Genome Center Offers NGS Applications to Support Microbiome Studies

Dr. Thomas Sutter selected the Illumina MiSeq® System and Nextera® XT kits to expand the capabilities of the University of Memphis genome center.

Thomas Sutter, Ph.D., is the Director of the W. Harry Feinestone Center for Genomic Research and the Feinestone Chair of Molecular Biology at the University of Memphis. He conducts research in the area of cancer causation and prevention in addition to running the university's genome center. Dr. Sutter opened the center with the goal of making a broad base of technology accessible to investigators across the campus. Last year, Dr. Sutter wanted to develop next-generation sequencing (NGS)-based genomic applications and data analysis that would assist researchers with discovery and in obtaining grant funding.

A typical project for the genome center involves resequencing small genomes (~5 Mb) such as microbiome samples and PCR amplicons. Some bacterial strains are difficult to culture making it challenging to obtain sufficient DNA for sequencing. Others are notorious for high nucleotide repeat regions, which can introduce bias in library preparation kits requiring enzymatic fragmentation. "We wanted a system that ensured correct loading of high-quality DNA and one that delivered the highest-quality results," explains Dr. Sutter.

Dr. Sutter and his colleagues evaluated the Illumina MiSeq® System and the Nextera® XT DNA Library Preparation kits. The MiSeq System's industry-leading data quality, accuracy, and streamlined workflows are ideal for resequencing small genomes and amplicons. When combined with Nextera XT kit's rapid library preparation and minimum hands-on time, sequencing results can be obtained in just a day and a half. By implementing these Illumina technologies, Dr. Sutter found that he progressed faster in reaching his goals than he expected.

Q: What challenges did you face when introducing NGS to the genome center?

Thomas Sutter (TS): Before we moved to NGS, we were mainly a microarray facility conducting gene expression analysis. It soon became clear that the field of genomics was moving to NGS. For our center to thrive, it was imperative that we quickly adapt NGS technology to meet the diverse needs of our researchers and center members, who range from evolutionary biologists to biomedical engineers. We wanted to provide applications to address a multitude of needs, and assist our investigators in obtaining grant funding.

Q: Why did you decide to go with the MiSeq System?

TS: We were new to the NGS area and wanted a system that we could learn to use and implement quickly. We looked at all of the existing technologies and wanted to avoid having to combine different platforms to get the output we desired. We chose the MiSeq System mainly for the quality of its reads and the high Q30 values. Because we wanted to develop all of the applications that the MiSeq can offer, we also liked that Illumina products provide a convenient, one-stop shop for NGS applications. The Illumina platform continues to deliver expanding read lengths and mate pair capabilities along with its ease-of-use. I believe that we made the right choice.

Thomas Sutter, Ph.D., is the Director of the W. Harry Feinestone Center for Genomic Research and the Feinestone Chair of Molecular Biology at the University of Memphis in Tennessee. With the MiSeq System and the Nextera XT DNA Library Preparation kit, NGS technologies were introduced into the university's genome center in less than a week.

Q: How long did it take you to implement the Illumina NGS platform?

TS: The transition to NGS went very smoothly. The Nextera XT kit was introduced when we bought the MiSeq System. The Illumina field application scientist (FAS) came in for a two-day training to help get us started. The following week, we were sequencing 2 million base pair bacterial genomes. In just two days, we were up and running. The MiSeq has given us a good opportunity to learn the Illumina chemistry, become proficient at library construction, and build the back end for data analysis and storage.

"Nextera XT allows our investigators to spend less time making libraries and more time making discoveries."

Q: What features led you to choose Nextera XT library preparation?

