Advancements in nanopore sequencing allow in-depth characterization of rAAV vector batches comparable to SMRT™ sequencing

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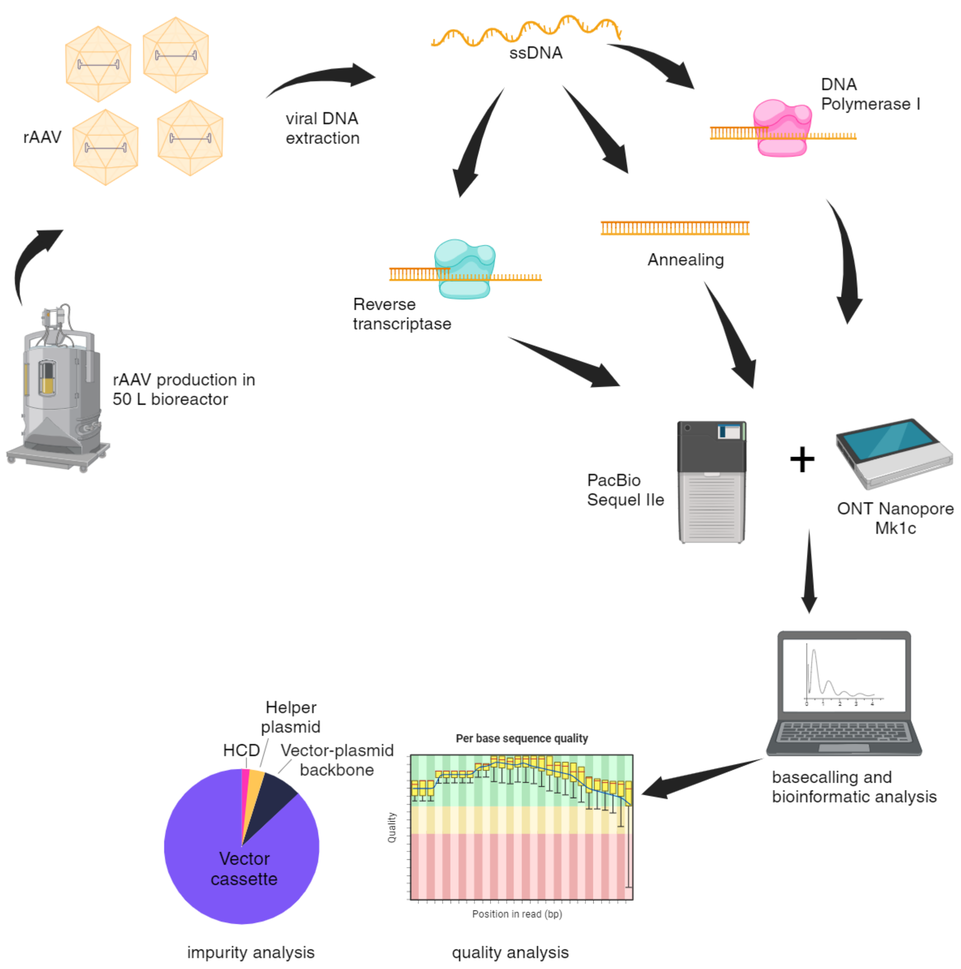
P0153

Abstract

Long-read sequencing is highly suited for thorough analysis of encapsidated DNA, which is crucial for optimizing gene therapy platforms especially with regards to safety. We utilized nanopore sequencing (Oxford Nanopore Technologies) to profile a rAAV9 batch produced in a 50-liter bioreactor using our proprietary EpyQ™ plasmid system. We evaluated three methods for converting single-stranded to double-stranded DNA, all resulted in consistent DNA impurity distributions. Additionally, we compared recent nanopore advancements, including V14 chemistry and dorado basecalling, with the established V9 chemistry and guppy basecalling, observing significant improvements in read quality and alignment accuracy.

Our nanopore data showed high vector quality (> 95% vector mapping) with minimal host cell DNA (HCD) impurities. Comparison with SMRT™ sequencing (PacBio) confirmed similar HCD and helper plasmid impurity levels, though SMRT™ sequencing detected more plasmid backbone reads, possibly due to differences in chimeric read assignment. Truncation analysis identified consistent vector hotspots, in agreement with an independent outsourced analysis based on the SMRT™ data.

