MinIon Review by Early Access User Suggests Technology Not Ready for Routine Use Yet

September 09, 2014

MinIon Review by Early Access User Suggests Technology Not Ready for Routine Use Yet

By Julia Karow

This article, originally published Sept. 4, has been updated with comments from other MinIon early access users.

NEW YORK (GenomeWeb) – A peer-reviewed opinion article by an early access user of Oxford Nanopore's MinIon from the Okinawa Institute of Science and Technology in Japan suggests that the data the platform has produced so far is not yet useful for most sequencing applications.

The paper, published online in *Molecular Ecology Resources* last week, is among the first in the scientific literature to review results from the MinIon. Two other recent publications, by researchers in England and the US and by a group in Scotland, focused on analysis tools for the platform. "The current iteration of the MinIon is not ready for routine use," the Okinawa researchers concluded.

Several other MinIon early-access users disagreed with the results and the conclusion of the paper, saying the researchers obtained particularly poor results because they used a chemistry that is already outdated and sub-optimal alignment tools.

The MinIon technology "is still very experimental, and it's perhaps years behind its competition," lead author Alexander Mikheyev, an assistant professor in the Ecology and Evolution Unit of the Okinawa Institute, told *In Sequence*. Based on his team's experience, "I can't think of any universal benefit to the MinIon at this point."

"I'm definitely optimistic that it will improve," he said, but it might be difficult for the technology to catch up to existing platforms. "In order to be a viable option, the MinIon has to compete in

terms of price and convenience with something that is already guaranteed to give you much better results."

"In my opinion, they have to improve by a couple of orders of magnitude in order for this to be a genuinely useful technology, or beat the competition at price point," Mikheyev said. Early access users pay about $2,000 per MinIon flow cell at present, he added, and "for that much money, you can easily buy a HiSeq paired-end run which generates a lot of data."

Mikheyev and his team were participating in Oxford Nanopore's MinIon Access Program until last week, when the company terminated their agreement, citing that the researchers' "objectives and outlook are fundamentally misaligned with those of the MAP program and the other participants," according to Mikheyev.

As part of their participation in the program, they first completed the mandatory burn-in phase to make sure the technology performed to the standards of the company in their laboratory. This entailed acknowledging that the performance they observed was appropriate for the purpose of their planned experiments.

They then proceeded with three experiments of their own: resequencing the 48-kilobase bacteriophage lambda genome, sequencing amplicons from a previously analyzed snake venom transcriptome, and *de novo* sequencing of an insect genome. The last experiment provided such poor data that it was not included in the publication, Mikheyev said.

Overall, about 90 percent of the MinIon reads had "no homology whatsoever" to the reference. Those that did had homologous stretches of a few hundred bases and an error rate of about 20 percent, he said.

Nick Loman, a researcher at the University of Birmingham in the UK and another early access user of the MinIon, said the performance reported by the Japanese group "falls far short of what we and others have seen." He pointed out that the group used an early version of the chemistry, R6, which has since been replaced by a newer one, R7, that he said is "much better."

Loman said his group is about to release several MinIon datasets from *E. coli* K-12 MG1655 on GigaScience's GigaDB repository "so the community can decide for themselves whether [the Japanese team's] conclusions are valid."

Earlier this year, Loman posted a single 8.5-kilobase MinIon read and commented on his experience with the platform.

The Okinawa researchers' paper "represents a snapshot of particularly poor runs very early in a fast-evolving process," said Keith Robison, a principal scientist at WarpDrive Bio and another early-access user of the MinIon.

According to Robison, there has been a wide range of success in the MinIon Access Program and Oxford Nanopore "is trying hard to both raise the top and get everyone on a more consistent footing."

The earlier chemistry, he said, provided fewer 2D reads – which use information from the top and bottom strand of the DNA – that were also of lower quality than the current chemistry. In addition, the basecaller has improved since the early access program started.

One reason Mikheyev's group obtained such poor alignment statistics is that they did not choose the best aligner for the task, Robison told *In Sequence*. Using another alignment scheme, he was able to map sixty to seventy times more bases, which resulted in higher coverage and a higher percentage of mapped bases per read, he said.

Others agreed. Mick Watson, a bioinformatics researcher at Edinburgh Genomics and the Roslin Institute in Scotland, and also a MinIon early access user, said a re-analysis of the Japanese researchers' data with different software and parameters showed that "their data, whilst below ususal MinIon standards, was usable and aligned to the whole lambda genome, calling 100 percent consensus accuracy."

According to Detlef Weigel, another early access user and a researcher at the Max Planck Institute for Developmental Biology in Tübingen, Germany, the results of the Japanese group fall "below the average performance of the whole community" of early access users, which he said has varied a lot. "Some labs had flow cells that looked phenomenal, while others had essentially no reads," and results have improved with the switch from the R6 to the R7 chemistry.

