

Sequencing to Inform Cancer Treatment

Dr. Andrew Fellowes, Department of Pathology at the Peter MacCallum Cancer Centre, uses TruSeq® Custom Amplicon and the MiSeq® personal sequencer to revolutionize tumor classification, making a dramatic impact on how cancer treatment decisions are made.

Andrew Fellowes, Ph.D., FRGSA, FFSC (RCFA) is Scientist in Charge, Molecular Pathology Diagnostic Development in the Department of Pathology at the Peter MacCallum Cancer Centre in Melbourne, Australia. Peter Mac is Australia's only public hospital solely dedicated to cancer and home to Australia's largest cancer research group. Peter Mac's vision is to provide the best in cancer care, accelerating discovery translating to cures. Working toward this vision, Dr. Fellowes is developing tumor profiling tests using Illumina next-generation sequencing technology. Peter Mac's next-generation sequencing program is funded by Therapeutic Innovation Australia through the Australian Government's Super Science Initiative as financed from the Education Investment Fund.

Andrew Fellowes (AE): Aca-Nation

Authorities, Australia (NATA)-accredited clinical diagnostic laboratory, we provide pathology reports for clinicians at Peter Mac and other hospitals for use in making patient treatment decisions. We are also interested in novel testing technologies and platforms for developing and transferring tests to the clinic. Turnaround time for these tests is critical. Speed can be of the essence when treating cancer, so this is especially important for our big research study, Cancer 2015.

AF: Cancer 2015 is a large

It involves collecting samples from 10,000 cancer patients, and will test the hypothesis that classifying tumors by their molecular profile can provide a better model of cancer diagnosis and treatment than traditional pathology. As a cohort study, it also collects epidemiological data and follows patient outcomes and subsequent quality of life. It may change the paradigm of traditional tumor classification and treatment to one based on a molecular profile.

introduce huge advantages in simplifying and streamlining the workflow, enabling us to deliver results within a clinically relevant time frame.”



next-generation sequencing technology and provides clinical diagnostics services at Peter Mac in Melbourne, Australia.

AF: When Cancer 2015 was first being designed, it was e

that we would run the Sequenom OncoCarta assay on these clinical study samples. OncoCarta has been a very successful research tool. But, thanks to great efforts of Illumina and those of us here at Peter Mac, we were instead able to bring a next-generation sequencing assay into the equation in time for the beginning of Cancer 2015.

AF: In cancer diagnostics, we generally sequence whole genes

change in those regions. A screening or a sequencing approach is the most popular paradigm for these sort of tests at the moment. For Cancer 2015 we needed to adopt a molecular profile assay that was easy to perform with a fast turnaround time, comprehensive in terms of covering all clinically relevant targets that predict success of a particular drug or prognosis, and useful for monitoring. We're only just beginning to develop these assays. With TruSeq Custom Amplicon we'll be able to profile tumor samples for a wider range of mutations and with greater speed and sensitivity than is possible with our current technology.

Q: Are there limitations with current technology?

AF: With current methods, we can't do much multiplexing. There are some tumors that are consistently negative; even when we throw all the screening tests at the tumor, they continue to not show a mutation. In those cases, clinicians often ask us to run a panel like the OncoCarta on it. By and large, we haven't had a great deal of success at finding driving mutations in those tumors. There's the possibility that the technology is underpowered, or too specific, or the assay requires more material. Basically, there's a mismatch somewhere between the assay and the real world situation.

Q: What makes the MiSeq system a good fit for clinical labs?

AF: We've done some pilot studies comparing the MiSeq system with a similar technology. In comparison, MiSeq provides a major step forward in terms of turnaround time in the laboratory. One of the most critical issues once a sample arrives in the laboratory is the complexity of the workflow. MiSeq and TruSeq Custom Amplicon introduce huge advantages in simplifying and streamlining the workflow, enabling us to deliver results within a clinically relevant time frame. We're confident we can now deliver a comprehensive tumor profile within one week.

Q: Can you discuss your experience preparing libraries for TruSeq Custom Amplicon and the MiSeq system?

AF: MiSeq has a lot of efficiencies with cluster generation that other instruments, particularly those based on emulsion PCR, are lacking, making it a very streamlined step. Because TruSeq Custom Amplicon is a single-tube assay, it's really easy to use. It takes only a few steps and can easily be achieved on a 96-well plate format with multichannel dispensing. Other library preparation methods require more input material to ensure sufficient amounts at the end of each step. In addition, we often have to normalize and balance quantities, particularly after pooling samples. In TruSeq Custom Amplicon this is achieved very elegantly with the use of magnetic beads, which intrinsically bind the same amount of material from each sample. Then we just pool volumetrically. We're confident that each sample then has the same amount of library in it. These nice innovations make it a very tidy, single-day workflow. A single-day library preparation workflow and MiSeq single-day sequencing go hand in hand.

Q: How was the observed uniformity and coverage?

AF: We would expect uniformity to vary across the amplicon panel because all the amplicons are in competition in one reaction, but the uniformity and coverage are really quite tight. The specification is there, 80% of the amplicons are within 0.2x of the mean. In fact, we observed better than that. Being able to process so many samples with so many targets, and doing it with relatively even coverage is impressive.

"Illumina has built on its experience... They have listened—we need rapid results and more on-board instrument functionality."

Q: What do you see as the benefits of the TruSeq Custom Amplicon assay?

AF: In my opinion TruSeq Custom Amplicon on MiSeq clearly raises the bar in the custom cancer panel space. It seems to be the best in terms of performance, design, and laboratory workflow, and it runs on what I believe to be the best platform.

Q: What do you see as the benefits of the MiSeq system?

AF: Illumina has built on its experience with the Genome Analyzer® II and HiSeq® systems and seems to have got it right. They have listened—we need rapid results and more on-board instrument functionality. It's not like using a capillary sequencer. There's very little to do on it. The Illumina chemistry progressing to longer reads gives us more information per run. The combination of TruSeq chemistry and MiSeq instrumentation works really well.

Q: Why do you prefer targeted resequencing over whole-genome sequencing?

AF: People say that whole-genome sequencing will become so inexpensive that it will be worth it to just do it. I am of the view that doing this buys a whole lot of trouble because our knowledge of genes, mutations, cells, and pathways is not yet mature enough to know how to interpret all the information we're going to get from whole genomes. I have no doubt that doing whole genomes on tumor biopsies and even plasma DNA will be possible in ten years time, but I really feel that we won't be in a position to comment sensibly on those findings. Clinicians need highly processed information so I believe targeted resequencing will continue to have a place for many years to come.

Q: How do you see clinical cancer genomics research changing with the introduction of MiSeq and TruSeq Amplicon?

AF: If studies such as Cancer 2015 have the anticipated outcomes, I believe molecular profiling is going to change the way tumors are classified and the way treatment decisions are made. It won't matter whether we're looking at lung or brain or colon, labs are going to be able to identify an EGFR, a KRAS, or a PI3K mutation and that's what clinicians will be able to base their treatment decisions on. It's potential is quite revolutionary.

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