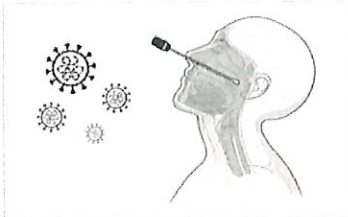


COVID- 22 April

## Coronavirus testing - how does it work?

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In New Zealand, we currently use a 'PCR' test to find out if someone is infected with the new coronavirus, SARS-CoV-2, which causes the disease called Covid-19.'

The PCR test is so precise that it can tell the difference between the seven coronaviruses that are known to cause diseases in humans.

### This virus:

### causes this disease:

SARS-CoV	SARS
SARS-CoV-2	Covid-19
MERS-CoV	MERS
HCoV-OC43	Common cold
HCoV-HKU1	Common cold
HCoV-229E	Common cold
HCoV-NL63	Common cold

The PCR test looks for the genetic material of the virus in a swab sample taken from a person's nose or throat. It involves making lots of copies of a piece of the virus's genetic material so we can see if it is there or not.

PCR is also one of the most important techniques that we use in our research at the Otago Department of Biochemistry.

But how does it actually work?

Here we explain what you do to figure out if someone has the virus or not.

### 1) Take a swab

What is happening with those long sticks up people's noses?



A healthcare worker about to swab someone for a coronavirus test.

To find out if a person has coronavirus, a healthcare worker uses a swab with a long shaft to gently scrape the back of the nasopharynx of that person. The nasopharynx is the upper part of the throat, right behind the nose. Not very comfortable!

This is where your nasopharynx is:

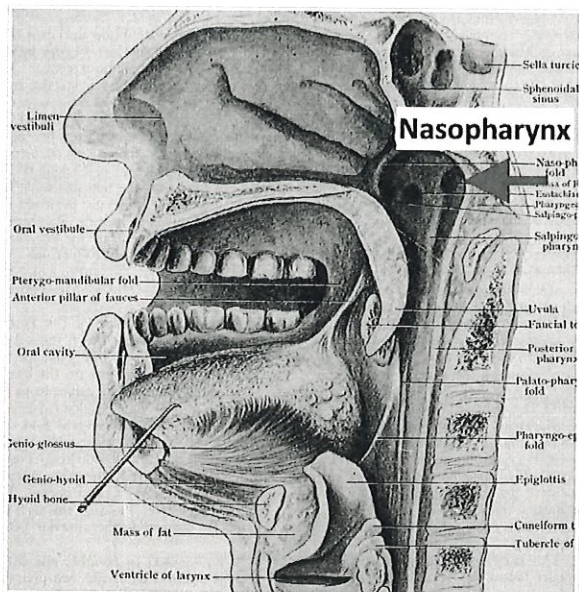


Image from "Human anatomy, including structure and development and practical considerations" (1911) on [www.flickr.com](http://www.flickr.com)

After scraping the nasopharynx, the end of the swab will have some of the person's cells and mucus on it, as well as any bacteria or viruses that were sitting back there.

The swab is quickly put into a tube containing a mix of protein and antibiotics that keeps any collected virus safe, then the tube is sealed and sent to a testing laboratory.

You can see how a swab is taken in this video made by the medical education company AMBOSS.

## COVID-19 Diagnostics: Performing a Nasopharyngeal and xxx



### Problems with swabbing

Usually, if someone is infected with the virus and is showing symptoms, the swab should be able to pick up virus particles from the nasopharynx.

However, sometimes the virus might be multiplying in places away from where the swab is, or there is not yet enough virus around for the swab to pick up.

So a negative test might mean that you don't have coronavirus, or that you have coronavirus and it just isn't detectable yet, or the wrong part was swabbed.

### 2) Send the swab sample to a laboratory

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Hopefully the courier is fast!