His own lab's initial experience "has been pretty much in line with the publication" in that the initial flow cells provided only low throughput, which he attributed to the old chemistry as well as issues with the flow cells, resulting from either their production or shipment. Experiments with new reagents are currently underway, "and we are hopeful that this will greatly improve things," he said.

Further variation between users' results might stem from library preparation issues, he said, noting that Mikheyev's paper seems to have had such issues, and from the use of different alignment tools. "From the blogs, it’s clear that some colleagues have been able to map at least 50 percent of the reads with an error rate of 20 to 30 percent, which seems much better than what the paper reported," he said.

Oxford Nanopore did not respond to a request for comment before press time.

To prepare their libraries, the Okinawa researchers used two kits from Oxford Nanopore, one for genomic DNA sequencing, the other for amplicon sequencing. Library prep, the authors noted, takes about half a day and is "of comparable complexity and cost to library preparation for other platforms." Mikheyev said that a kit currently costs on the order of $500 and can prepare several samples.

A MinIon run can take up to 48 hours, the lifetime of a flow cell, according to the authors, and the MinIon must be refilled with additional library every four hours for optimal yield.

For the lambda genome, the researchers generated data for 36 hours and mapped the reads to the reference using BLASR, a tool that was originally developed to map reads from the Pacific Biosciences platform, and BLASTN.

The data were analyzed using either the 1D workflow, which uses data from a single DNA strand, or the 2D workflow, which combines data from the top and bottom strands of double-stranded DNA, linked by a hairpin adapter, in order to increase accuracy.

The lambda run generated more than 150 megabases of sequence data with a read length peak around 5 kilobases and was "largely able to reconstruct the sequence of the 48-kb lambda phage by mapping," the authors wrote.

Using BLASR, about 10 percent of reads mapped to the reference genome, and those reads had identities of just 2.2 percent for the 1D workflow and 8.9 percent for the 2D workflow. With BLASTN, about 25 percent of reads mapped but the identity of those reads was only 0.4 percent for the 1D and 1.1 percent for the 2D workflow.

Many of the mapped reads had only short stretches of similarity to the reference, though "a number of" 7 to 10-kilobase reads matched "along much of their length."

The major error types were insertion and deletion errors, "particularly insertions that introduce spurious data," they wrote. Most errors were not systematic, and the researchers were able to call the consensus sequence "in most cases" from 16x coverage data.

The snake cDNA amplicon data "were useless for any practical purpose," they wrote. That run lasted 24 hours and generated only about 1 megabase of data. Fewer than 25 reads mapped to a reference assembly of the snake transcriptome using either mapping tool. The researchers tried to correct errors in the nanopore data by mapping previously generated Illumina GAII reads to the MinIon reads but "this was not a successful strategy," they wrote.

In terms of applications, the MinIon, since it is portable, may be useful for certain diagnostic field applications where data from an unknown sample need to be matched to a reference, they wrote.

One benefit of the platform is that it does not require PCR amplification of the template DNA and could thus be used for samples that are difficult to amplify. However, the current low number of reads and relatively high DNA input requirements of about 1 microgram "offset any benefits of eliminating PCR," the authors wrote.

For genotyping applications, "because of its extraordinarily high error rates, the current iteration of the Oxford Nanopore technology is close to useless," they said, noting that those applications "account for much of genomics research" today.

Combined with more accurate short-read data, MinIon data "may assist genome assembly through scaffolding," similar to how researchers have combined PacBio data with Illumina data, though "our attempt to do this has not been successful, because large regions of a MinIon read have no homology to the template."

"The yield and accuracy of the MinIon will need to increase dramatically to work for large genomes, where scaffolding is most needed," the authors wrote. Scaffolding could even be possible with the current low accuracy, "but reads would need to contain multiple regions homologous to the template to be present," they added.

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.

Related Stories

- Oxford Nanopore Presents Details on New High-throughput Sequencer, Improvements to MinIon

September 16, 2014 / In Sequence

- Oxford Nanopore Shows off MinIon at ASHG; 'Hundreds' to be Shipped for Early-Access Program
  October 29, 2013 / In Sequence
- MinIon Early Access Users Demonstrate De Novo and Hybrid Assemblies, SNP Calling, Outbreak Analysis
  September 16, 2014 / In Sequence
- Oxford Nanopore Says 50 Kb 'Easily Obtained' from Single Reads; Addresses MinIon Error Types
  March 18, 2014 / In Sequence
- Oxford Nanopore Opens Registration for MinIon Early Access; 'Robust and Diverse' Response so Far
  December 3, 2013 / In Sequence