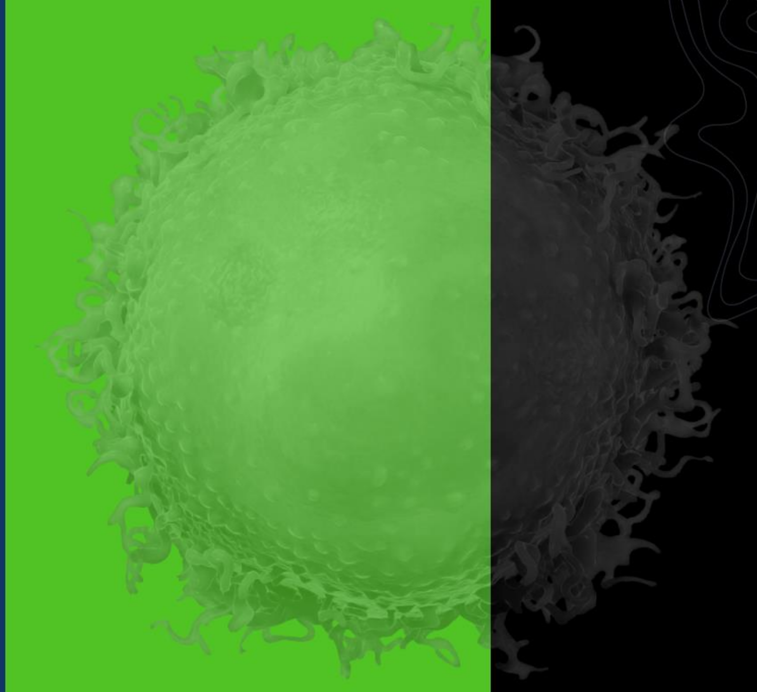
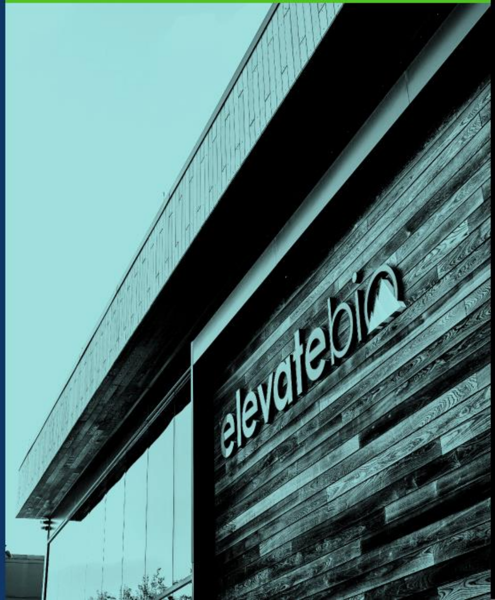
Advanced Sequencing Approaches for Comprehensive AAV Vector Characterization

