Sequencing DNA with Linux Cores and Nanopores

January 24, 2019

D-N-A: It's in our blood!

It's in our food. It's even in our jeans.

Tēnā kotou i tenei ata.

Ko Kurahaupo te waka
Ko Tapuae-o-uenuku te maunga
Ko Wairau te awa
Ko Rangitāne te iwi
Ko Huataki tōku tupuna
Ko Moa tōku whanau
Ko Rawiri Ekeru tāku ingoa

No Reira
tēnā kotou, tēnā kotou, tēnā kotou katoa

Thank you all for coming to hear me talk about DNA Sequencing with Linux Cores and Nanopores.

My name is David Eccles, and I am a Research fellow in Bioinformatics at the Malaghan Institute of Medical Research in Wellington. Part of my work at the Malaghan Institute involves using genetics to work out how cancer cells interact with mitochondria, but I'm not here to talk about that work today. I'm here to talk about DNA. In other words, this talk is my own, and opinions are my own.

1 The Sequence of a Three-Course Meal

I hope you'll forgive my informality. I've turned up with genes in a T-shirt, and I'm using a crutch of a talk transcript to help me through my anxiety and the talky bits of my demonstration. But there's a silver lining to that: I'll put my talk transcript into the zenodo repository indicated on this slide, and you can visit that afterwards to see all the data that I'll be producing today.

So... anyway, I call myself a theoretical geneticist. What that

practically means is that I spend a lot of time behind computers thinking about DNA, but not much time doing practical experiments.

Today I want to change that a bit. I want to show the Linux enthusiasts what DNA looks like in a few different ways, both as a physical thing, and as a constructed string on my laptop here. Hopefully today will give you a much better idea of what it looks like, both in real life, and in the symbolic life on a computer that we know and love.

For those who haven't read my abstract, what I'm going to do is *attempt* a full workflow of DNA sequencing, from food to figures.

In preparation for this, I'll break oen the seal on this flow cell packet, load it into the sequencer, and get it warmed up for sequencing.

2 Part 1a - DNA Extraction

On this slide are my instructions. What I'm going to *try* to do is extract DNA from some food that I got at a cafe on campus.

I was originally going to try a 3-course meal, but the food at the speakers dinner wasn't quite right for DNA extraction using this method. This here is a chickpea salad.

Because I don't have much time on this stage, I'll be throwing a bit of standard DNA extraction practise out the window. This means there's a chance that things won't work, but I've got a few backup options in the event that it all turns to custard.

Right. Let's get to it.

3 Food Squishing

On the table there, there's a mortar and pestle, and the chickpea salad. I'll put the food into the mortar (that bowl thing), and grind it up with the pestle. What we're trying to do is make some food juice, the more the better.

What I'm is doing is grinding up and breaking open cells in the food. Cells are usually pretty resilient, but I'm hoping that enough will be broken up to get out visible amounts of DNA. DNA is present in every living thing, and most things that were recently living.

Okay, great, that looks *just* like chickpea custard. I'll put in a bit of water and some salt to try to encourage the DNA out of the cells.

4 Straining Credibility

Now also on the table there's a cup with a tea strainer in it. I didn't get a strainer with a small enough mesh for me, so I've put a cut up old sheet in the top of it. The juice should drip out the bottom into the cup.

I'm going to squish it inside the the sheet to see if I can pull out a bit more of the juice.

5 Meth to the Madness

The next step involves some alcohol. I'm going to get kitted up in preparation for that. I'll put on a lab coat, some gloves, and some safety goggles.

I'm going to slide some methylated spirits over the top of the food juice. Wikipedia tells me that denatured ethanol can be used for precipitating nucleic acids, so let's do that and see if it works.

If this does work, you'll start seeing bubbles forming in the upper spirit layer. I'm not quite sure about the chemistry behind it, I just know that bubbles are a good thing.

This forms what is affectionately known in the DNA sequencing world as a DNA jellyfish.

6 Toothpick Fishing

Now it's time to go fishing for DNA. I'm going to take one of those toothpicks and break it into a hook at the end, so it looks like a J shape.

This hook can be used to fish the jellyfish out of the liquid. Once it's out, I'll stir it around in this shallow dish of water. If I've done this right, then the DNA will dissolve in the water, leaving a bit of extra stuff hanging around.

