Analysis of recombinant AAV (adeno associated virus) vectors using long read sequencing.

Genomics

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The University of Pennsylvania's Gene Therapy Program utilizes recombinant adeno associated viral (rAAV) vectors in the pursuit of engineering vehicles to deliver therapeutic genetic material, such as a working gene, directly into a cell. For consideration of an IND (Investigational New Drug) filing status, the FDA requires complete sequencing of the vector genome. We currently have a rigorous, short-read sequencing assay in place, but short read sequencing cannot detect every type of variant due to the need to align short fragments. Utilizing long read sequencing, the Nucleic Acid Technologies Core present the results of our efforts to sequence the entire 4500 bp genome of rAAVs using the MinIon from Oxford Nanopore. By comparing against our existing short read assay, we examine how well long read technology performs. Although the library preparation protocol is significantly easier with long read sequencing, we report the challenges that remain to incorporate this technology to our overall assay, in particular supply chain issues. Long read sequencing has the potential to improve the regulatory science for manufacturing these potential drug products, but it is still a developing technology.