WILL ARNOLD PHD

elevatebio





Executive Summary

Background and Existing Methods

- Background on AAV
- Existing Analytics and Limitations

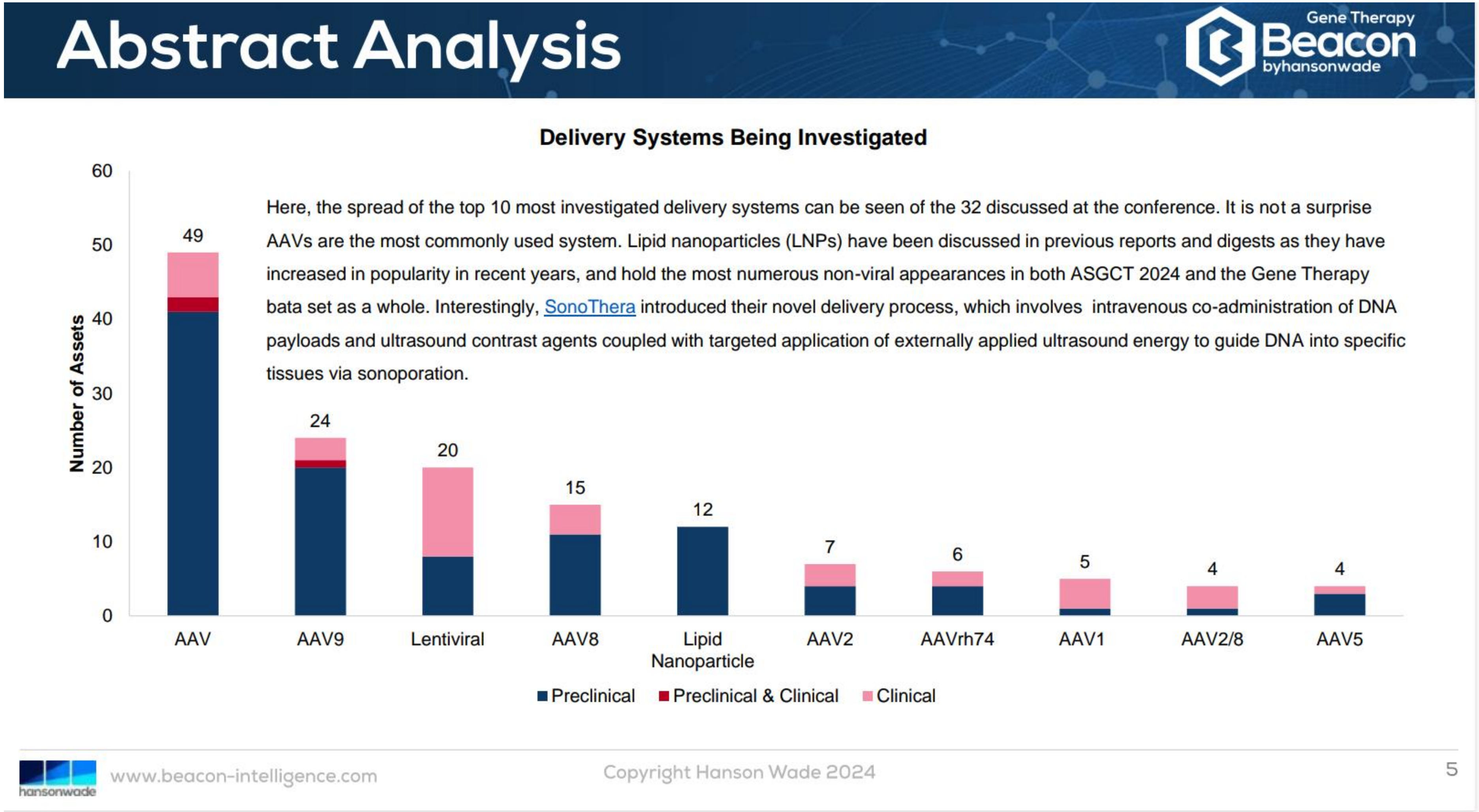
ElevateBio's Application of NGS

- 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

Gene Therapy Beacon byhansonwade

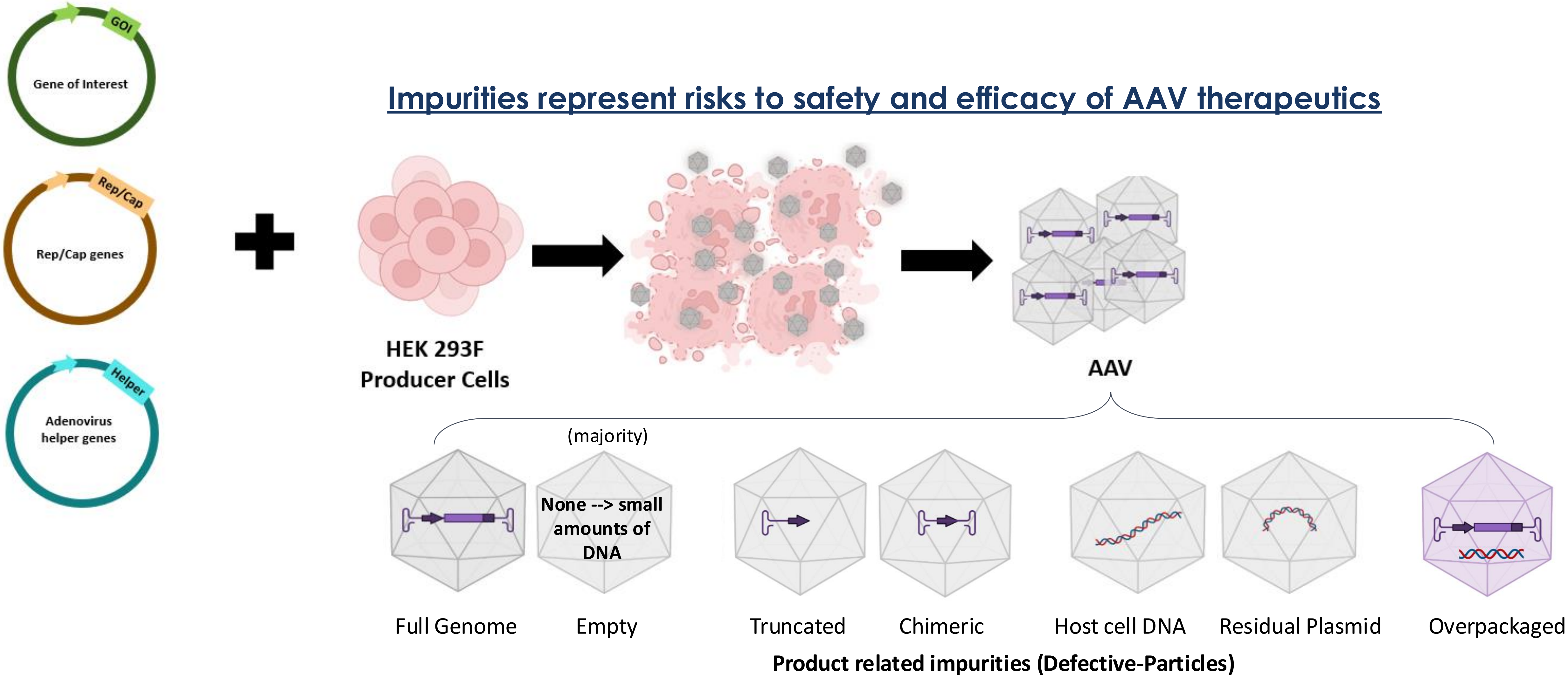
Delivery Systems Being Investigated

Here, the spread of the top 10 most investigated delivery systems can be seen of the 32 discussed at the conference. It is not a surprise AAVs are the most commonly used system. Lipid nanoparticles (LNPs) have been discussed in previous reports and digests as they have increased in popularity in recent years, and hold the most numerous non-viral appearances in both ASGCT 2024 and the Gene Therapy bata set as a whole. Interestingly, SonoThera introduced their novel delivery process, which involves intravenous co-administration of DNA payloads and ultrasound contrast agents coupled with targeted application of externally applied ultrasound energy to guide DNA into specific tissues via sonoporation.

hansonwade www.beacon-intelligence.com

AAV Particles Package DNA Impurities

Impurities represent risks to safety and efficacy of AAV therapeutics



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All Analytic Methods Have Specific Advantages and Shortcomings

AAV Plasmid Synthesis

2L Shake flask (1L production)

Lysis and Endonuclease Treatment

Clarification

TFF

Capture Affinity Chromatography

Ion-Exchange Chromatography

TFF

Sterile Filtration

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.*

VG Titers (ddPCR)

%Full (AUC)

ssAAV quality (TapeStation)

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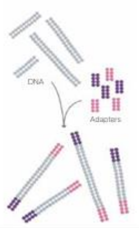
All NGS Methods Have Specific Advantages and Shortcomings

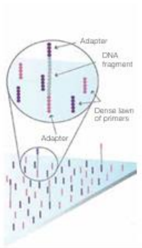
Illumina

Figure 2: Prepare Genomic DNA Sample



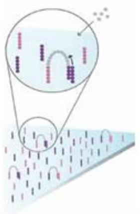
Randomly fragment genomic DNA and ligate adapters to both ends of the fragments.

Figure 3: Attach DNA to Surface



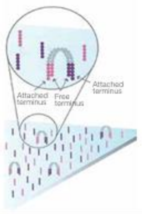
Bind single-stranded fragments randomly to the inside surface of the flow cell channels.