Okay, so that's the DNA extraction and concentration done. I'm going to transfer the liquid from the dish into two tubes, put the tubes on opposite sides of this hand-crank centrifuge here and give it a little spin by turning the stick thingy. Okay, maybe a big spin. What I'm trying to do here is bring the bits that didn't dissolve down to the bottom of the tubes, because the DNA we want has dissolved into the water.

This didn't actually achieve anything when I tried it yesterday, but *today*... it gives me an opportunity to show off some of my 3D printing.

So there we have it: a very faintly whitish liquid, hopefully containing slightly purified DNA.

7 Preparing for Sequencing

Now it's time to start using commercially-produced sequencing kits. This one is from a company called Oxford Nanopore Technologies, and calls itself the rapid barcoding kit. This kit allows me to attach a DNA barcode to a sample, so that I can run two samples at the same time and tease them apart on the computer side of things.

In this case, I prepared some other DNA in a lab last week, and I'll be adding that in together with what we've prepared today, just in case our extraction didn't work. Today's sample will get barcode #1, and last week's mish-mash will get barcode #2.

8 Fragmentation

The first step is to break the DNA up and add the barcodes. For that, I need seven and a half microlitres of each sample. To give some idea of how much that is, an average dripping drop of water is about fifty microlitres, so this is about a seventh of that amount. I then add two and a half microlitres of fragmentation enzyme mix to each tube. Those samples are left for about a minute to stew and think about their life.

9 The Heat is On

Next up, I need a source of heat, something that's about 80 degrees celsius. I borrowed one of the hot water containers from outside, and I think that will do just nicely. They need at least a minute to make sure that the fragmentation enzymes are suitably dead and won't do any funny business later on.

10 Adaptation

Now that the fragmentation mixes are inactivated, I can pool both samples together into one tube. I'll take five microlitres from each and put them into a new tube. I then add one microlitre of rapid adapter, which clicks into to the prepared DNA and gives it the equipment it needs to sequence itself. Putting on that equipment takes about five minutes, so while that's happening, I'll move on to preparing the flow cell for sequencing.

11 The Flow of DNA

This is my DNA sequencing setup. This tiny pocket-sized thing that you see here is a mini DNA sequencer. Inside the sequencer is a consumable device called a flow cell. It's got a few holes inside it. If I rotate this cover, you can see the inlet channel. I can push this flushing buffer through the inlet channel, and it moves over the sequencing matrix, displacing other liquid which leaves the outlet channel.

In a few minutes, I'm going to put the adapted DNA into this device as well by dropping it directly over the flow cell matrix. There's a rubber bung that's not shown in the picture on the slide which allows me to do that. Underneath the flow cell matrix is another reservoir of liquid, but things like DNA will only be encouraged to get there by the application of a very mild electric current. But there's a catch: the way is blocked by a polymer cover (or membrane), and the only way to get through that cover is via nanopores that have been loaded into the polymer membrane. These nanopores are electrically connected to exquisitely sensitive ammeters; electrical current sensors that are able to detect very subtle changes in the chemical structure of the molecules that go through the nanopores. Those picoamp signal levels are sent over the USB3 connection to be decoded by software on the computer.

12 Fuel Throttle

So, let's go back to the prepared sample. Now that there's a bit of attachment that's gone on, I add in some more water, and some fuel mix to allow the DNA to kick into gear. This mixture gets added to loading beads, which hold onto the DNA and help it to sink down to the bottom of the flow cell matrix area, near where the nanopores are.

Now I'll lift up the SpotON bung above the flow cell matrix, and put a little bit more flush buffer into the flow cell. I think this provides a bit of liquid flow to encourage the sample to be drawn down into the matrix area. The sample is then dripped into the SpotON area, hopefully covering the entire sequencing matrix with loading beads. Once that's done, I replace the bung, close the inlet channel cover, and I can breathe a big sigh of relief now that all the messy experimental side of things is done.

It's time to go off script and start sequencing. How much time have we got?

13 Additional Notes

- Last MinKNOW update was last Thursday (17th), to address issues associated with slow basecalling
- `/opt/ONT/MinKNOW/conf/app_conf:`
- `[guppy] 3 threads, 1 runner`