A passing storm or climate change: What competitors think of Oxford Nanopore's technology

The Right Size

Oxford Nanopore Technologies, founded in 2005, have found the holy grail: a device that can deep read DNA in real time, costing less than €1,000 and scalable down to the size of a USB stick. But will their new "Gridion" system, due for release later in 2012, really deliver on its promise? And if it does, what are the consequences for the multi-million dollar sequencing industry and for your humble lab?

It was in Florida that a small British genomic company amazed the sequencing world. In February this year, at the Advances in Genome Biology and Technology Conference (AGBT), Oxford Nanopore Technologies awed the sequencing world with the data that can be generated by their new pore-based technology.

That data included DNA reads of tens of kilobases measured in real-time. Even better, no amplification of the message is necessary – you literally put the sample (which can be any DNA preparation you like) into a cartridge, insert the cartridge into a gadget they call "Gridion" device, and watch the sequencing happen on your laptop.

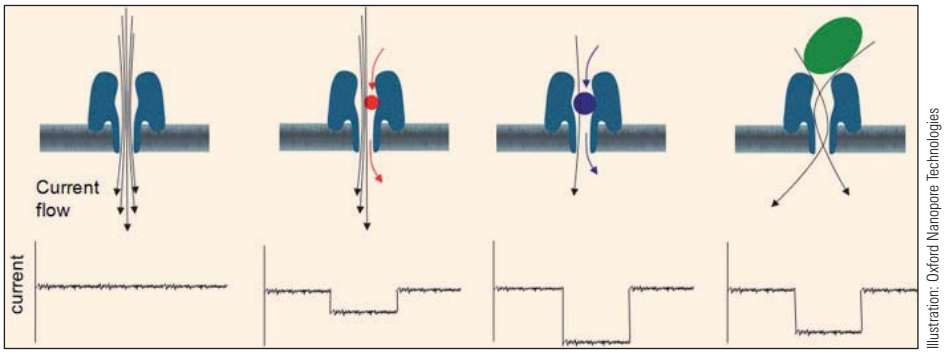

Illustration: Oxford Nanopore Technologies

When molecules (i.e. a strand of DNA) pass through a nanopore that is set under voltage, characteristic changes in the magnitude of the current occur. Thus, single DNA molecules can be sequenced without the need for PCR or chemical labelling steps.

How does it work?

How does the technology work? Hagan Bayley, professor of Biological Chemistry at Oxford University and co-founder of Oxford Nanopore Technologies (ONT), explained it to your *Lab Times* editor, "I was interested in how I could use a protein called α-hemolysin. I thought, permeabalise cells? Kill tumours? Then it occurred to me: stochastic sensing!" – Stochastic sensing? Bayley explains: Biological pores – ion channels – provide electrical continuity across electrically insulating membranes. As such, they have a specific electrical conductance that can be measured directly in real time. Nothing new there: after all, that is how patch-clamp electrophysiology work won Bert Sakmann and Erwin Neher their Nobel prize in 1991. Ion channels open and close in response to changes in their environment. But the subtle difference that Bayley exploited is not how these channels open and close, but how small molecules passing through them partly reduce their conductivity.

Photo: private

Hagan Bayley, born in 1951, has studied transmembrane pore-forming proteins and chemical signal transduction. He co-founded Oxford Nanopore Technologies in 2005.

Getting the pore's size right

As you might expect, a small molecule passing through the pore causes a small change in conductivity whereas a larger molecule completely blocks it. Get the size of your pore just right, and you can detect, which molecules are passing through by the characteristic effect they have

on the channel's conductivity. Now, if your molecule happens to be, say, a strand of DNA, it will create a signature of electrical signals depending on the sequence of bases. If you can just get that pore to stay on an artificial membrane then you are in business.

Hooking the membrane up to a chip

As anyone who has tried putting ion channels into membranes will tell you, it is not easy to keep them there. The team of scientists and engineers who wanted to make ONT a reality found ways of fixing the protein into artificial silicone-based membranes and hooking the membrane up to a computer chip, leaving a trail of valuable intellectual property rights along the way.