Figure 4: Bridge Amplification



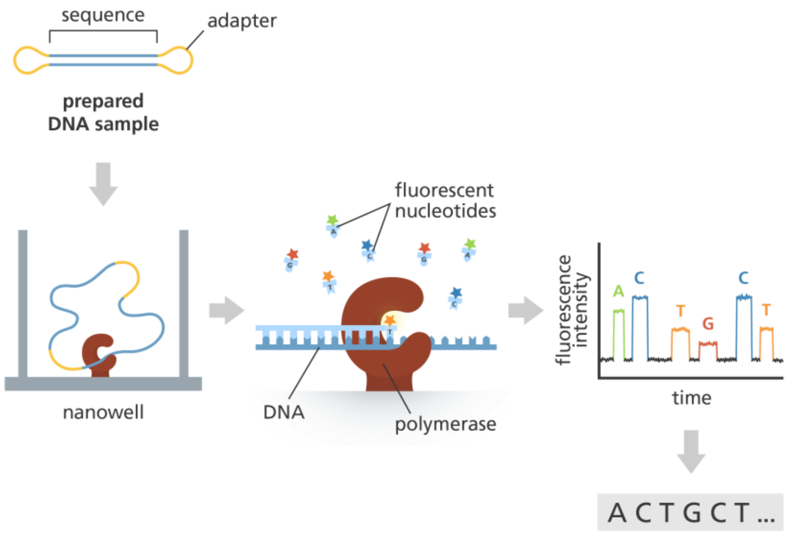
Add unlabeled nucleotides and enzyme to initiate solid-phase bridge amplification.

Figure 5: Fragments Become Double Stranded

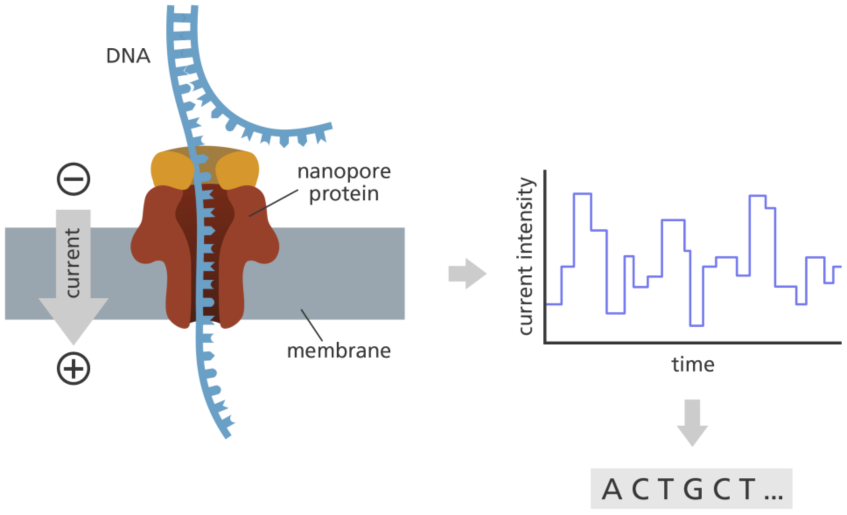


The enzyme incorporates nucleotides to build double-stranded bridges on the solid-phase substrate.