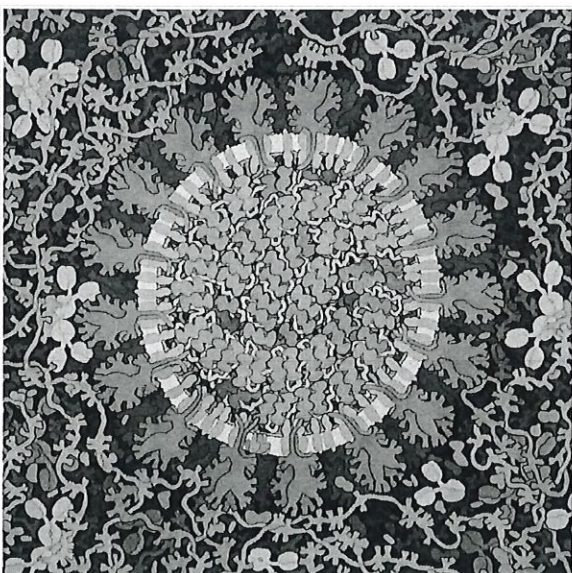
### 3) Get the RNA genetic material out and clean it up

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#### What we're dealing with:

The coronavirus is made up of two main parts: an oily membrane around the outside, studded with proteins that stick out of the surface, and genetic material called RNA on the inside, with more proteins tightly wrapped around it.

Here is a drawing of a coronavirus particle cut in half so you can see the inside. The proteins sticking out of the outside membrane are drawn in pink. Inside you can see the thin, squiggly RNA wrapped up in purple proteins.



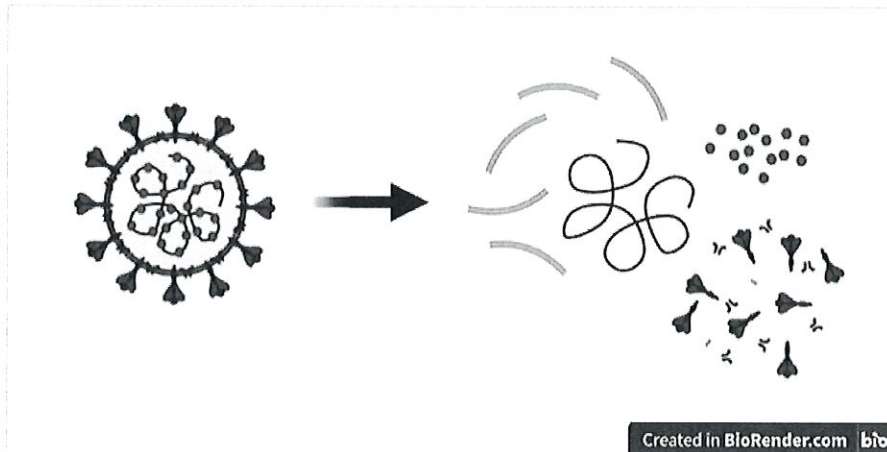
To do the test to check for coronavirus, we have to break open the virus particles to get out the genetic material. We also need to get rid of everything else in the sample that could stop the test from working.

The swab sample will have lots of stuff in it, including mucus and human cells as well as viruses. The human cells are also made up of proteins, membrane, DNA, and RNA.

That means we will need to get rid of the parts of the virus that we don't need for the test (proteins and oily membrane) and everything else in the sample – the proteins, oily membranes and DNA from the mucus and human cells.

So... break up the molecules we don't want...

We break open the virus particles using some chemicals – a detergent and something called a chaotropic salt.

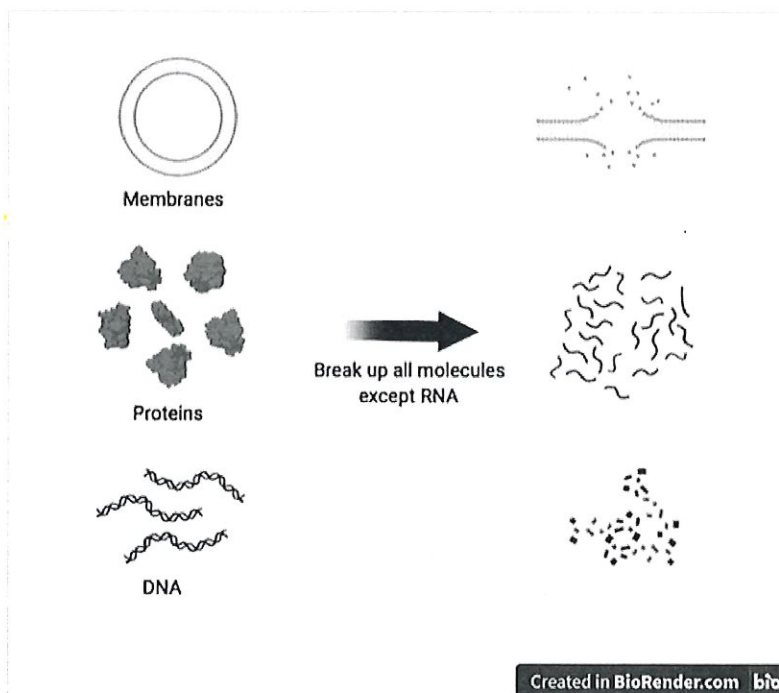


*Breaking the virus into bits releases its RNA (the squiggly line in red).*

The detergent helps to break open the membranes of cells and viruses, which are both made up of oily molecules.

