

Next-Generation Sequencing Used for Biological Quality Control in BioPharma Production

Dr. Emiliano Toso and his team at Merck Serono use Illumina next-generation sequencing systems for cell line genetic stability testing and biosafety in-process monitoring.

Introduction

Emiliano Toso, Ph.D. is Head of the Molecular Biology Group of Merck's Biological Quality Control (BQC) department. His team is responsible for the development and validation of methods to ensure that the company's pharmaceutical production is compliant with GMP guidelines. Biopharma quality control applications include genetic stability testing of recombinant cell lines, viral and mycoplasma safety testing, and microbial identification. These tests are based on *in vivo* and *in vitro* cell-based assays and biomolecular methods, which are required by regulatory agencies to register and release a new bioproduct.

Dr. Toso is a pioneer in the development and implementation of molecular biology techniques in pharma quality control. In 2000, he spearheaded the implementation of PCR-based methods and in the intervening years has introduced a broad range of technologies into the department, including FISH, blotting techniques, Sanger sequencing, and real-time PCR. More recently, he oversaw the addition of Illumina next-generation systems (NGS) into Merck's quality control testing technology portfolio.

iCommunity spoke with Dr. Toso, Chiara Modena, Ph.D. (Head of the Genetic Stability Lab), and Fabio La Neve, Ph.D. (Head of the NGS Lab) about how they developed and implemented a complete NGS quality control testing pipeline, from sample preparation to data analysis.

Q: Why did you consider NGS for biopharma production quality control?

Emiliano Toso (ET): We started exploring NGS in 2010 when we realized that this technology was a powerful tool for biosafety and risk monitoring. These are areas where we need fast and reliable methods



Led by Dr. Emilano Toso (center), the pioneering Head of Merck's BQC department, Dr. Chiara Modena and Dr. Fabio La Neve implemented an NGS quality control testing pipeline based on use of Illumina sequencing systems.

to avoid large-scale contamination. NGS combines the benefits of traditional methods with a broader detection range, faster response time, and higher sensitivity.

Q: What made you choose the HiSeq[®] System to meet your biosafety requirements?

ET: Among all the NGS platforms available in 2011, the HiSeq System was the only commercially available system that guaranteed the level of sensitivity required to detect viral contamination in cell lines and bulk harvests. Other technologies, such as real-time PCR, require specific assay design to detect already known viral targets. In contrast, NGS on the HiSeq System delivers a large amount of highly accurate data for the unbiased and reliable detection of DNA and RNA viruses.

Q: Why did you expand your NGS portfolio to include the MiSeq[®] System?

ET: Thanks to the flexibility of the technology, we realized that NGS could be used in other application areas such as cell line genetic stability testing. We evaluated different benchtop NGS systems available on the market to see which one fit our needs. Thanks to its faster turnaround time, longer read lengths and paired-end sequencing, higher sensitivity, and overall reduced running cost, we chose the MiSeq System. It is the most suitable system to obtain reliable and accurate genotypic characterization of our cell lines and, ultimately, to become a standard routine technology for cell bank validation.

Q: What are the benefits of running both the MiSeq and HiSeq Systems?

Chiara Modena (CM): Having access to NGS technologies with different technical specifications, offers multiple advantages. Biosafety monitoring and cell bank characterization have completely different requirements related to the amount of data generated, read length, and turnaround time. We now have the flexibility to use either platform, depending on our experimental needs.

Merck's BQC also performs other applications, such as transcriptome analysis, which previously were outsourced to a service provider. We can now perform these tests in-house, reducing turnaround time and overall cost. Ultimately, the joint use of the two systems provides the flexibility to work on organisms very different in terms of size and complexity, from viruses to mammalian cell lines.

Q: Can you describe the NGS workflows implemented in your lab?