Pacific Biosciences



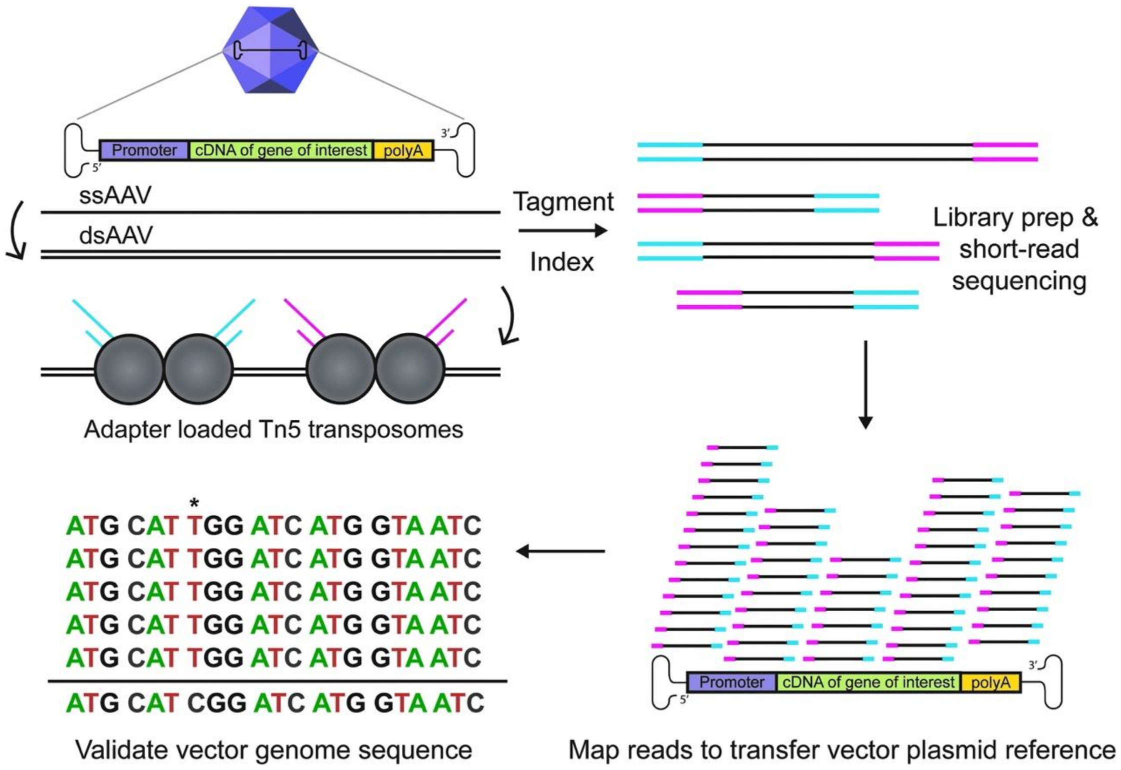
Oxford Nanopore



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

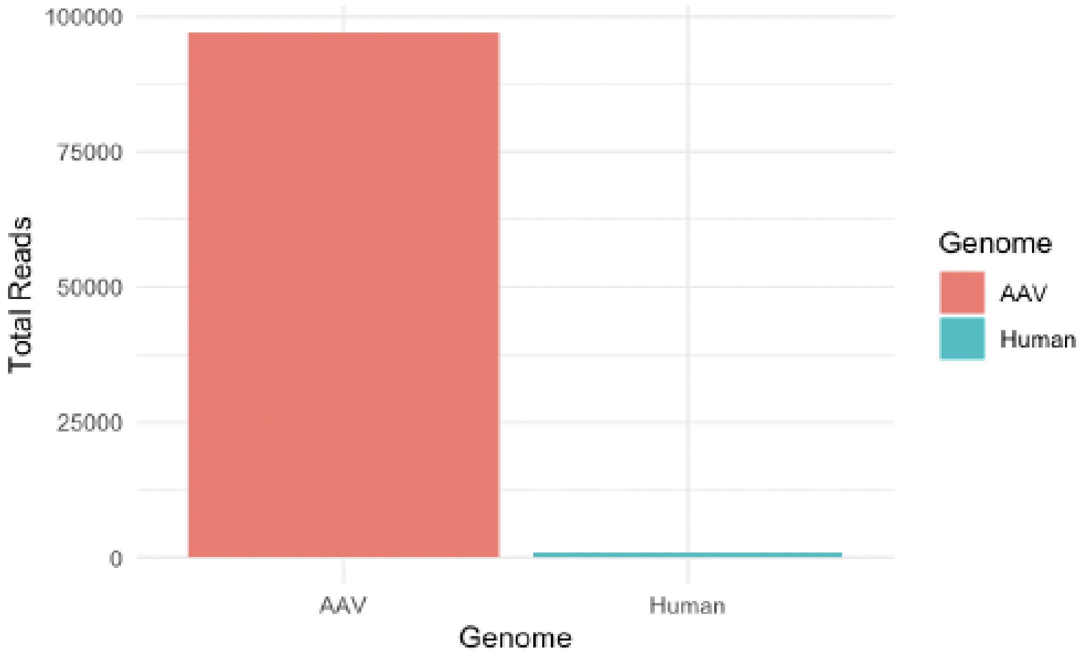
Value of Short Read Sequencing for Sequence Identity

Library Prep



Maynard et al. 2019. Human Gene Therapy Methods.

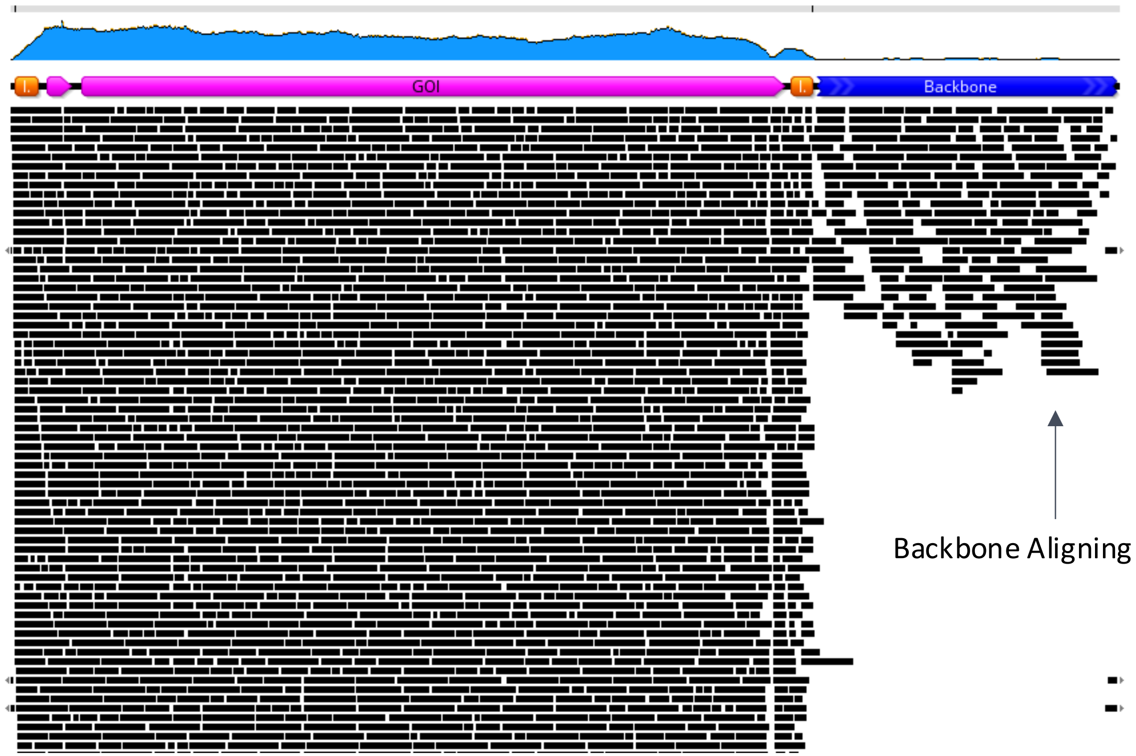
Quantification of Read Mapping



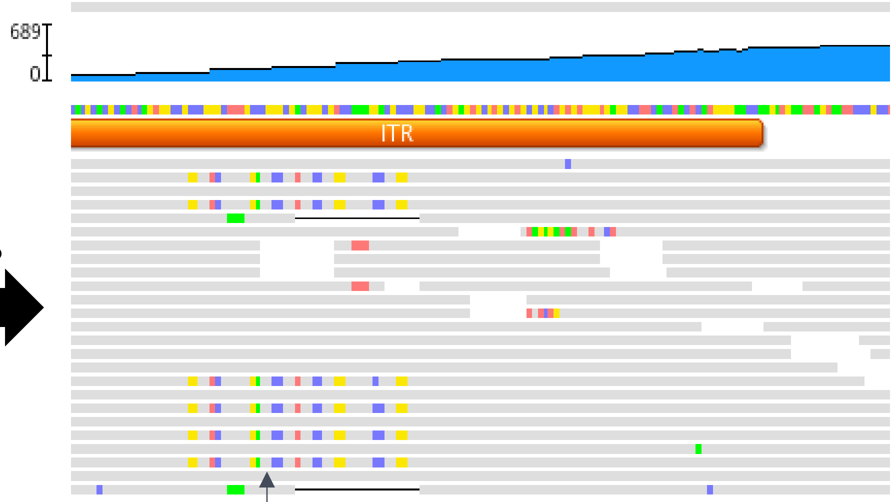
Value of Short Read Sequencing for Sequence Identity