The chaotropic salt has several roles. It denatures, or unravels, the many different proteins in the sample, stopping them from working, and helps separate the RNA from any proteins wrapped around it.

We chop up the proteins in the sample into bits using an enzyme called a protease, and we chop up the DNA in the sample into bits using an enzyme called a DNase.



...Then get rid of everything except the RNA.

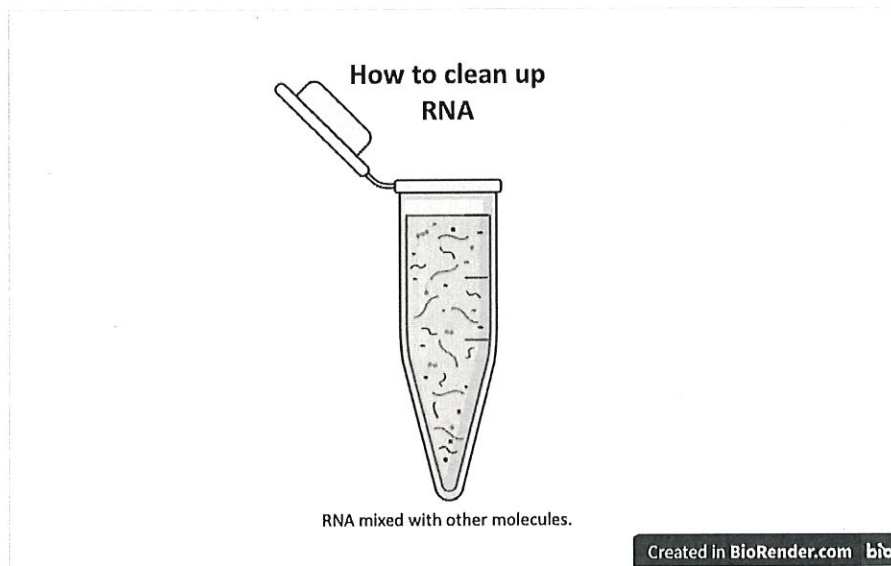
Now we have a tube with lots of different types of broken-up molecules in it, mixed in with RNA. We need to get rid of everything except the RNA.

To make sure we keep the RNA and not wash it away accidentally, we add little silica (glass) magnetic beads. The RNA sticks to the silica beads, helped by the chaotropic salts. Then we put a magnet on the outside of the tube. The beads (and therefore the RNA) stick to the inner wall of the tube, held there by the magnet on the outside.

With the magnet holding the beads and RNA in place, you can easily wash all the remaining bits of unwanted molecules away without losing the RNA. Adding liquid with some more chaotropic salt will help to do this, then we can wash the salt away using some alcohol.

Once the RNA is clean, we can unstick it from the magnetic silica beads simply by washing the beads with water. When the RNA is in the water, and not on the beads anymore, we can get rid of the beads using the magnet again.

This gif shows you the steps we use to clean up the RNA:



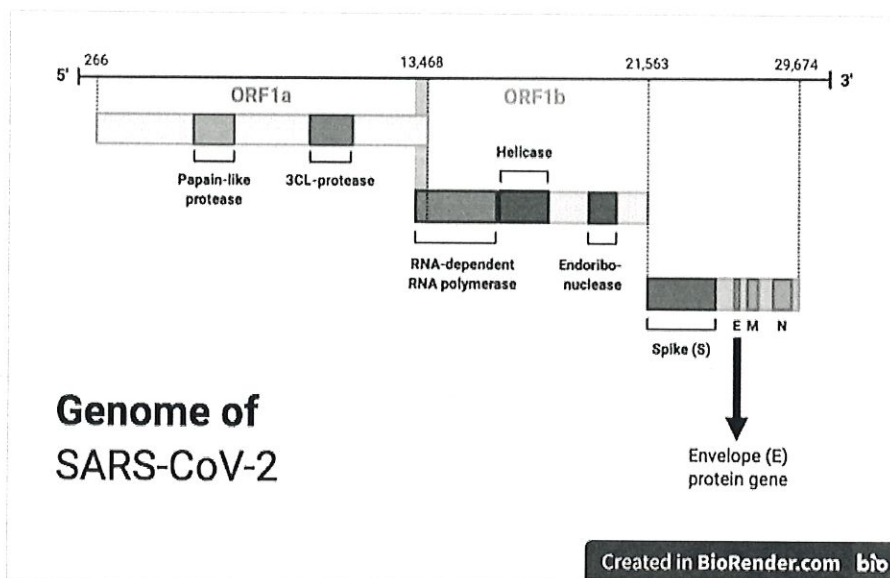
#### 4) Make sure the test will only detect the new coronavirus