CM: The genotypic characterization of cell lines requires a specific sample preparation protocol to amplify the region of interest based

on long-range PCR, followed by a library generation method (Illumina commercial kit) to prepare the samples for sequencing. Through this workflow, both the transgene and the flanking regions can be characterized simultaneously without the need of time consuming individual primer design typically associated with Sanger sequencing.

Biosafety monitoring to detect viral contamination requires high sequencing coverage to reach the level of sensitivity needed to detect adventitious contaminating agents. For this purpose, we developed a new library preparation protocol and data analysis pipeline for the unbiased and sensitive detection of any DNA and RNA viruses in our production line. We are also implementing a GMP validation for both application areas.

"NGS enabled us to reduce the overall time-to-answer by 50% at a significantly reduced cost compared to traditional methods."

Q: What have been the main challenges in implementing NGS workflows into your daily routine?

Fabio La Neve (FLN): We evaluated different strategies and methods to identify the most suitable sample and library preparation protocol to work in concert with a specific data analysis pipeline. The workflow had to be flexible and robust enough to work on different matrices and sample types based on a validation procedure that is unique compared to the clinical or academic settings. At the same time, we were looking at hardware and software qualifications to be compliant with FDA regulations and requirements.

In general, the use of NGS in a strictly regulated environment is challenging. In fact, its use for pharmaceutical quality control depends upon the ability of companies and NGS manufacturers to meet the most stringent Health Authorities regulations.

First, the sequencing and data analysis software needs to be designed to fit the most stringent requirements from regulatory authorities worldwide, such as 21 CFR part 11. Another crucial point is related to the instrument validation and maintenance. The IQ/OQ offered by providers must be integrated with internal test to be in compliance with the Health Authorities needs. Finally, auditors need to be educated about NGS and how to inspect data produced by this innovative technology.

Q: Describe some of the results you have generated with the HiSeg and MiSeg Systems? Did they meet your expectations?

CM: The HiSeq system gave us new insights into the monitoring of large-scale contamination in bioproduction. For example, using viral DNA and RNA genomes to artificially spike mock samples, we were able to detect 10-20 viral genome copies/cell at sensitivity as low as 10 titration units/ml.

For transgene resequencing, the MiSeq System has enabled us to reach much higher levels of sensitivity compared to Sanger sequencing. The limit of detection (LOD) has increased by 5-fold (from around 25% to 5%). Moreover, this system enabled us to reduce the overall time-to-answer by 50% (down to 8 weeks including experimental design, pilot study, and final reporting) at a significantly reduced cost compared to traditional sequencing methods.

The combined use of HiSeq and MiSeq Systems also enables us to perform de novo sequencing and assembly of our cell banks, thus obtaining an unbiased and fully annotated reference genome for subsequent functional annotation of cell lines.

Q: Are you adding new NGS systems to your technology portfolio?

ET: To exploit the flexibility provided by Illumina systems, we are adding a NextSeq[®] 500 System to our NGS portfolio. Introducing this new platform will allow us to match our sample throughput requirements more efficiently, achieving reduced costs while maintaining the same data quality and sensitivity. The NextSeq 500 System can be used as a backup technology with the HiSeq and MiSeq systems.

Q: What is your strategic vision for the Merck BQC department?

ET: The Health Authorities approach concerning authorization and use of new technologies is guite critical. PCR took decades to be fully accepted for mycoplasma detection, demonstrating that a non-structured approach could lead to technology and requirement misunderstandings. The early implementation of a state-of-theart technology allows us to be proactively involved in regulatory requirements and expectations. The introduction of NGS in pharma quality control enables the development of new applications that deliver results that cannot be achieved with conventional technologies.

Thanks to its flexibility, NGS has the potential to be adopted soon for routine testing in the BQC, integrating with, and eventually replacing, conventional methods such as Sanger sequencing and others. This is not only applicable in cell bank validation and control processes, but also in the development of a new host cell line and a new process, offering the biopharma industry a generic tool to revolutionize development and manufacturing divisions.

"NGS can now offer the biopharma industry with a generic tool to revolutionize development and manufacturing divisions."

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