Speed of MiSeq® System Critical to Clinic Use

Professor Graham Taylor is using the MiSeq system to move genomic sequencing into the future of diagnostic tools.

Dr. Graham Taylor has worked in diagnostics and genomics for over 20 years. He currently runs the Translational Genomics Unit at Leeds University, supporting research groups who are mapping genetic disease. Part of his team's mission is to translate technologies and applications into a diagnostic setting.

Dr. Taylor's main priority is pushing next-generation sequencing technology into a usable, diagnostic framework. He shared with us important considerations when moving genomic sequencing into a clinical setting.

Q: How will you use the MiSeq system in translational research?

Graham Taylor (GT): We need a method that we can use to sequence regions of interest in cancer patient genomes that are likely to be relevant for clinical utility and to inform treatment, ultimately arriving at a more personalized cancer therapy. MiSeq allows the speed of data generation that fits into a clinical treatment context. We'll be able to get results in a matter of hours or days, or certainly within a week. It will allow us to get enough data to robustly call mutations in heterogeneous tumors that may be important in relapse. Researchers at the Cancer Research Institute (CRI) would like to pick up those mutations earlier so that the appropriate therapy can be decided.

> "MiSeq allows the speed of data generation that fits into a clinical treatment context."

Q: What makes the MiSeq system amenable to a diagnostic setting?

GT: Turnaround time is critical. The sequencing process we've been using for the past six months takes about three days to run the analysis. In a clinical setting, doing tumor testing in real time, this wouldn't meet the requirements of the clinicians. We need a system that can generate the same data in less time. The MiSeq can perform that function, so we started looking at it. We wouldn't even it consider it if we couldn't turn the data around in close to a day. That is absolutely critical. The accuracy of sequencing is also important. There are other small-scale personalized genome sequencers around at the moment that work on an intermediate scale of half a gigabase to two gigabases a sequence that are generating data of considerably lower base calling accuracy. This reduces the sensitivity of the test and you will need to have more reads to get the same equivalence of data. In some cases, the increasing read numbers wouldn't overcome the low quality of the sequencing. Of the currently available personal sequencers, MiSeq is the only one we've found to produce the level of quality output we need.

In addition to running the Translational Genomics Unit at Leeds University, Graham Taylor, Ph.D., FRCPath, is President of the Human Genome Variation Society, a small scientific community that is responsible for formalizing the naming of mutations.

Q: What is important in the workflow for a diagnostic?

GT: A very important feature of the MiSeq is that it has a very simple workflow. Library preparation is integrated into the machine. In most other situations you have a library preparation step that's separate from the sequencing step. Preparation of emulsion PCR from titrations is very time consuming, quite demanding, and relatively error prone. It may take the better part of a day, in some cases two or three days, which obviously means that the workflow wouldn't be quick enough for real-time clinical diagnostics where drug decisions need to be made and acted upon promptly. We don't lose any time preparing emulsion PCR libraries with MiSeq. The library preparation can be undertaken by competent technical staff. It's not a high-end scientific application; it's straightforward. I don't think that is quite the case with emulsion library preparation at the moment.

Q: How do the data you received back compare to data you're accustomed to?

GT: We are comparing the MiSeq performance specs to our Illumina Genome Analyzer™ *IIx* data. In my case, I'm not really interested in reads that have quality scores of less than 30. Based on those expectations, I thought we would get about half the number of reads that we actually are getting. The number of reads is ahead of the quoted values for the MiSeq system. I've compared the qualities of the reads based on the Illumina Q values for over 100 cycles and the MiSeq is as good as other Illumina systems. I'm doing some quality analysis to look at the actual error rates in the reads. They're pretty low. Most of the errors derive from the PCR process, not from sequencing. I had made some projections on what our testing costs would be based on the Q values we get. The data we have so far is exceeding those expectations and we are fairly optimistic in terms of what our tests will end up costing. We think the capacity and the quality of the MiSeq will meet our requirements.

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Q: If you weren't able to do this study on a MiSeq, what other method or technology would you use?

GT: Other available small-scale sequencers have got sequencing quality issues that I think are going to be very limiting, especially for analyzing tumors, and so I don't think we would have taken the sequencing approach. I think we would have gone for things like real-time PCR, possibly high resolution melting, and possibly Sanger sequencing, all of which are a lot more expensive, even given the new nanoliter volume real-time PCR technologies. I think we would have gone in that direction and it would have been a lot less flexible. I'm pretty confident that if we can keep the reagent costs under control and we can get the economies of scale by batching samples and doing multiple tests, sequencing is going to be very hard to beat.

Q: What advice do you have for scientists considering next-generation sequencing?

GT: Make sure you're comfortable with the informatics and that you can prepare the samples in a way that will be suitable for sequence analysis. Operating the machines, especially something like a MiSeq, isn't going to be a problem at all.

Learn more about the MiSeq system at www.illumina.com/miseq

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