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September 16, 2014

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By Julia Karow

NEW YORK (GenomeWeb) – Oxford Nanopore Technologies is working on a high-throughput nanopore sequencing platform called PromethION, along with updates and new applications for the MinION, according to a company official.

During an online conference called "Balti and Bioinformatics" this week, Oxford Nanopore Chief Technology Officer Clive Brown also presented some of the company's MinION results and provided an update on the MinION early-access program. The half-day meeting, which was webcast, was organized by Nick Loman, a researcher at the University of Birmingham and an early MinION user.

The PromethION will use the same core technology as the MinION. It will combine several flow cells into a single system, with up to 100,000 nanopores or channels in total, and allow users to run large numbers of samples in parallel. For example, users could choose to run 96 individual microbial samples or a single human sample on the instrument, Brown said. For comparison, the current MinION has 512 nanopores per flow cell and can sequence a single sample at a time.

Samples are loaded into the PromethION from the top, and the system will be compatible with automated pipetters and other laboratory automation equipment.

The system will have an estimated throughput of 300 to 400 gigabases per day, based on the current speed of the enzyme that feeds the DNA through the pore. With faster chemistry and a larger number of channels, throughput could increase to more than a terabase per day and, with run times of several days, the platform "should be a multi-terabase machine," Brown said.

Oxford Nanopore plans to showcase the PromethION, which appears to be the size of a small desktop computer and will be "pavestone-heavy," according to Brown, at the American Society of Human Genetics annual meeting in San Diego next month.

The company has also been working on short-term and long-term updates to the MinION platform, which is currently in early access testing.

Internally, Oxford Nanopore has achieved up to 1 gigabase of data per run from its best MinION runs, and Brown said he believes several gigabases per run can eventually come from a MinION "in its current form." Early access users have achieved up to about 550 megabases per run so far, he added.

The next MinION generation will likely be able to generate up to tens of gigabases, enough to sequence a human genome "at reasonable coverage," he said.

"There is still room for expansion, both in speed and number of pores, and the numbers are getting very big very quickly," Brown said.

Up to 80 percent of pores are active at a time in the company's MinION runs, whereas early access users have seen about 50 percent of pores active, he said. The company has also obtained reads exceeding 50 kilobases, where the read quality looks similar throughout the length of the read.

Brown showed what he said is a typical read from the R7 chemistry, which it recently started shipping to early-access customers.

That read was about 7.9 kilobases and had a total error rate of 13.7 percent, consisting of 5.2 percent substitution errors, 3.7 percent insertion errors, and 4.8 percent deletion errors. "What we tend to get are chunks of good data interspersed with bad data," he explained.

Errors depend in part on the parameters of the base caller, he said, adding that the base caller version currently available to early access users does not have all features available yet.

A "slightly more updated chemistry", version R7.X, produces better data, he said, showing a 6.3-kilobase read with a total error of 9.2 percent.

The company has already replaced the R6 chemistry that early access users initially received with the R7 chemistry, which contains a reengineered nanopore that improves the signal-to-noise ratio and increases the current range of the signal. The latest iteration of the chemistry — version R7.3 — in combination with updates to the base caller "should get most people a lot closer to where we are internally" in terms of performance, Brown said.

Oxford Nanopore is also working on a new version of the chemistry, R8, which will use another type of nanopore that generates a different current signal or "squiggle shape" than the R7 chemistry. R8 will be more amenable to GC-rich DNA, and will generate better 2D data, where information from the top and bottom strand of DNA is recorded. Users will be able to use the R8 chemistry either on its own or in combination with R7. "I'm predicting that things like SNP calling will be significantly boosted by using the two-pore system," Brown said.

The company is also developing a revised version of the MinION hardware to replace the

current one, which Brown said is more of a beta system. The update will have a number of "fixes and tweaks," including a "slightly different looking" flow cell, he said.

In addition, the company plans different MinIon versions for separate markets, including "non-traditional markets," such as consumer self-quantification.

Library prep for the MinIon currently requires several DNA manipulation steps off the instrument. To eliminate those, the company is working on a system called SpotOn-T, a modified flow cell in which users load DNA from the top, a transposase contained in a matrix tags the DNA with adapters, and the prepared DNA diffuses onto the nanopore array. "You literally put sample on the consumable and it does everything for you," Brown said.

A new MinIon application the company is working on is direct RNA sequencing. According to Brown, company researchers have shown proof of concept that the nanopores can generate sequence-specific data directly from RNA. "We imagine that in the near future, there will be a direct RNA kit," he said.

Finally, Oxford Nanopore is looking to further develop its cloud-based Metrichor data analysis service into "a software service with vertically integrated devices," Brown said. "Where we see that going is in non-traditional areas outside a lab," he said, such as food production, biosecurity, and self-quantification. Early-access users currently upload their raw data to Metrichor, which translates it into base calls.

The MinIon Access program started this spring and currently includes approximately 500 laboratories in about 20 countries around the world, a number that will likely increase over time, Brown said. The company received applications from several thousand interested users for more than 20,000 MinIons, he said. As previously reported, several large-scale genome centers were not selected to participate.

"MinIon is clearly not designed for genome centers and core facilities," Brown said. "It's designed for individual PIs who want to do everything, from experimental design to sample prep to analysis, so most of the people who are in MAP are like that."

Most early access users appear to be located in Europe and North America, but the program includes sites in Asia, Australia, New Zealand, South America, and Africa.

Brown acknowledged that shipping reagents abroad has caused "a considerable number of issues" and that the company had trouble delivering shipments to New Zealand and other places, a problem that he said has been resolved.

"Because it's an early system, we've had quite a bit of heterogeneity in what we shipped," he said, including problems with defective enzyme, and those issues "impacted a lot of the early runs." Many of those problems have been fixed with the latest reagent and software releases, he said.

To support early access users, Oxford Nanopore currently manufactures several hundred flow cells per day, and tens of thousands per month, and the MinIon system has been "designed to be highly manufacturable," Brown said.

Early-access customers have already been receiving software and chemistry upgrades, and are supported through an online portal, an online community, and a company team that responds to questions within 24 hours.

"I know we have haters and we have lovers, and in the middle is a huge wave of silent majority who are just waiting to see how this pans out," Brown said.

Julia Karow tracks trends in next-generation sequencing for research and clinical applications for GenomeWeb's *In Sequence* and *Clinical Sequencing News*. E-mail Julia Karow or follow her GenomeWeb Twitter accounts at @InSequence and @ClinSeqNews.

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