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In Sequence

The Inside Read on Genome Sequencing

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Q&A: Mount Sinai's Milind Mahajan on Running a CLIA-Certified Genomics Core Facility



Name: Milind Mahajan

Position: Director, genomics core facility, and associate professor of genetics and genome sciences, Institute of Genomics and Multiscale Biology, Mount Sinai School of Medicine, since 2011

Experience and Education:

Associate director, Yale Center for Genome Analysis, Yale School of Medicine, 2009-2011; Scientist, Yale School of Medicine, 1997-2009; Research fellow, Colley's Anemia Foundation, 1995-1997; PhD in biochemistry, Indian Institute of Science, Bangalore, 1993; BS in biology, Osmania University, India, 1983

SINCE HE BECAME DIRECTOR of Medicine's genomics core facility in 2011, Milind Mahajan has significantly grown the lab's sequencing capacity, adding several types of platforms.

The facility currently has three Illumina HiSeq 2000s, two HiSeq 2500s, one MiSeq, one Pacific Biosciences RS, and one Ion Torrent Proton on site.

In addition, the lab is equipped with an Applied Biosystems 3730xl Sanger sequencer, an ABI 7900HT real-time PCR machine, and Illumina HiScan and BeadXPress microarray platforms.

For sample preparation and handling, the lab has a Covaris

sonicator, two Tecan liquid handling robots for Bead Array hybridization, an Agilent Bravo for Illumina sequencing library preparation, a Beckman BioMek FX for Sanger sequencing and RT PCR preparation, as well as three Agilent BioAnalyzers and an Agilent 2200 TapeStation for DNA and RNA analysis.

Under Mahajan's leadership, the genomics core facility last year became a CLIA-certified laboratory and expects to provide a variety of next-gen sequencing-based diagnostic tests to Mount Sinai patients in the future.

In Sequence visited Mahajan last month and spoke with him about the recent developments. Below is an edited version of the conversation.

Why did you decide to obtain CLIA certification for the genomics core facility?

The main reason is that next-generation sequencing will enter into the clinical diagnostic field, and pretty soon, it will be routine to take a patient's sample, run it through next-generation sequencing and report the results to the patient. Sanger sequencing was used previously and is the gold standard, but that is one test at a time. With next-generation sequencing, you can do many tests in one go. That's the big advantage, and we want to lead in this field. For diagnostic work, we need the CLIA [certification].

Also, clinicians want to use patient samples for clinical research and sequence them under the CLIA regulations, and we have the facility to do that.

Third, in the case of cancer, I foresee that [based on sequencing of tumor biopsies], we can [help] develop personalized therapies.

What were the greatest challenges in becoming CLIA certified, and what did you have to do?

The challenge was that there were no clear-cut guidelines

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about what it takes to run next-generation sequencing as a CLIA laboratory. Even for the New York State Department of Health it was new. We initiated the process sometime in 2011, and at the end of January 2012, we had a meeting with state department representatives.

Lisa Edelmann runs the genetic testing laboratory here at Mount Sinai, which is a CLIA facility, and we became a CLIA lab under their umbrella. That was possible because their building and our building are connected through an underground tunnel, because there needs to be a physical connection. The advantage of that was that we did not need to apply for a fresh CLIA certificate. But we had to train our technicians and explain to them what the rules and regulations of CLIA are, and we set up all the working protocols and all the paperwork according to the regulations by CLIA. In the laboratory, there is a separation between the pre-PCR and post-PCR facility, and we also have designated areas in the lab for research.

How much of your work is research, and how much is diagnostic? Who are your users, and what kinds of projects do they conduct?

Actually, most of it is research right now. We are in the initial stages of getting into CLIA.

Almost all departments use our facility, around 60 PIs this year. Our first priority is Mount Sinai researchers, but we are open worldwide, because Mount Sinai scientists have collaborators in Europe and other places, and we sequence those samples, too.