The RNA left in your sample will be a mixture of human RNA and RNA from any bacteria or viruses in the nasopharynx of the person who was swabbed.

To make sure we only detect the coronavirus RNA, and not the DNA or RNA of any other organism, we need to find a little bit of the coronavirus RNA sequence that is unique to the coronavirus, and is not shared by any other living thing.

The RNA of the new coronavirus was sequenced by scientists early in the outbreak. There are nearly 30,000 bases (letters) in the coronavirus genome, containing the instructions for making 29 different proteins.

Here is a diagram of that genome:



We need to make two short pieces of DNA ('primers') that will only stick to somewhere on that genome sequence.

Scientists chose two short pieces of the sequence in gene E, 22 and 26 bases long, for the PCR test. The E (envelope) protein encoded by this gene helps to form the oily membrane of the virus.

The scientists made DNA primers that only stick to those pieces of gene E and nowhere else.

(By the way, DNA and RNA do stick together, if they have the right sequences.)

Here is the RNA sequence of the E gene (RNA is made up of G, C, A and **U** bases):

```
AUGUACUCAUUCGUUUCGGAAGAGACAGGUACGUUAAUAGUUAUAGCGUACUUCUUUUUCUUGCUUUCGUGGUAUUCUU
GCUAGUUACACUAGCCAUCCUACUGCGCUUCGAUUGUGUGCGUACUGCUGCAAUAUUGUUAACGUGAGUCUUGUAAAAC
CUUCUUUUUACGUUUAUCUCUGUGUAAAAAUCUGAAUUCUUCUAGAGUCCUGAUCUUCUGGUCUAAACGAACUAAAUA
UUAUAUUAGUUUUUCUGUUUGGAACUUUAAUUUUAGCC
```

And here are the sequences of the two DNA primers used in the test (DNA is made up of G, C, A and **T** bases):

Forward primer: ACAGGTACGTTAATAGTTAATAGCGT

Reverse primer: ATATTGCAGCAGTACGCACACA

The primers will only stick to the E gene on the parts underlined here:

```
AUGUACUCAUUCGUUUCGGAAGAGACAGGUACGUUAAUAGUUAUAGCGUACUUCUUUUUCUUGCUUUCGUGGUAUUCUU
GCUAGUUACACUAGCCAUCCUACUGCGCUUCGAUUGUGUGCGUACUGCUGCAAUAUUGUUAACGUGAGUCUUGUAAAAC
CUUCUUUUUACGUUUAUCUCUGUGUAAAAAUCUGAAUUCUUCUAGAGUCCUGAUCUUCUGGUCUAAACGAACUAAAUA
UUAUAUUAGUUUUUCUGUUUGGAACUUUAAUUUUAGCC
```

This is how the test will only detect the RNA of coronavirus, and not the RNA of anything else.

## 5) If the virus is there, make DNA!

### Time for some RT-PCR

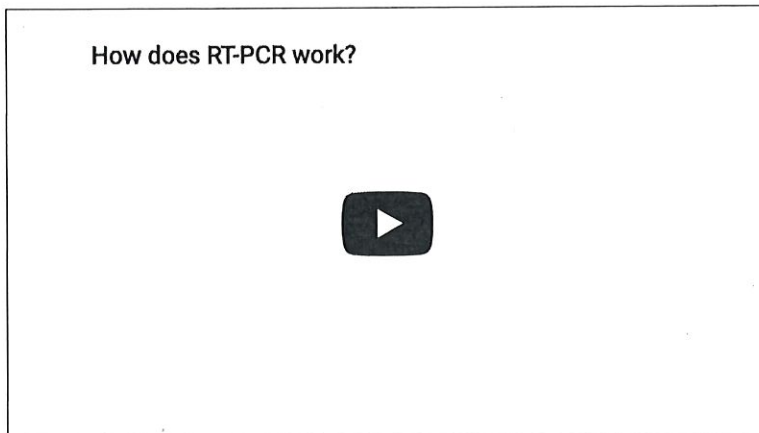
To detect any coronavirus RNA present, we need to make lots and lots of copies of it, but we can't easily make lots of copies of RNA directly.