There is no mistaking it, the product that ONT are launching later this year is a robust, off-the-shelf machine. It consists of a reading device and a disposable, single-use cartridge. The reading device does the computational part and the chemistry is in the cartridge. That cartridge, which looks rather like an old-fashioned VHS video tape and is about the same size, houses the pore-bearing membranes and the electronics needed to measure resistance. There are actually hundreds of separate membranes in each cartridge, each bearing a single nanopore so that the readout from each nanopore can be measured independently by the on-board electronic hardware. This is all done in parallel and in real time – all you have to do is to watch the sequence roll off.

Real time feature

And that "real time" feature is an important one. It means you can stop the experiment when you have enough data. For instance, once you have determined you have statistical confidence that an SNP is present in a sequence, you can stop the reading, flush out the cartridge and pop in another sample. Come to that, why do it yourself? The Gridion cartridges can take 96 well plates, which means you can load any combination of samples and decide, even during the course of a read, which well to do next, depending on what data are coming in.

If you have the cash, you may even think about buying a whole stack of Gridion devices. Why? Because they talk to each other. You can network them together, and each experimenter can run his own project on one or more of them, controlling them from a networked laptop. Or you can get them to work on a common project, communicating with each other and prioritising reads. This is one of the big advantages to real time sequencing or analysis: you can change the course of your experiment depending on what you find. Let the computer do the experiment.

Photo: BCG

Richard Creager of Beckman Coulter Genomics is, "closely watching the interesting nanopore technology".

Hang on a second...

Or perhaps you think small is beautiful? Well, ONT has something for you, too. As well as scaling up, you can scale down. That is what the "Minion" does. It has a single membrane and literally plugs into a USB port on your laptop. Hang on a second whilst I sequence my genome...

With all this hype, it is no wonder some 6% of shares have been wiped off the sequencing industry following ONT's dramatic leap onto the world stage in February this year. Surely this means curtains for sequencing services? I put this to Richard Creager, Senior Vice President of Molecular Diagnostics at Beckman Coulter Genomics, who have a major stake in the custom sequencing market, "The nanopore technology is interesting and we're closely watching its progress", admits Creager.

Photo: OGT

Oxford Gene Technology's James Clough is sure that there will be, "always a requirement for bioinformatic analysis".

But like many other companies that are making a living out of doing other people's sequencing, Beckman are keen to point out that there is more to sequencing than just sequences.

"We work with our customers," adds Creager. "We help them optimize their study designs, prepare their sequence libraries, process the sequencing run and interpret the results. In other words, turning data into information."

Indeed, Creager tells me that Beckman Coulter Genomics might well add ONT's Gridion to their services in the future.

OGT's remarks

James Clough of Oxford Gene Technology takes the same line. "Current sequencing technology is already providing an overwhelming amount of data for internal academic bioinformatics resources. And the sheer diversity of genomic research means that there will always be a requirement for bespoke bioinformatic analysis".

Chris Hebel, VP of LC Sciences, strikes an even more positive note. "I see a brighter future for service providers."

Really?

"When next-gen sequencing came along, many predicted it would be the

Photo: Nigel Chapman for Oxford Nanopore Technologies

A single-use, self-contained cartridge is inserted into an Oxford Nanopore Gridion device. Each cartridge includes all the reagents required to run an experiment.

demise of the microarray. But instead it has been a springboard for our array service because all the sequencing discovery work has added new content to put on our arrays for high-throughput expression analysis."

Okay, so some companies will just absorb ONT's "3rd generation" sequencing technology into their armoury. But surely there will be a significant number of researchers who, able to do their own sequencing quickly, easily and cheaply, will desert them? The sequencing companies may well buy a Gridion, but researchers might just buy a Minion.

Photo: LC Sciences

Chris Hebel from Houston-based genomics company LC Sciences, sees, "a brighter future for service providers".

Hebel thinks not. "There are plenty of researchers who would just rather have someone else do the work. There is a steep learning curve and most don't want to become experts in the analysis methods, they just want data so they can focus on their own research."

Maybe, although emphasising customer care does make it look like sequencing companies are on the back foot.