They work on different projects, for example skin cancer, cardiovascular disease, inflammatory bowel syndrome, autism, different brain and mental disorders.

What types of sequencing do they require?

Mostly, it is whole-exome and transcriptome sequencing. In the last three or four months, people have been asking for targeted capture sequencing, which will become very popular in 2013. For example, they don't want to do the entire exome but zero in on certain areas of the genome, and then they use a large cohort and investigate only that area. The inflammatory bowel and Crohn's disease researchers do 16S sequencing for microbiome studies.

How do you advise your users on what sequencing platform to use for their projects?

Some of the users know what they want, but there are also many users for whom we do consulting. As far as sequencing

is concerned, we ask them what kind of depth they want, and how fast they want their results. If they want greater depth, we advise them to go with HiSeq. If it is 16S sequencing or amplicon sequencing, we suggest going with the MiSeq because it is fast and the lower throughput is enough.

What kinds of bioinformatics services do you provide?

For RNA-seq, for example, we do TopHat and Cufflinks and then Cuffdiff analysis and then give them a list of the differentially expressed genes. With gDNA and exome sequencing, we run the data through the GATK pipeline and give them the VCF files, the list of all the genes with all the SNPs and index. And then the PIs use those data for their discovery.

We also have the Institute of Genomics and Multiscale Biology here, and we facilitate collaborations between lab researchers and our bioinformatics researchers. Many active collaborations have been established this year.

How is the core facility funded?

We are becoming fairly big now, so we have to become a self-sustaining core facility, and for that, the model is fee for service. We are not expected to make a profit, we are non-profit, but we are expected to not run at a loss, also.

How do you decide to bring in a new technology?

It depends on whether there is a paradigm shift in the technology. For example, we decided to get the Ion Torrent Proton when they announced they would have a \$1,000 genome in a day's time. That was a big attraction, because in a clinical setup, time is important. I thought it would be good to go with this machine, test it, and see whether we can actually deliver around 90 Gb to 120 Gb of human genome data in a CLIA facility. That would be great, because if there is an emergency case, sample preparation for gDNA takes one day, immediately we can load it onto the sequencer and get the data in two days.

Would that be an advantage over Illumina's HiSeq 2500?

We thought it would also be a good idea to have the 2500 [fast-run] facility on our HiSeqs and then see which one works better. We need good quality data to get the best Q30 values so that we don't get false positives. That technology will prevail – we will be using that routinely for the CLIA work. If both perform well, we will be doing sequencing on one platform and the other platform will be for validation.

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In a presentation you gave in the summer of 2012, you mentioned Oxford Nanopore under “future plans.” What’s your thought on that?

Oxford Nanopore is a fantastic technology; it looks very good on paper. The major bottleneck with current sequencers is the sample preparation; cost remains high because of sample preparation. If you eliminate sample preparation, cost will come down significantly, and Oxford Nanopore, as far as I know, does not require or requires very minimal steps of sample prep. Also, it is a small device. When it comes to market, we’ll go for it. But I don’t know when it will become available, in a real sense.

How is the genomics core facility interacting with the New York Genome Center?

The interaction is very nice in the sense that our model is to develop genomic medicine and next-generation-based diagnostics for patients at Mount Sinai. We have a very strong in-house facility, and our presence will improve the

genomics know-how in research as well as at the clinical level. For example, we are working with people at Mount Sinai on developing techniques for difficult, non-routine samples, for example FFPE samples, or low-throughput samples, or samples that are partially degraded. That’s why our presence is very important here at Mount Sinai.

The New York Genome Center is a fantastic facility for high-throughput, big projects. We will also be working with the New York Genome Center in research and technology development. And if there are any high-throughput projects where we can’t handle the volume, we will get it done at the New York Genome Center.

Is there anything you wish to add?

Our goal and our philosophy has been that we should be a central hub of genomics activities at Mount Sinai and develop clinical genomics at Mount Sinai. We are not a routine core laboratory, we moved from the routine core laboratory to a genomics facility.