Therefore, we first have to make a DNA copy of the RNA. This is called **reverse transcription** (RT), which we do using an enzyme called reverse transcriptase.

Once we have the DNA copy, then we can make loads of copies of that using the **polymerase chain reaction** (PCR) and an enzyme called DNA polymerase.

We use the E gene primers in both steps so that only RNA from the virus is copied, and nothing else.

This video shows you how RT-PCR works.



How do we find out if the virus RNA has been successfully copied?

The testing laboratories do 45 "cycles" of PCR on a Covid-19 test sample, which takes a little over half an hour. After this time, a single SARS-CoV-2 RNA molecule will have become 17 million million identical DNA molecules. These are so small that you STILL can't see them with your eyes.

To see how much DNA there is, and to find out if the test is positive, the PCR reaction mix includes a special tag that glows when it is cut up.

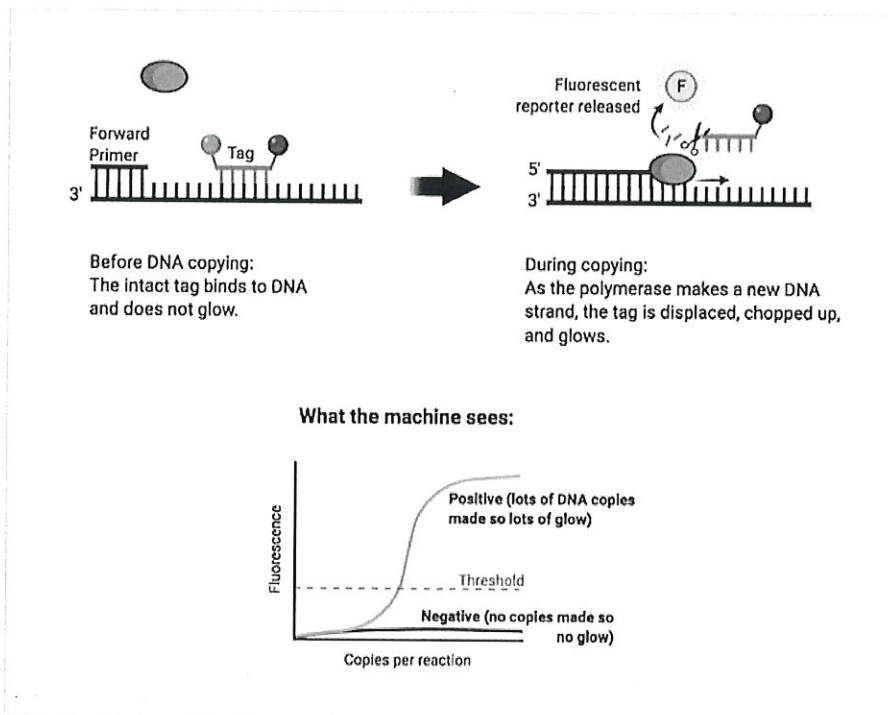
The tag sticks to the same piece of DNA as one of the primers.

When the DNA gets copied, the tag is knocked off the DNA and gets chopped up. The unstuck, chopped-up tag then starts to fluoresce (glow).

These tags can be 'seen' and measured by a machine that detects light. The more fluorescence detected from a PCR reaction, the more DNA copies have been made.

So when there is a lot of glowing, you know that coronavirus RNA was in the swab sample and lots of DNA copies were made, and therefore that the test is positive.

If there is no glowing, there wasn't any coronavirus in the swab sample, no copies of DNA could be made, and the test is negative.



