Revolutionize Translational Research

Uppsala’s Ulf Gyllensten on How Long Reads Give Access to New Areas of the Genome

In an interview with Theral Timpson — part of Mendelspod’s series on long-read sequencing — Ulf Gyllensten, a professor in Medical Molecular Genetics at Uppsala University, spoke about using PacBio® technology for HLA typing, human genome studies, transcriptomics, and more.

Based in the medical genetics and genomics department, Gyllensten focuses on two areas: using systems biology to study biological variation in human physiology, and studying the epidemiology of human papilloma virus and its genetic link to cervical cancer. He also works with the National Genomics Infrastructure, a core facility in Sweden for genotyping and DNA sequencing, where he has access to all commercially available sequencing platforms.

In the podcast, Gyllensten spoke about advances in screening for HPV, his predictions for the widespread use of genome sequencing in the clinic, and applications using Single Molecule, Real-Time (SMRT®) Sequencing for human genome studies.

Unambiguous HLA Typing

“PacBio is really revolutionizing HLA typing,” Gyllensten said, noting that long-read sequencing addresses the ongoing challenge of linking polymorphisms in distant parts of the HLA genes and distinguishing alleles. “I have been in that field for quite a while. ... Finally, we have a technology that will resolve all the ambiguities in HLA typing, which will have a huge impact.”

Gyllensten said the major advantage of SMRT Sequencing for the HLA region is its ability to completely sequence all HLA genes (both class 1 and class 2), getting all the introns and exons for each in a single long read. He believes PacBio sequencing, with its rapid turnaround time, will ultimately become “the key technology” for matching donors and recipients in organ transplantation.

When asked whether it’s really possible to achieve 100 percent accuracy for these complicated regions using SMRT Sequencing, Gyllensten replied that it was.

“The fact that you can sequence a single allele — that is, a single chromosome by itself and then the other chromosome in the individual — and separate them down to the single base is really the most accurate way you can ever do HLA typing,” he said.

Applications in Human Genomics

Gyllensten said that his team had expected the primary use of PacBio sequencing to be for smaller genomes, such as getting complete de novo assemblies for pathogens. While they do routinely handle those projects, he was surprised to find robust demand for using the sequencer to analyze larger genomes — including human — as well. “Before having the PacBio instrument installed and running we hadn’t thought about some of these things,” he said. “But it’s opening a lot of opportunities.”

He noted that clinical research, in particular, is a good fit for SMRT Sequencing. “Focusing in on particular regions actually suits clinical genetics and clinical immunology because they don’t want the whole genome. They have their favorite genes, favorite targets,” Gyllensten said. “Those can then be accessed through the PacBio [system], and the information that

The full interview can be found at www.Mendelspod.com
is coming out is really information that could not come out of any other sequencing technology at this point.”

Researching treatment resistance and cancer biology in individuals with leukemia is one example of where the PacBio platform can make a difference. SMRT Sequencing can more accurately cover the fusion gene that is responsible for the nature of the leukemia and its development, Gyllensten said. In addition, he believes PacBio’s technology offers the potential for early detection of new mutations linked to treatment resistance. Reliable early detection could one day make a difference in clinicians’ ability to change a patient’s therapy at the earliest sign of resistance, he noted.

“It all has to do with the long read because you need to sequence maybe 2 or 3 kb around the particular breakpoint in this patient to figure out whether they have a resistance mutation or not,” Gyllensten said, “and there is no other technology that can do that.”

A View Into Genomic Dark Matter

Gyllensten remarked that as people begin to figure out how much important information is being missed in genome sequences, they will move to a platform that offers more complete views of biology. Transcriptomics is one place where SMRT Sequencing makes a real difference. “Very few studies have been done on complete transcriptome data,” Gyllensten said. “I think when people start to see that, they will eventually move into ... long-read [sequencing].”

A comprehensive view of the human genome will also motivate people to move away from short-read sequencing. At some point, he said, scientists will look at all the short-read data that has been amassed for human genome studies and “realize that a lot of the questions will still not have been answered. They will ask, is the answer hidden in that 15-20 percent of the genome we still haven’t covered with the present technology?” Gyllensten said. “Then there will be a rush to understand the remaining [portion] of the genome.”

Validating Other Technologies

According to Gyllensten, whose core facility still runs a number of Sanger sequencers, PacBio sequencing has been gaining ground as the preferred technology for validating results found by short-read platforms. “We are seeing more and more requests to do it not with the Sanger, but with the PacBio [sequencer],” he said. “You need to validate [with] different technology and PacBio is really well suited for that.”