Advanced Sequencing **Approaches for** Comprehensive AAV Vector Characterization WILL ARNOLD PHD



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Executive Summary

Background and Existing Methods

- Background on AAV
- Existing Analytics and Limitations

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ElevateBio's Application of NGS

- Background on Sequencing
- Application of Short Read
- Application of Long Read

Findings and Summary

- Identifying Variants
- Characterizing Structural Isoforms
- Proposed Use Model

AAV is The Dominant Delivery Modality for Gene Therapies

Abstract Analysis



Delivery Systems Being Investigated





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AAV Particles Package DNA Impurities



All Analytic Methods Have Specific Advantages and Shortcomings



Current Methods:

- AUC: Distribution of capsids of various masses.
 - Does not assess identity of DNA.
- ddPCR: Assess molar quantities of specific components of viral genome.
 - Does not assess full genome.
- TapeStation: Characterize size and proportion of DNA after extraction.
 - Does not assess per capsid or identity of DNA.



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All NGS Methods Have Specific Advantages and Shortcomings



	Technology	Capital Cost	Time to Data	Accuracy	Read Length	Output (Gbp)
	Illumina	\$\$ - \$\$\$\$	Days	++++	Short	10's - 1000's
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	Oxford Nanopore	\$ \$\$\$	Hours	+	Long	10's - 100's

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Value of Short Read Sequencing for Sequence Identity



Quantification of Read Mapping



Maynard et al. 2019. Human Gene Therapy Methods.

Value of Short Read Sequencing for Sequence Identity



Long Read Sequencing Provides an Additional Avenue to Understand AAV





Extraction Parameters Influence Vector Integrity



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ElevateBio AAV Pipeline Enables Efficient, Reproducible, and Scalable



Diverse Sequences are Packaged within Capsids



Frequency Distribution of Assigned Types Across AAV Samples

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Not All Long Read Sequencing is Equal

Challenge: No in-house ٠ PacBio



- <\$5K for entry level instrument
- Results: •

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- Similar ssAAV % _
- Slight variability in _ contaminant profiles detected.
- Truncation hot spots are still identified.
- Continuing to optimize identification of ITR-ITR length reads.



Contaminant Profile



2500

5000

7500

10000

Start-End Position (500 bins)

12500

15000

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ElevateBio's Application Schema for NGS & AAV



 Characterize identity, truncations, and contaminant profiles.

Potential for all-in-one sequencing for identity, truncations, contamination profile, and % full length.

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NGS Complements Existing AAV Analytical Tools

- 1. AAV is a critical delivery modality for Gene and Cell Therapy.
- 2. A comprehensive profile of the nucleic acid content of AAVs is critical to safety and efficacy.
- 3. Short read sequencing offers a rapid and affordable route to vector identity.
- 4. Long read sequencing offers novel insight to the composition of packaged DNA molecules.
- 5. Analytic and process optimization remains an evolving and critically important space.

ddPCR	AUC	TapeStation/Electrophoretic	Short Read Sequencing	Long Read Sequencing
VG Titers	Capsid Content Mass	Nucleic Acid Sizing	Sequence Identity Sequence Contaminants	Sequence Identity Sequence Contaminants Sequence Species

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*Presenting