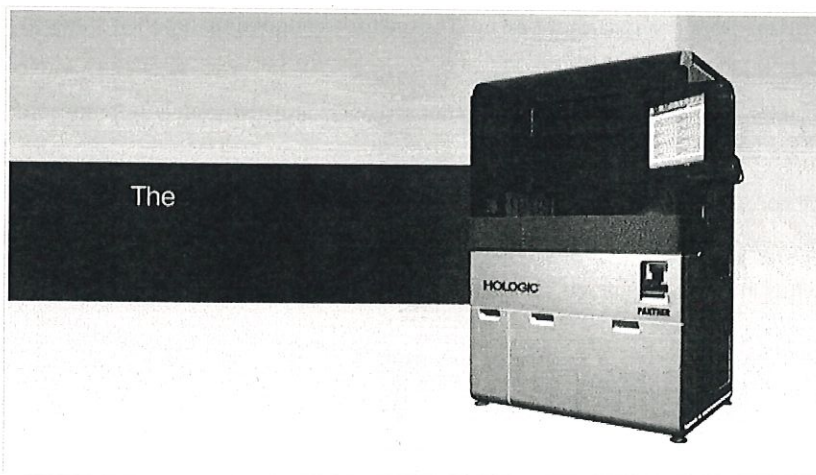
Do many tests all at the same time (Get in the robots!!!)

A really important part of testing for coronavirus is that we can carry out many tests as quickly as possible.

Our testing laboratories do this by getting robots to do the RNA preparation and the reverse transcription PCR.

One person can process maybe 100 samples by hand each day, but a robotic machine, once it has been set up correctly, can carry out as many as 1000 tests in 24 hours, and is not as likely to make mistakes.

This is one of the robot testing machines that we use in New Zealand:



## What happens if we run out of test kits from overseas?

Our friends next door in the Otago Department of Microbiology and Immunology have helped to get this test up and running in New Zealand. They have designed it to work with enzymes and other reagents that we can import from overseas.

But what happens if there are shortages of some of those reagents from overseas, and we can't import them for a while?

Health workers, scientists and the media have highlighted this as a problem that we need to think about. (Read the article on RNZ: Covid-19: Limited testing kits has scientists searching for generic alternative.)

We can make important parts of the test here in New Zealand if we needed to.

This is where the skills and equipment in research institutions like Otago Biochemistry can help out.

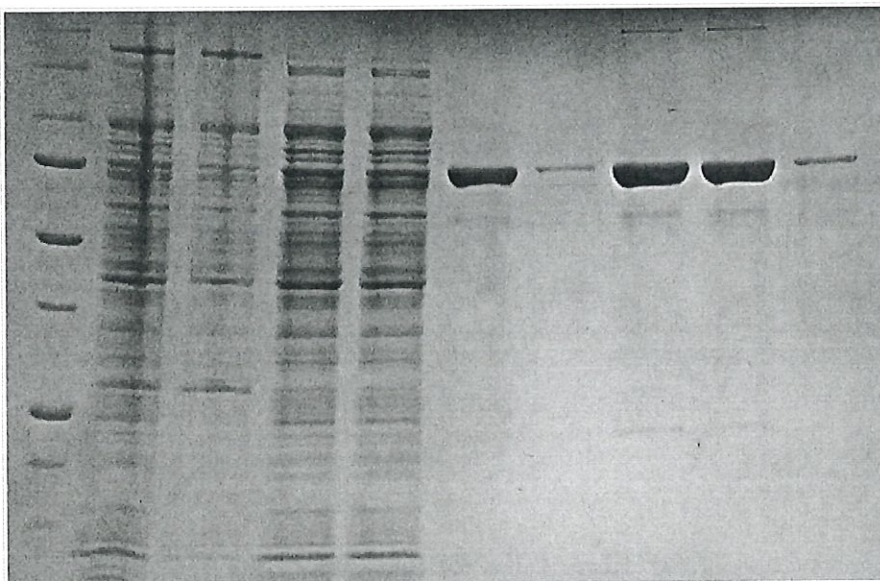


*Sam Jamieson purifies reverse transcriptase enzyme in the Mace lab.*

In Otago Biochemistry we have already been practicing making enzymes just in case. Associate Professor Peter Mace's research group has already made the reverse transcriptase enzyme.

They put the gene encoding the enzyme into some bacteria, got the bacteria to make the enzyme, and then extracted the enzyme out of the bacteria.

Scientists often make proteins in this way so that we can study them and find out how they work.



*A photo of a gel that shows the enzyme at different stages during purification. The big blobs on the right are samples of pure reverse transcriptase enzyme.*

There is an amazing amount of research being carried out on the new coronavirus around the world. We have put together a collection of links to useful websites that explain what the virus looks like, how it works, how to test for it, what treatments and vaccines are being developed, and more on our Useful information about the new coronavirus page.

You can read and watch more stories about research at Otago Biochemistry on our School Resources page.

Contact us with any suggestions to improve or add to this page at [biochemistry@otago.ac.nz](mailto:biochemistry@otago.ac.nz).

