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Following Feasibility Study, UK Registry Plans to Implement PacBio for HLA Typing by Year's End

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NEW YORK (GenomeWeb) – The UK's Anthony Nolan Research Institute plans to start using Pacific Biosciences' sequencing technology routinely for HLA typing by the end of this year after scientists from the institute and PacBio published a feasibility study this spring.

The institute is part of Anthony Nolan, a UK charity that runs a stem cell and bone marrow registry with more than 550,000 potential donors.

The long reads of the PacBio technology allow the researchers to phase polymorphisms in HLA genes at high resolution, which could lead to better matches between donors and recipients and better transplant outcomes.

A year ago, PacBio cited HLA typing as a growing market for its technology after both Anthony Nolan and US-based HistoGenetics purchased two RS II sequencing systems each and presented results at a conference.

This spring, Anthony Nolan scientists published a feasibility study in PLOS One, for which they sequenced three HLA class I genes in seven DNA samples. In the meantime, they have been working on sequencing HLA class II genes and on reducing costs through higher multiplexing.

"The idea is to use this technology in the future for all our HLA typing, for all the donors we recruit to our registry, and also for all of the patients where we do clinical HLA typing for a number of transplant centers in the UK," Steven Marsh, director of bioinformatics and deputy of research at the Anthony Nolan Research Institute, told GenomeWeb.

For their published study, he and his colleagues sequenced the HLA-A, -B, and -C genes, which are all class I genes, in donor DNA from two blood samples and two saliva samples and in DNA from three cell lines with well-characterized HLA genes. For comparison, they also genotyped the three genes in the donor samples using Luminex LABType sequence-specific oligonucleotide typing kits.

PacBio sequencing of PCR-generated full-length amplicons of the three genes, using one single molecule real time (SMRT) cell per sample, generated at least 150-fold coverage per allele and allowed for accurate allele calling. Each experiment took three days in total, including two hours of sequencing. "In combination, these factors make the SMRT DNA sequencing method amenable for use in a high-throughput HLA typing laboratory," the authors wrote.

In particular, they noted, the technology produced accurate sequence data for homopolymer regions, which can be a problem for other sequencing platforms. The PacBio sequencer also identified four novel alleles in the HLA genes, which were confirmed by Sanger Sequence Based Typing (SBT). Finding so many novel alleles in such a small number of samples "highlights the extensive polymorphism seen in the HLA genes outside of the routinely typed exons, much of which may as yet be unknown," according to the researchers.

For clinical HLA typing, they would also need to sequence HLA class II genes, Marsh said. His group has been developing primers for those genes, which are more challenging because they are much longer than class I genes and because few full-length sequences for HLA class II genes currently exist in the databases, he said.

To bring down assay costs to the level of current technologies, which generate lower-resolution data, the team has increased the level of multiplexing and now routinely sequences the three HLA class I genes in 48 samples per SMRT cell, Marsh said. Also, PacBio earlier this year introduced barcoding kits to increase the number of samples per run.

"Before we go live using this routinely, we are looking at ways of multiplexing to the greatest degree, so we have [sufficient] depth of coverage of the genes we're sequencing, but also at an economically viable cost," Marsh said. He declined to reveal what that cost would be per sample, citing commercial sensitivity.

The institute, he said, is in the process of streamlining the PacBio HLA sequencing pipeline, with the goal of providing routine clinical HLA typing on the platform for class I and class II genes by the end of this year. One of the biggest challenges is the bioinformatic analysis, he said, and his team is working closely with PacBio in this area.

While the capital cost of the PacBio instrument is high, no other technology can currently match its long reads, Marsh said, "and if you have [genes] which differ solely at the ends of the genomic sequence, then it's impossible to assign phase across the whole gene with only short reads."

He said his group is keeping an eye on other emerging long-read technologies, such as Oxford Nanopore's, but believes they are still "a little away" from HLA typing.

In particular, the PacBio technology could help discover new polymorphisms in regions of the HLA genes that are not usually typed but might contribute to complications such as graft-versus-host disease. This could "result in considerable improvement in survival rates post-transplant," the authors wrote.

"We've been largely ignoring this because it's been impossible to sequence the whole genes," Marsh said. "What we do know [is that] somebody who has an HLA-identical sibling still does better in many scenarios than people who have an unrelated donor. We believe that at least some of those differences are going to be between the patient and the donor in the regions of the molecule that we would be able to sequence when we have this technology routinely used in our laboratory."

In addition, his team found that the PacBio technology sometimes reveals that a donor has a rare rather than a common pair of HLA alleles, which could also impact the outcome of a transplant. "What you really want is to have a patient who is perfectly matched with their donor," Marsh said.