PacBio Releases Chemistry Update Raising Average Reads to 10K-15K Bases

Oct 21, 2014 | Molika Ashford

Premium

NEW YORK (GenomeWeb) — Pacific Biosciences last week announced the release of its latest updated chemistry and software, which the company says increases the performance and output of its RS II sequencing platform by about 45 percent.

The new chemistry, P6-C4, represents the company's sixth generation of polymerase and fourth generation of associated base pairs, reagents, and buffers. It further extends the PacBio RS II's average read length to between 10,000 and 15,000 bases depending on the library used, with the platform's longest reads exceeding 40,000 bases.

According to PacBio CSO Jonas Korlach, with a good sequencing library users of the new chemistry should see about a "60 to 70 percent increase in read length compared to the previous chemistry."

Moreover, improvements to both the sample and library preparation steps also mean that the read quality of the technology is now higher, yielding a higher proportion of reads that can actually be used, according to the company.

"If you take it all together, the yield of reads that are usable and that go into downstream analysis is increased by almost a factor of two," Korlach told *In Sequence*.

"In any sequencing project you acquire sequencing reads and then they are mapped to the location of the genome where they came from, either in re-sequencing or as part of *de novo* assembly. So any technology has a dependency on coverage with respect to final accuracy," he explained.

"With the new chemistry you only need about a coverage of 30-fold to get to 99.999 percent accuracy, or less than one error in 100,000 bases," he added. "That's much less than the previous chemistry … so it's another substantial decrease in the amount of sequencing needed for a particular project."

In addition to updating chemistry, the company has also improved its software. The new version, 2.3, improves the overall efficiency of sequence analysis and mapping by a factor of two, Korlach said.

"Really, these are multiplicative improvements, so if you put it all together you have almost a [four-fold] improvement in effective throughput overall," he said.

According to Korlach, the improvement is in line with the company's goals and timeline. "We communicated to the research community at the beginning of the year that we would have a factor of

four improvement and would be getting up to a gigabase of throughput per smart cell run, and we are achieving that."

Though the updates do not necessarily open up novel applications for PacBio's sequencing, Korlach said that it certainly makes current applications more efficient.

With the company's previous chemistry, P5-C3, PacBio saw an initial penetration into some new areas of the human genome market, including isoform sequencing and contributions to *de novo* human genome assembly, in addition to the plant and animal sequencing markets.

With the newest upgrade, Korlach said the company expects these application spaces to open up further. "We will be going from feasible to really ready for prime time," he said. "And we are certainly hoping for a lot of adoption and people using this more frequently."

One early user of the new chemistry has been Korean sequencing firm Macrogen, which recently purchased two PacBio systems that it is using for*de novo* assembly of a Korean human genome as part of its larger Asian Genome Project.

Korlach said that the laboratory of Gene Meyers at the Max Planck Institute in Dresden, Germany has also had early access to the new chemistry.

Luke Tallon, of the University of Maryland's Institute for Genome Sciences, who has also been working with the P6-C4 update, told *IS* in an email that though his lab is early in its testing of P6-C4, the group's initial results have been "impressive, with the average SMRT Cell yielding more than one gigabase."

"We are also pleased with the reduction in loading material required per cell, meaning a much higher overall yield for each library. We are eager to move this new chemistry into production on our projects," he wrote.

Moving forward, Korlach said PacBio intends to continue its periodic updates to hardware or software on a timeframe of about every six months. The company is also continuing its work to increase the efficiency of loading into its SMRT cells.

In a paper in *Nano Letters* last month, researchers from Northeastern University and PacBio showed they were able to improve the capture of DNA into the PacBio zero-mode waveguide by two to three orders of magnitude compared to the current diffusion-based approach by drilling a narrow solid-state nanopore into the bottom of the well.

"I think there is another factor of two we are hopeful we'll be able to achieve there," Korlach said. "At this point we are very excited about what the new chemistry will enable, but there is no reason why we are at any fundamental limit of what the technology can provide any time soon," he added.