TS: There are several features that attracted us to Nextera XT. The accommodation of low DNA input down to 1 ng was of particular interest to us. No matter how easy it is to obtain samples, they're always precious and we never want to use more than we have to. We particularly liked the Nextera bead-based normalization. The bead step eliminates the real-time PCR step and the validation with a Bioanalyzer that's necessary for normalizing the samples to ensure proper DNA loading and high-quality runs. With Nextera XT, when we load bar-coded samples onto the MiSeq flow cell, the reads are uniform and consistent, both within and between preps. Nextera XT has cut down on our library preparation headaches, and our experience with the kit has been extremely positive.

> "With Nextera XT, we consistently get 20 million reads and upwards of 5 billion base pairs from a single MiSeq run."

Q: What applications have you developed?

TS: We've focused our applications on bacterial genomes because a number of our investigators are interested in virulent and disease-related microbes. It is a rapidly emerging area. Thanks to Nextera XT, we've been able to process more microbiome studies. Microbial genome sequencing is relatively straightforward for us now.

We've also started using the Nextera Mate Pair Library Preparation kit with both the gel-free, and the gel-plus protocols, and have been successful in assembling complete, single-chromosome genomes. Having larger mate pair libraries has greatly facilitated the assembly of our genomes. The Illumina approach is cost-effective for whole-genome sequencing, and the Nextera Mate Pair gel-plus protocol has made a significant difference for us in applications requiring a specific mate pair size. We're amazed at what we've been able to accomplish in less than a year.

Q: How has Nextera XT improved your workflow?

TS: In terms of processing time, you can't beat the turnaround time of Nextera XT. Instead of taking one or two days, we can produce 96 libraries in less than a day, and load the MiSeq flow cell at the end of the day. We're a small core facility so our volumes aren't necessarily rate-limiting for us. Nextera XT provides more time for researchers who produce the libraries to work on other applications. Nextera XT allows our investigators to spend less time making libraries and more time making discoveries.

Q: How have the Nextera XT kits improved your data results?

TS: We typically sequence 10 microbial genomes at a time. With the Nextera XT kit, we can process multiple samples up to 96 quite easily. One of our evolutionary biologists is interested in beetles, which have 200–300 million base pair genomes. We were able to generate a complete sequence of an insect for him using the MiSeq System and Nextera XT. The increase in read length and the number of reads make that possible. We consistently get 20 million reads and upwards of 5 billion base pairs from a single MiSeq run. That's amazing when you think about it.

Q: How do you measure the quality of the sequence data?

TS: The Q30 value is important to us and we've obtained long read lengths with high Q values using the Nextera XT kit. Generally, we get one error in 1,000 base pairs, which is consistent across most of the read length, especially on the first read. We also look at the distribution of the library. When we sequence genomes that have a reference genome, we simply align our reads to that reference sequence. Bacterial genomes can be difficult to sequence due to high nucleotide repeat regions. Because the Nextera XT fragmentation process reduces enzymatic bias in repeat regions, we get better library diversity, producing a distribution that randomly covers the genome without introducing sequence or technology bias. Our initial alignments and quality of data have given us high confidence in the Illumina technology.

Q: What applications do you plan to introduce next?

TS: We'd like to move away from smaller amplicons so we're interested in optimizing long-range PCR. Coupled with the efficiency of Nextera XT, long-range PCR would be an ideal application for resequencing projects and studies involving repetitive sequencing for taxonomy or evolutionary purposes. We've already worked out the Nextera XT protocol and the amplicon sequencing is coming along nicely.

Q: What advice do you have for researchers considering Nextera XT Kits?

TS: Each application brings its own nuances. The Illumina FAS team is really adept at helping to troubleshoot those. After you learn the basics, the rest comes easily, and an Illumina FAS is there to provide training at every step. The Nextera XT kit has been particularly useful for sequencing our microbial genomes and has been a great experience for us. I highly recommend Nextera XT for microbiome studies.

To learn more about the MiSeq Desktop Sequencer, visit www.illumina.com/miseq.

For more information on the Nextera XT DNA Library Preparation kits, visit www.illumina.com/products/nextera_xt_dna_sample_prep_kit.ilmn.

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