Variant

Coverage

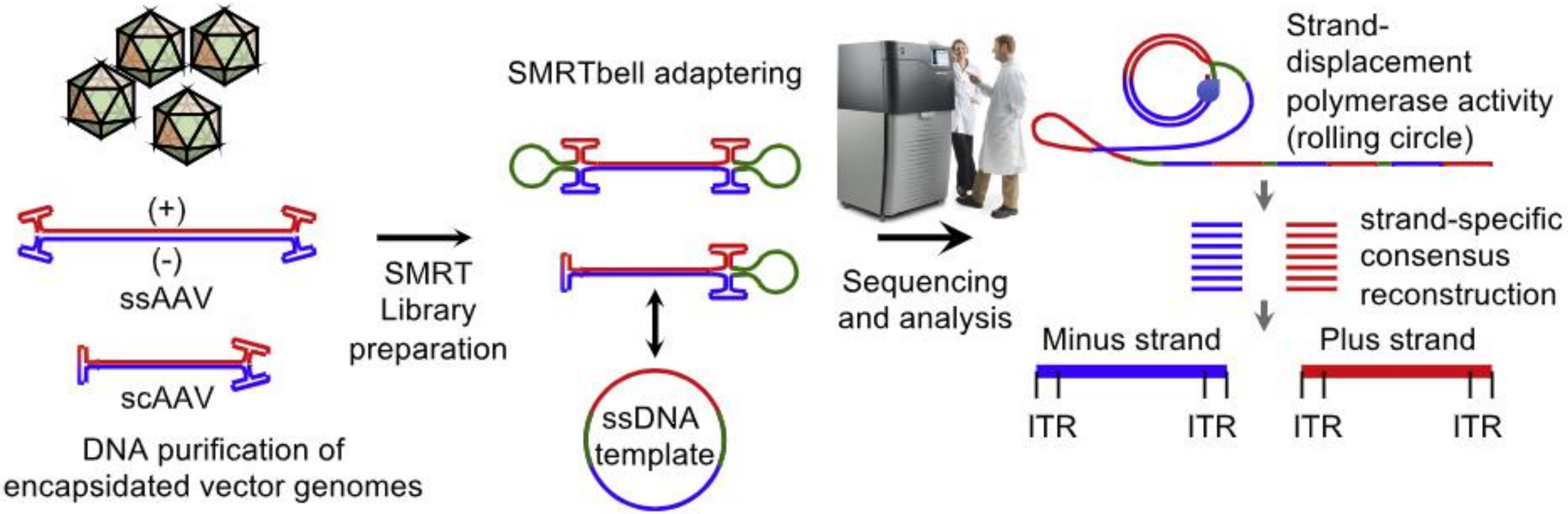


Backbone Aligning Reads



Variant

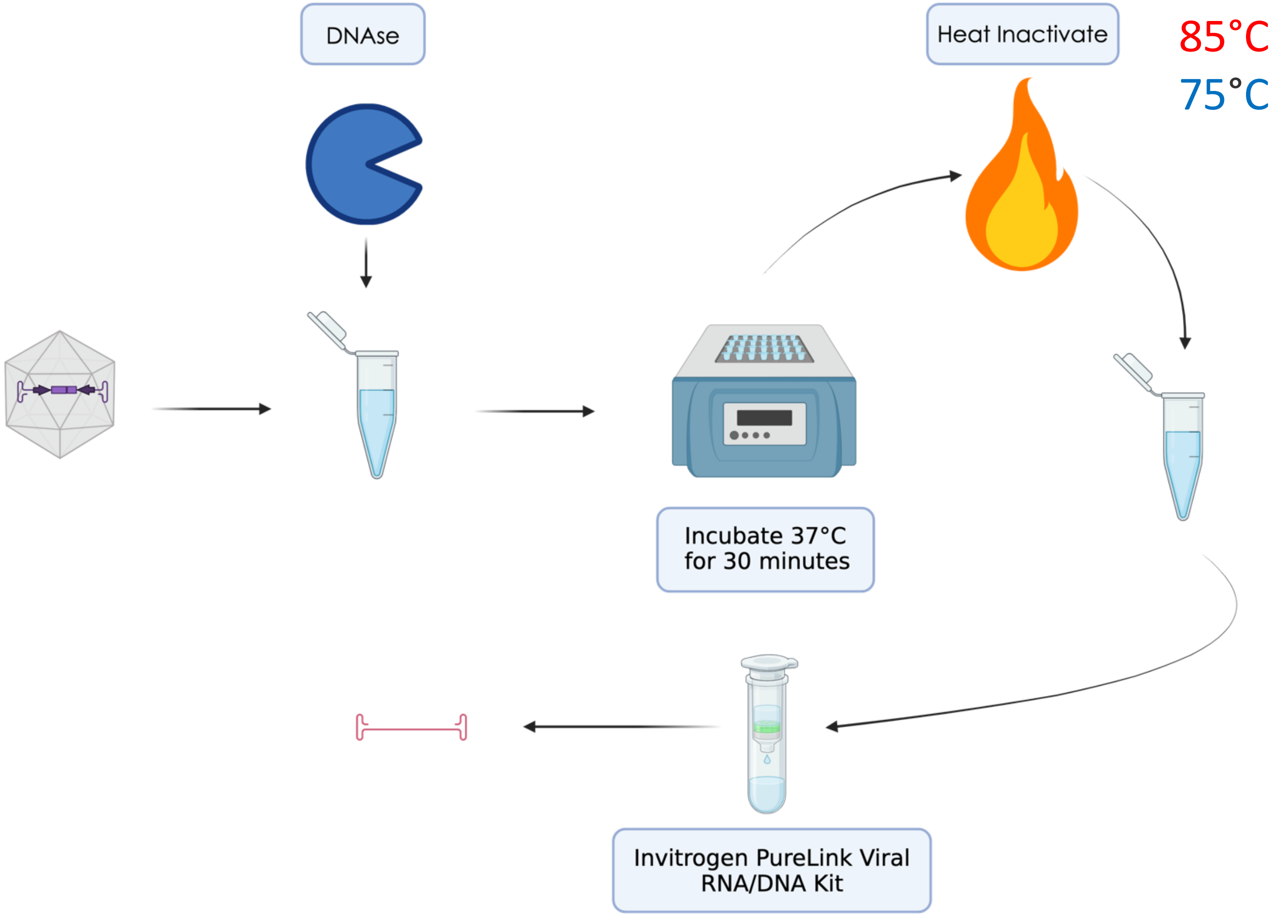
Long Read Sequencing Provides an Additional Avenue to Understand AAV