Workflow

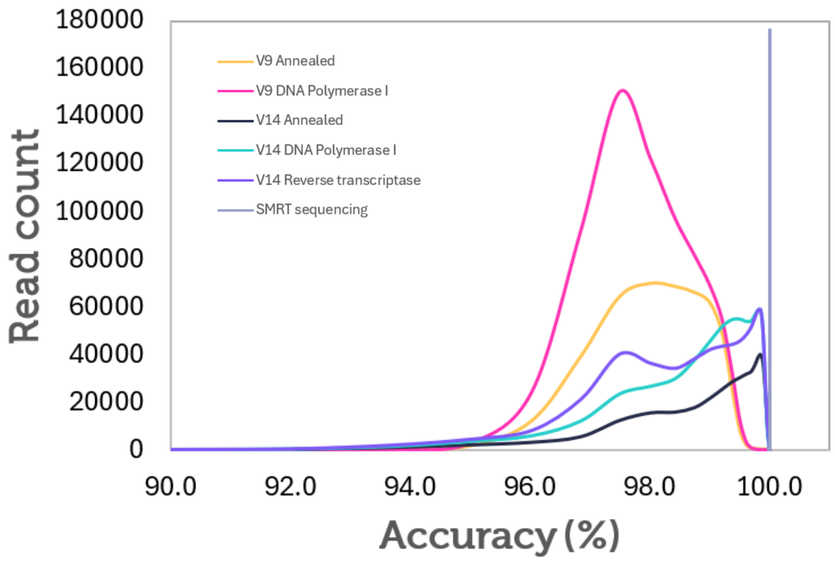


Conversion of ssDNA to dsDNA with

- Annealing
- Reverse Transcriptase
- DNA Polymerase I

For comparison of conversion methods see ASGCT 2023 #1428

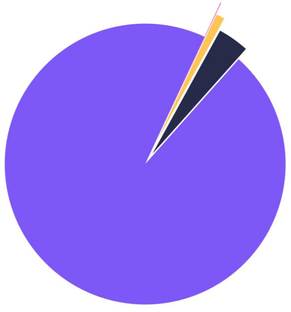
Accuracy of raw reads in Nanopore & SMRT sequencing



- Increased accuracy through improved chemistry and basecalling models for Nanopore technology
- SMRT sequencing unveils remarkable accuracy of raw reads
- SMRT technologies relies on sequencing the same molecule multiple times increasing the accuracy significantly while in comparison ONT relies on a single molecule read

In-depth analysis of Nanopore and SMRT sequencing data shows higher vector backbone impurities for SMRT

Nanopore
AAV9-SEAP annealed



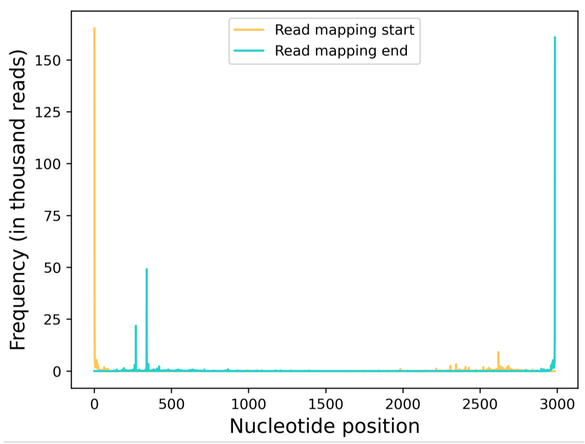
SMRT
AAV9-SEAP annealed

Reference sequence	Nanopore		SMRT	
	Absolute reads	Mapping %	Absolute reads	Mapping %
AAV vector genome	478561	95.24	496852	90.91
Vector plasmid backbone	18536	3.69	41825	7.65
Helper plasmid	5056	1.01	7606	1.39
Host cell DNA	326	0.06	268	0.05
Total	502479	100	546551	100