Fast is fine, but accuracy is everything

But ONT aren't going to have everything their own way – they have something of an Achilles heel when it comes to read accuracy. At the Florida AGBT meeting in February, the British company admitted to a 4% error rate, and that worries some people.

"Nanopore's product might well reduce the need for the current high-throughput sequencing platforms", admits Amy Liao, President & COO of global contract research organization, Genewiz. "But for that to happen, the accuracy issue has to be resolved first."

Your *Lab Times* reporter raised this concern with a representative at ONT, who insisted that they aren't losing any sleep over it. "At the conference we drew attention to this because we wanted to be up front. And it really isn't a problem: the reads give a confidence score which means you aren't going to be misled. And in any case, we expect to have this figure right down to perhaps 1% by the release date. Besides, the deep reads more than make up for it."

Granted, but that won't wash when it comes to diagnostics, as Beckman Coulter's Creager points out. "As the technologies move towards diagnostics and our customers are moving to more regulated services, the accuracy of output is critical", he argues. "Sure, error rates are improving. But they're not yet accurate enough for true diagnostics. I don't think the additional sequence coverage makes up for the error rate. And at this early stage, it's unclear how and when this might be resolved."

Pores "for nearly everything"?

But let's get this into perspective. ONT don't claim that their nanopores do everything – they only claim that they can do a lot. And what a lot. It doesn't stop at DNA sequencing. As Bayley pointed out, in principle there is almost no limit to what you can design pores for: "You could design nanopores for all kinds of things, not just DNA. Make one for explosives and you can have an instant screen at airports. Make one for bacterial compounds and you have an instant screen for food quality".

ONT are looking into the potential for protein analysis, even protein sequencing.

However, that is for the future. For the present, ONT are concentrating on DNA, and that is where the competition is coming from. Ion Torrent, the company that brought us the "Personal Genome Machine", are promising to release the hyped "Ion Proton" this year, which they claim will sequence a human genome in a day for less than $1,000 (€770). And Illumina don't want to be left out. They are promising their own genome-in-a-day contender later this year: the HiSeq2500.

Photo: University of Oxford

Chris Ponting (MRC Functional Genomics Unit, University of Oxford) thinks that science needs, "better computing".

ONT aren't saying what the Gridion is going to cost, but it is likely to be much cheaper than their rivals. Factor in the versatility afforded by real-time sequencing and you can see why ONT don't seem that worried about their critics.

Bring on the data flood

So if ONT are right, the €770 (or less) genome will be ready by Christmas, and it'll be on your very own lab bench. Isn't this a climate change in the sequencing world?

With some 16 *Nature* articles on his CV, Chris Ponting of the MRC's Functional Genomics Unit is a recognised leader on the world's sequence analysis scene. Your *Lab Times* reporter asked Ponting how significant he sees ONT's new technology.

"Retrospect is a great thing, and we have seen great ideas come and go", says Ponting, "but there is a lot that is very exciting about Nanopore. The ability to do such long reads is really important. For starters, we can look at full-length transcripts – something you can't really do with short reads."

Ponting believes that the effects of getting faster, cheaper and more convenient sequencing go beyond taking a few customers away from custom sequencing companies. According to Ponting, what we need right now is not just faster sequencing, but better computing. "Just what are we going to do with all that data?" he asks. Major infrastructural concerns like the European Bioinformatics Institute are soaking up taxpayers money, but who will have the incentive to keep this data accessible in a future when we won't bother storing sequence data any more, but will just sequence on the fly? Ponting fears an expensive data graveyard:

"What we really need is research centres with not just the right people to know how to use the data, but also with a whole order of magnitude of computing power. If the data deluge outstrips Moore's law, we've had it."

Analysis is the bottleneck

So, perhaps when companies like Beckman Coulter Genomics defend themselves by crying up customer service, they're not so much on the defensive, after all. Perhaps they are a step ahead. "There is consensus that analysis is the rate limiting step for next-gen sequencing and there's already a massive back log of data waiting for analysis", says Hebel of LC Sciences. "A new technology like ONT is only going to make backlog worse."

Maybe we should find a different way to store sequence data altogether. Maybe we could package it as a molecule. A molecule that can replicate itself...

Steven Buckingham