Tran et al. 2020. Molecular Therapy Methods & Clinical Development

Extraction Parameters Influence Vector Integrity

DNAse Treatment



DNAse

Heat Inactivate

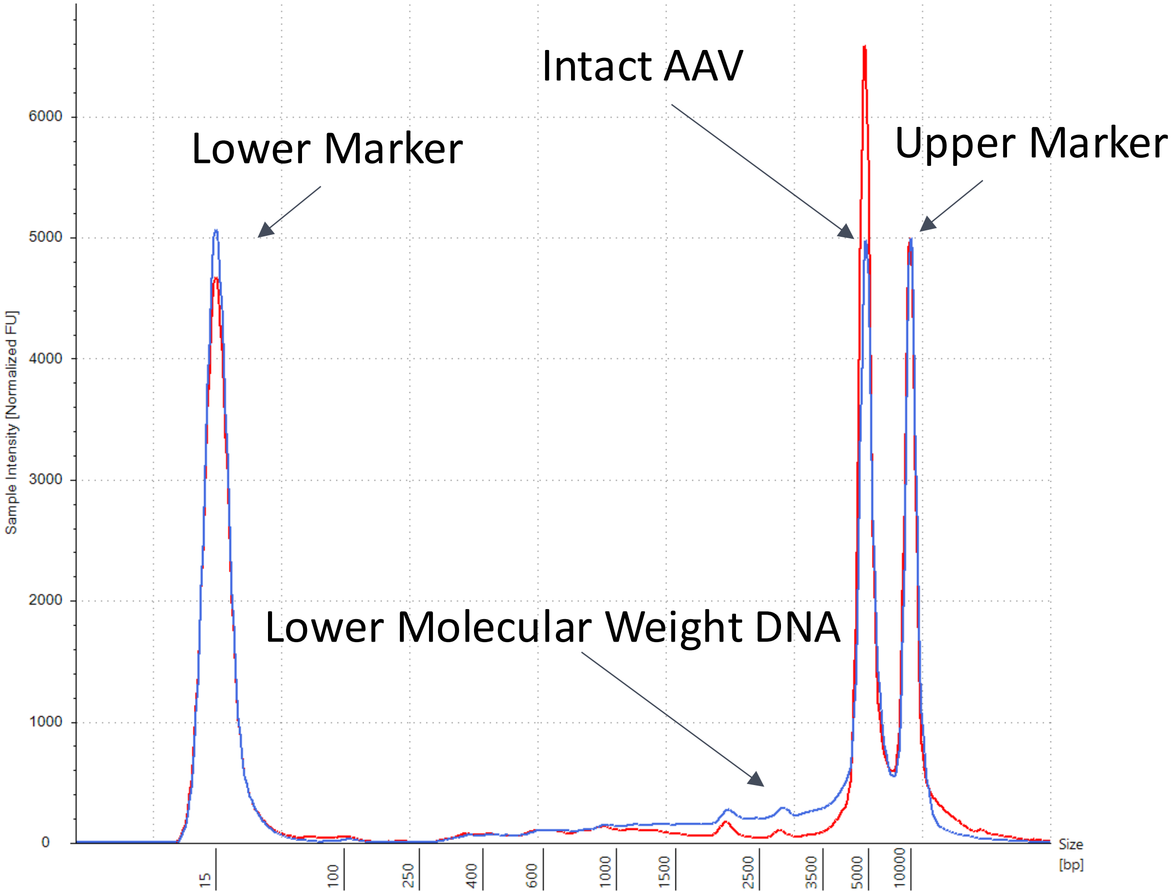
85°C

75°C

Incubate 37°C for 30 minutes

Invitrogen PureLink Viral RNA/DNA Kit

Tape Station Results



