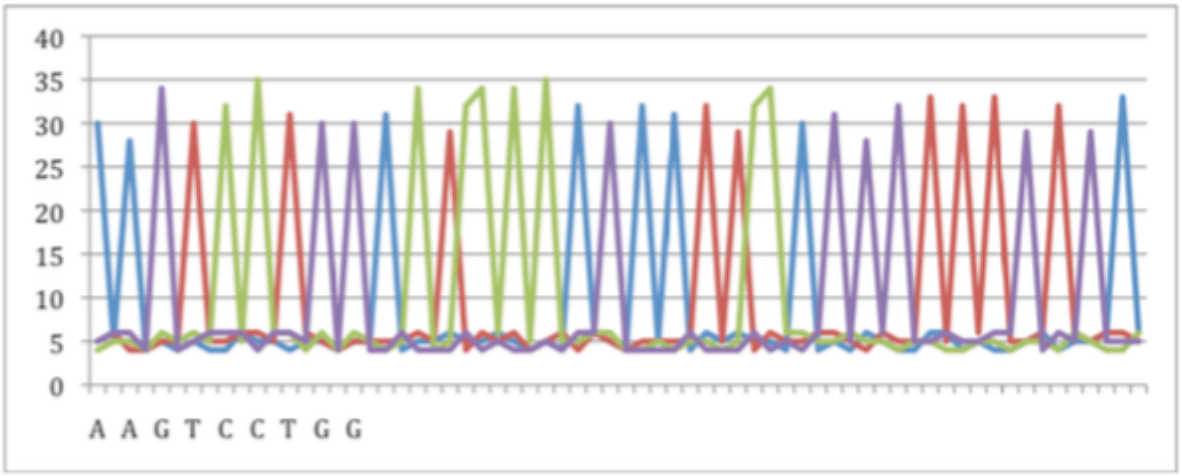
Date: 3/9/11

Exhibit E

Using DNA technology, investigators have extracted, amplified and sequenced a small region of DNA found on the kiwi. You need to compare the sample DNA to the reference samples to see which species of animal you think is most likely to have attacked the kiwi.

SAMPLE SEQUENCE

Complete the table below using the sequence data given. A blue peak means there is an Adenosine (A) at that position; purple means Guanine (G); red means Thymine (T) and green means Cytosine (C). The size of the peak is not important, only the colour. Read the sequence from left to right, the first nine bases have been done for you.



A	A	G	T	C	C	T	G	G			
Start …											
…											
…											.
											… End

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Copy your sample sequence to the table below and compare the sample sequence with the reference sequence for the kiwi.

Sample	A	A	G	T	C	C	G	G								
Kiwi	A	A	G	C	A	T	C	G	A	C	G	C	A	A	G	...

Sample																
Kiwi	G	C	A	G	G	A	A	G	T	C	T	T	A	A	C	G	A

Is the sample from the attacker or from the kiwi?

_____

Now compare the sample sequence with the reference sequences shown below.

Sample	A	A	G	T	C	C	G	G								**...**
Human	A	A	G	T	A	G	C	G	A	C	T	C	T	A	G	...
Stoat	A	A	G	T	C	C	C	T	A	T	T	C	C	C	A	...
Dog	A	A	G	T	C	C	G	G	A	C	T	C	C	C	A	...
Cat	A	A	T	A	T	G	C	G	C	A	A	T	C	G	A	...
Rat	A	A	G	T	C	C	C	G	A	C	G	G	C	A	T	...
Possum	A	A	G	T	A	A	A	C	G	A	T	C	C	C	A	...

Sample																
Human	G	A	A	T	T	C	A	G	G	C	T	T	G	A	T	G	A
Stoat	G	A	A	G	G	G	A	G	A	T	T	C	C	C	A	C	C
Dog	G	A	A	T	T	C	A	G	G	G	T	C	T	G	T	G	A
Cat	T	C	G	T	A	C	A	G	T	A	T	A	A	G	T	G	A
Rat	T	A	C	T	T	C	A	G	G	G	T	T	T	T	G	A	C
Possum	G	A	A	G	C	G	C	G	C	A	A	T	A	C	T	G	A

Which reference sequence is the **closest match** to the sample sequence?

