Rapid Detection of Drug Resistance Mutations in Microbial Pathogens

Dr. Corbeil at Laval University uses the MiSeq® personal sequencer and Nextera® XT DNA Library Preparation Kits to analyze low-frequency mutations in microbial pathogens.

Dr. Jacques Corbeil is Full Professor and Canada Research Chair in Medical Genomics at the Infectious Diseases Research Center, Faculty of Medicine at Centre Hospitalier Université Laval (CHUL) in Quebec, Canada. His lab uses next-generation sequencing to decipher host–pathogen interactions such as those observed in bacterial and viral infections. They seek to understand the molecular mechanisms of infection in order to improve diagnostics and contribute to the development of new therapies and vaccines.

Q: What are the goals of your research?

Jacques Corbeil (JC): I've been studying the host–pathogen relationship of infectious diseases most of my career, primarily in HIV. More recently, I've become interested in microbes like the parasite *Leishmania*, the bacterium *Streptococcus pneumonia*, and a few others that are important to public health. I've also done some metagenomic research, primarily looking at the effect of antibiotics on the human microbiome. Furthermore, I have looked at bacterial mats in the Arctic and Antarctic to see what bugs were common, what bugs were different, and the similarities in the functions they performed.

Q: How do you see the MiSeq system advancing your research?

JC: MiSeq is the right size for us in the microbial world because we deal with genomes much smaller than the human genome, millions versus billions of base pairs. Because the output of the MiSeq is so great, I can sequence 20, 24, or 96 microbes at a time. It allows us to look at drug resistance mutations and population dynamics between wild-type and resistant microorganisms in blood, nasopharyngeal, and cerebrospinal fluids. MiSeq gives us a handle on low-frequency mutations because it can sequence to such high depth. For instance, with the Nextera XT kit, you can ask questions about a particular base pair at one position. You can sequence it 30,000 times and easily detect very low-prevalence mutants, those present at 1–2%. You have confidence that they're really there, not just the result of sequencing errors or biases introduced by PCR amplification or other processes.

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Jacques Corbeil, Ph.D., author of 88 articles in international journals, works extensively in the infectious diseases and diagnostic fields to understand host–pathogen interactions.

Q: What samples have you sequenced with the Nextera XT kit and MiSeq system?

JC: In collaboration with our public health institute in Quebec, we looked at plasma of individuals infected with HIV. We sequenced three proteins from the *pol* gene—reverse transcriptase, protease, and integrase—and monitored the mutations. Using correlations between certain genotypes and drug resistance, we could inform which drug to give the patient. For instance, if we knew that the HIV strain a person carried was resistant to Zidovudine, we could recommend another drug.

Q: What technologies did you previously rely upon and how do they compare to the MiSeq system?

JC: We used Sanger sequencing, but it was painful. Some applications we didn't do because it was just too cumbersome. MiSeq changed the scales. With Sanger sequencing I could pick up a 50/50 mutation, 50% wild-type or 50% mutant, maybe 25/75, but I could never pick up a minor population around 2% like I can with MiSeq.

For pathogen detection we designed qRT-PCR assays to determine the presence of the most common mutations, but with PCR you have to know what you're looking for. MiSeq is sensitive and quantitative so you'll see all the mutations you know about and also may learn about new ones. That's quite important. MiSeq is really changing how we

do experiments, enabling us to do projects we couldn't do before. In the microbe world it's starting to have a big impact. New technology brings new knowledge and that's what we're going after right now.

Q: Can you discuss your experience with the Nextera XT kit?

JC: The Nextera XT kit worked the first time we used it, provided reproducible results, and fit well in our workflow. There's a vast improvement in speed. Using the Nextera XT kit, our process went from several days to only half a day. That's just 4 hours for 96 samples. People in our lab love it.

We can start a small program and sequence 5 to 10 samples from a patient, get a perspective, and then easily move to sequencing samples from 200 to 300 patients on the HiSeq® system. In microbiology, it's easy to scale up from a retrospective study. For a microbiome, you can go from the MiSeq to one or two HiSeq runs and do complete studies very quickly.

Q: How is the quality of the data from the MiSeq system?

JC: The data quality is good. We obtained the promised specs. MiSeq output is over 1 billion base pairs right now, moving to 7 billion by the end of summer, and hopefully up to 15 billion by the end of 2012. I will be able to do most of my experiments on microbes on the MiSeq instrument.

Q: How does the MiSeq system compare to other next-generation sequencing technologies that you have used?

JC: We used 454 for my metagenomic project, sequencing bacteria in the Arctic. While it gives longer reads, we had issues with homopolymer stretches. This is something that is common with serial addition chemistries, but we don't see it with Illumina SBS chemistry*. Since we were looking at largely unknown microbes, we couldn't correct it. For true *de novo* sequencing, we don't want anything that has too much of a systematic error in it, which is why we are so excited about using the HiSeq and MiSeq systems.

Q: How will MiSeq enable major changes to pathogen detection and microbial sequencing in public health surveillance?

JC: I can see this being helpful in public health surveillance because now we can study new generations of mutants that may emerge. We can look at microorganisms and see if new strains appear, like H1N1 for influenza, and assess treatment. We can monitor mutations pretty rapidly with the Nextera XT kit and MiSeq.

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For example, we vaccinate children to prevent *Streptococcus pneumonia* respiratory infections. *S. pneumonia* has 92 serotypes which are identified using antibodies. We devised a PCR microarray assay to recognize positive serotypes using genotypic information. But we can't do it perfectly, we can only recognize 51 serotypes very specifically. When Prevnar 7 was introduced in 2001, all of the strains in the vaccine disappeared from the population, but were replaced by others. The new vaccine, Prevnar 13, includes all of the previous strains, so that they don't return, plus new ones that have emerged. Now, instead of doing a PCR microarray to identify serotypes, I think I'll use sequencing to match the serotypes to genotypes and monitor the niche emergence of other *S. pneumonia* strains.

Now, using the HiSeq system, we are investigating happens to a patient's microbiome, both aerobic and anaerobic flora, when an antibiotic is given.

Q: What advice do you have for researchers currently try to choose one sequencing technology over another?

JC: When you're making your choice, test the instrument, or go see the instrument work in somebody else's lab, and see how suitable it would be for your own experiments. You can't believe everything that the suppliers tell you. I'm not saying that about Illumina, I think they're pretty good. Most scientists won't believe claims until they're proven anyway.

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

* Sequencing by synthesis (SBS) chemistry is the basis of the technology used by all Illumina sequencing systems.

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