Intact AAV

Lower Marker

Upper Marker

Lower Molecular Weight DNA

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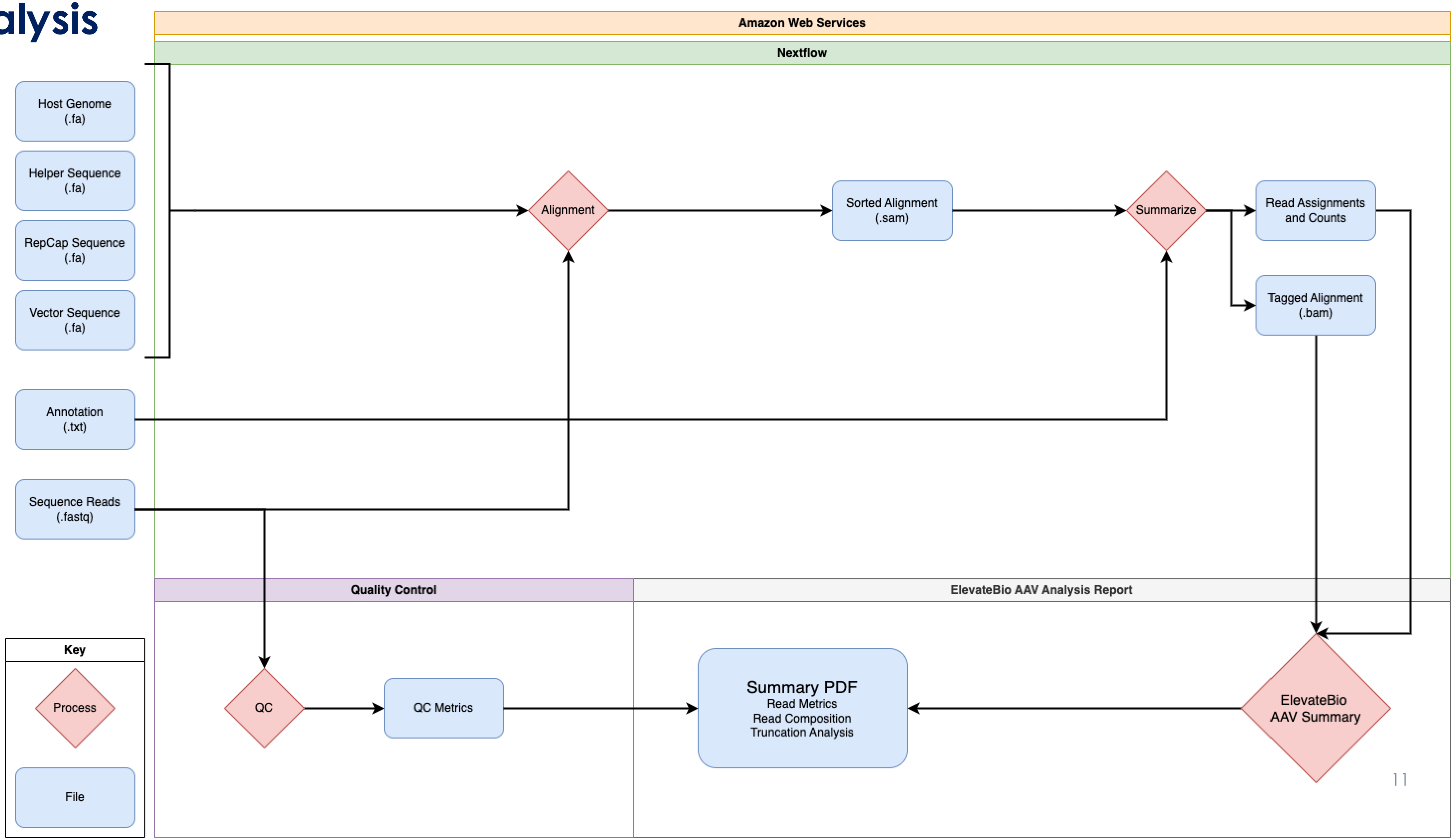
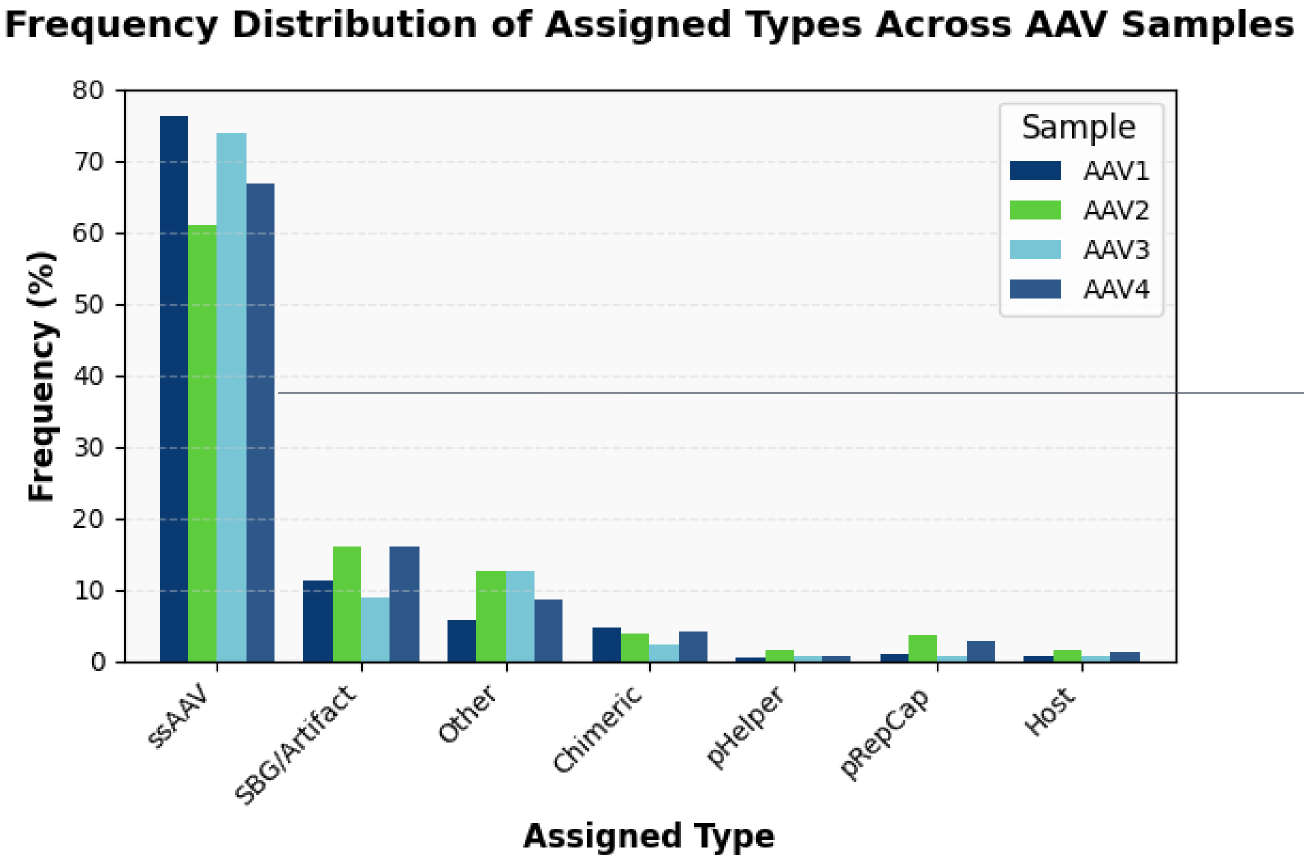
ElevateBio AAV Pipeline Enables Efficient, Reproducible, and Scalable AAV Analysis