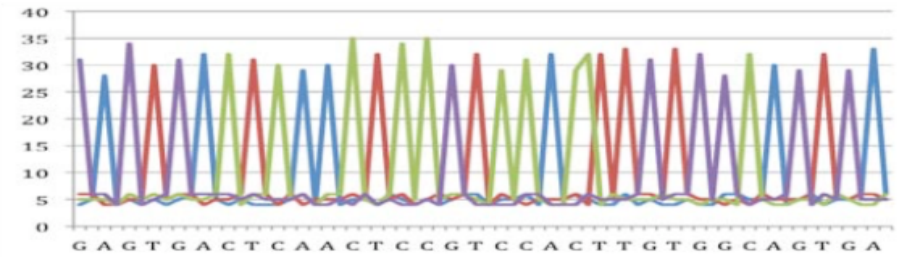
_____

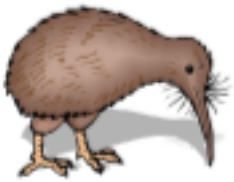
The kiwi is likely to have been attacked by: _____

Extension Exercise

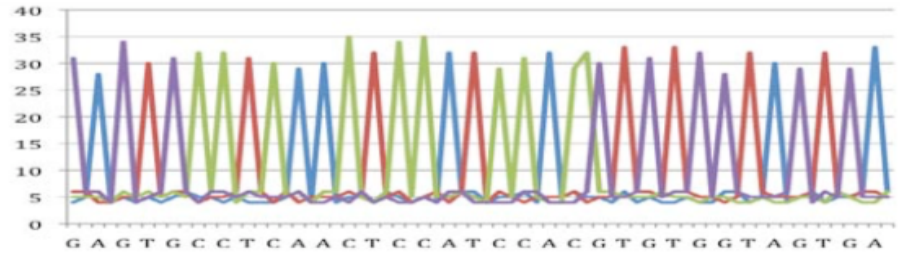
The DoC worker suspects that the dog responsible is one of three possible candidates. The investigators have taken samples from each dog and need to compare them against the sample taken from the kiwi. A DNA sequence which shows differences between individuals of the same species was used. It was extracted, amplified and sequenced in the same way as the previous sample but used different primers so that the PCR reaction "copied" this different section of the DNA. The results are as follows:

Kiwi DNA Sequence

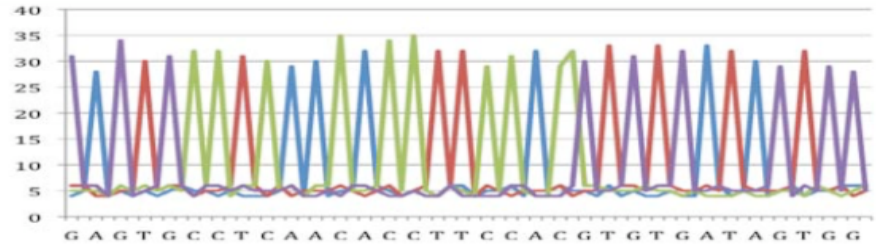




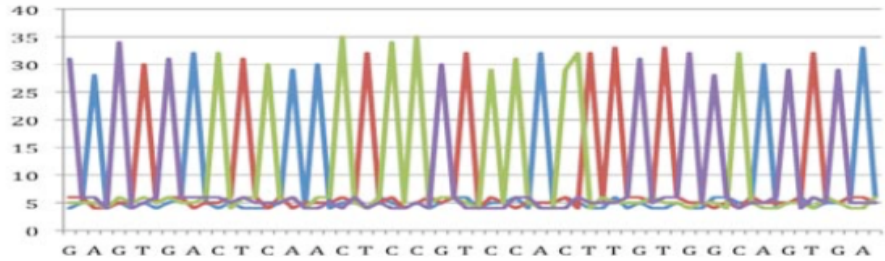
Dog A Sequence



Dog B Sequence



Dog C Sequence



Which dog attacked the kiwi? _____

ACKNOWLEDGEMENTS

This lesson plan is based on the work of Warren Agnew of Black Trakka and Dianne Gleeson (and team) at Landcare Research. Where possible we have stuck to the information that they have provided about how these techniques are used in real life wildlife forensics.

We hope you have enjoyed this lesson. Feedback is very welcome to:
go@otago.ac.nz