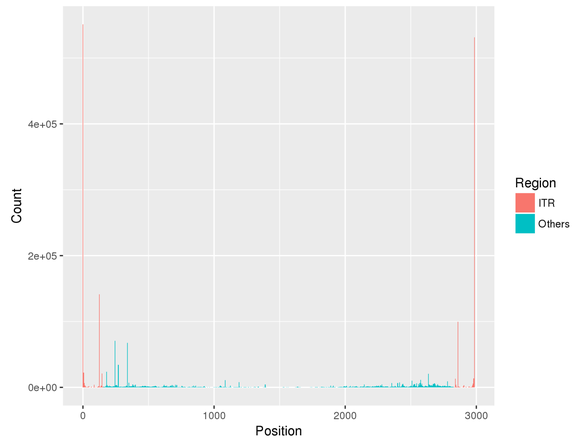
- No great difference was observed for different conversion methods (data not shown)
- Comparable results between Nanopore and SMRT sequencing for main impurities (HCD and helper plasmid impurity levels similar)
- Higher vector backbone impurity of SMRT sequencing possibly due to chimeric read assignment

Truncation hotspot analysis of both sequencing technologies shows only little difference

Nanopore



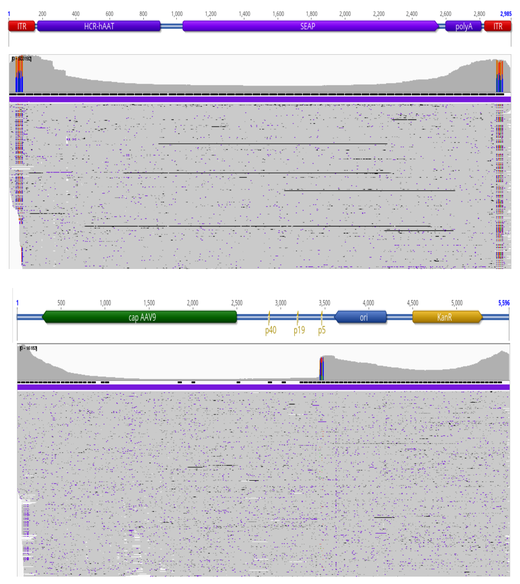
SMRT



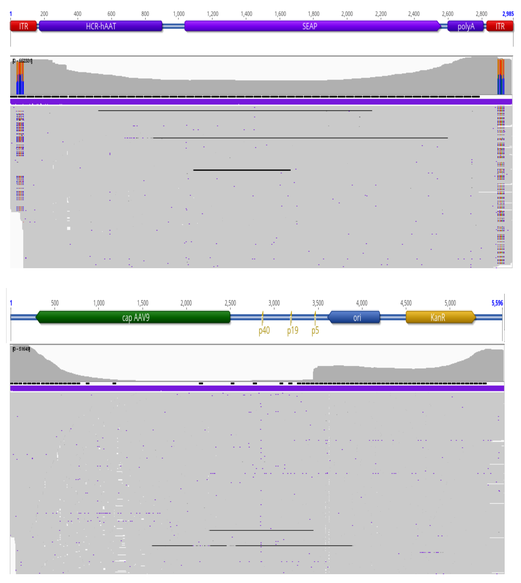
- Very few intermediate read start and end events throughout the AAV vector genome
- Weak truncation hotspots in a highly structured promoter region
- Outsourced SMRT sequencing shows comparable results for rAAV transgene integrity

Coverage plots of AAV vector genome & vector plasmid backbone are comparable

Nanopore



SMRT



Despite a strong reduction in single nucleotide mismatches in SMRT sequencing, the overall mapping coverage pattern was nearly identical

Summary

In summary both sequencing methods provide very similar data that prove the high vector quality of the EpyQ™ manufacturing system. Our research highlights the advantages of nanopore sequencing for the comprehensive analysis of DNA encapsidated in AAV particles. Profiting from recent technological advancements, as well as our internal bioinformatics pipeline, we produced data comparable to SMRT sequencing.

However, nanopore sequencing bears substantial benefits, especially during early development as it requires far lower DNA input and has a very quick turn around time. This makes nanopore sequencing an optimal choice for in-depth characterization of AAV-packaged DNA, combining efficiency and precision in a streamlined workflow.

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