International Alliance for Biological Standardization

4th Conference on Next Generation Sequencing for Adventitious Virus Detection in Biologics for Humans and Animal

Frankfurt, Germany
December 3-5, 2024

Comparison of Long- and Short-Read Sequencing Methods for Recombinant Adeno-Associated Virus Sequence and Adventitious Agent Identification in a GMP Environment

Authors:
- **Megan Gura**
- **Patrick Blatt**
- **Sriramana Kanginakudru**
- **Marissa Martinek**
- **Morgan Hewes**
- **Tyler Dion**
- **Chris Remillard**
- **Devika Torvi**
- **Ping Li**
- **Steven Davis**
- **Jared Richardson**
- **David VanHoute**

Regeneron

Recombinant adeno-associated virus (rAAV) is an emerging class of treatment within the cell and gene therapy field that requires additional testing to ensure the delivery of safe and effective medicine to patients. Under Good Manufacturing Practices (GMP), the Critical Quality Attributes (CQAs) of an rAAV therapeutic must be assessed, including ensuring that the genomic sequence of the rAAV is complete and matches the reference sequence. High-throughput sequencing (HTS) approaches can address multiple CQAs in one experiment such as testing for exogenous contaminants introduced by the manufacturing process. We have developed a bioinformatics pipeline that can accept both long- and short-read HTS data to evaluate CQAs when sequencing rAAV. Using this pipeline, we evaluated both long- and short-read sequencing methods using the Illumina NextSeq 2000 (short-read) and Oxford Nanopore Technologies PromethION (long-read) systems, respectively, to evaluate their strengths and weaknesses. The main goal of these experiments was to determine the sequence identity of the rAAV target(s) tested and identify any sequence variants in our samples. Additionally, we tested if using adaptive sequencing on the PromethION, which can selectively enrich or deplete a chosen sequence, improved the results of long-read sequencing. Secondary goals of these experiments were to evaluate other CQAs such as determining ITR sequence identity and contaminant testing such that this pipeline could be applied to both CQA testing and adventitious agent detection within one HTS method. Multiple sample and library preparation approaches were also tested to gain an understanding of the optimal approach for sequencing rAAV for CQA and contaminant detection. This presentation will provide a comprehensive overview of our findings.