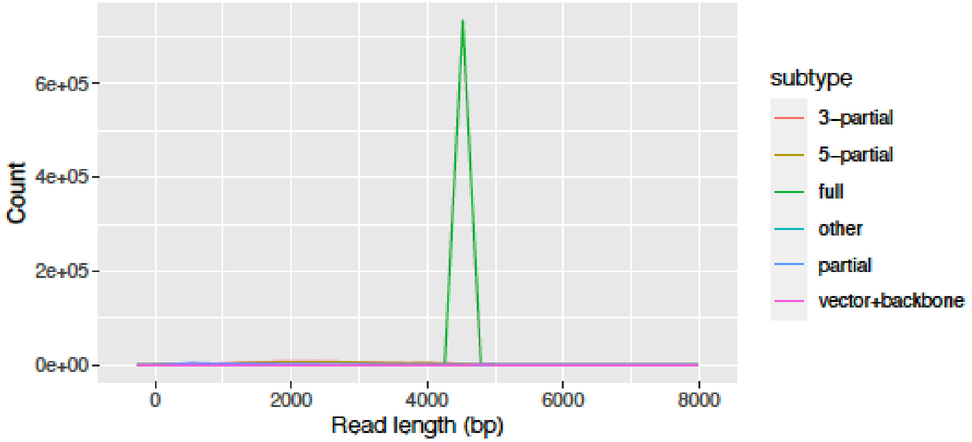


Diverse Sequences are Packaged within Capsids



Frequency Distribution of Assigned Types Across AAV Samples

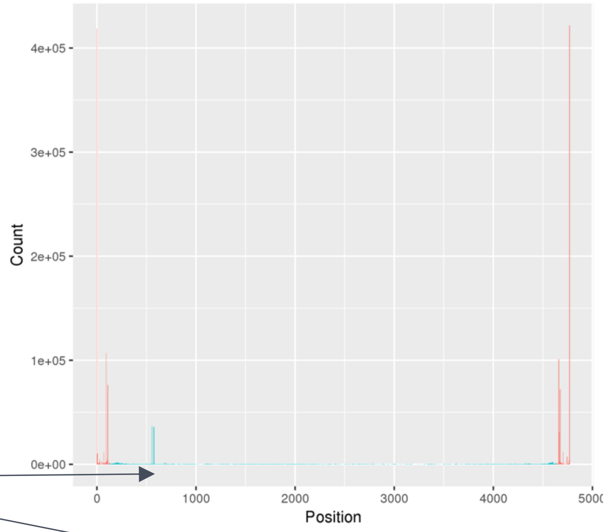
ssAAV Subtypes



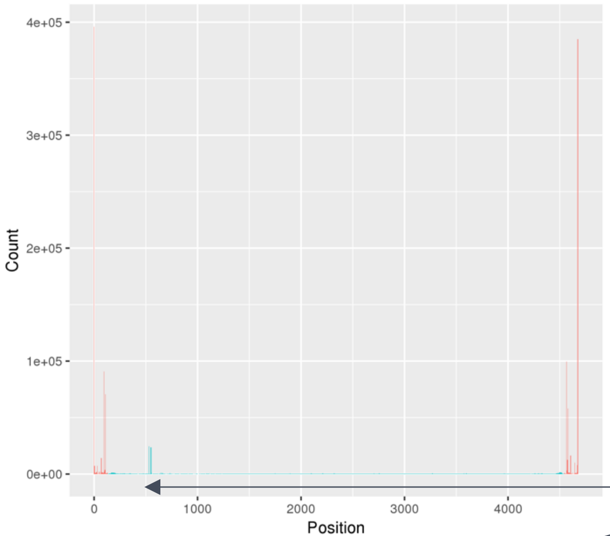
Approximately 67% of ssAAV reads are ITR-ITR

Sequence Motif Derived Truncations are Reproducibly Identified

AAV1

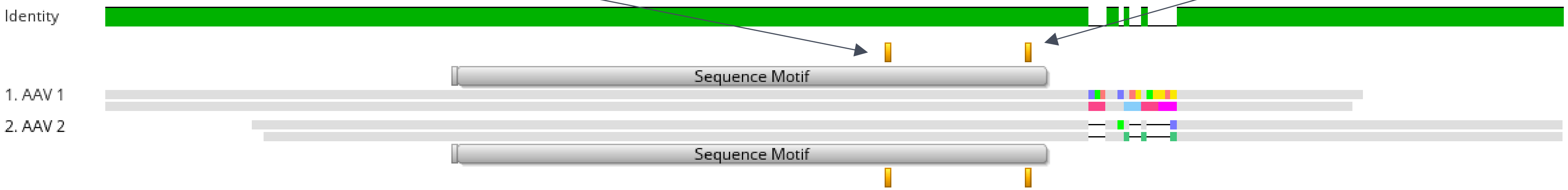


AAV2



Truncations

Truncations



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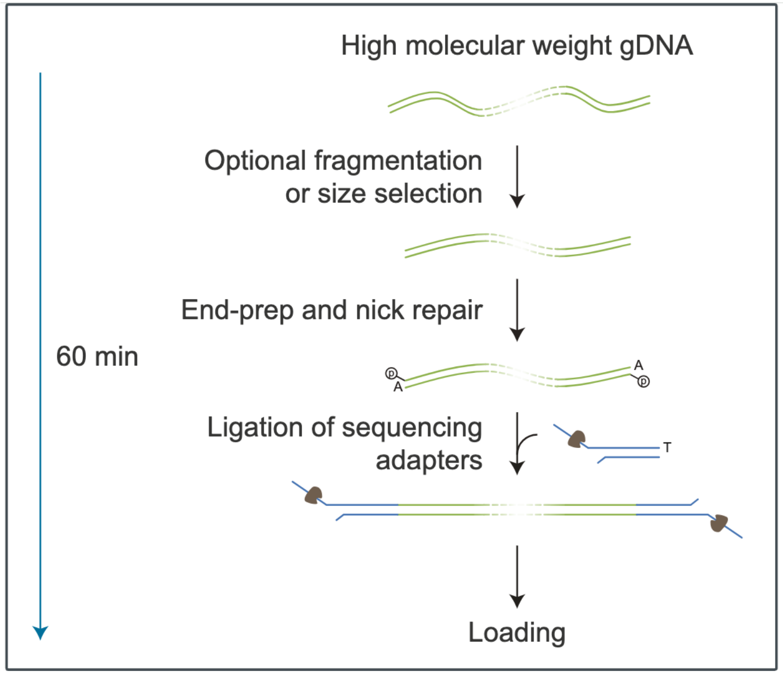
Not All Long Read Sequencing is Equal

- **Challenge**: No in-house PacBio
- **Solution**: Oxford Nanopore offers inexpensive entry into long-read sequencing through the MinION.
  - <$5K for entry level instrument
- **Results**:
  - Similar ssAAV %
  - Slight variability in contaminant profiles detected.
  - Truncation hot spots are still identified.
  - Continuing to optimize identification of ITR-ITR length reads.



High molecular weight gDNA

Optional fragmentation or size selection

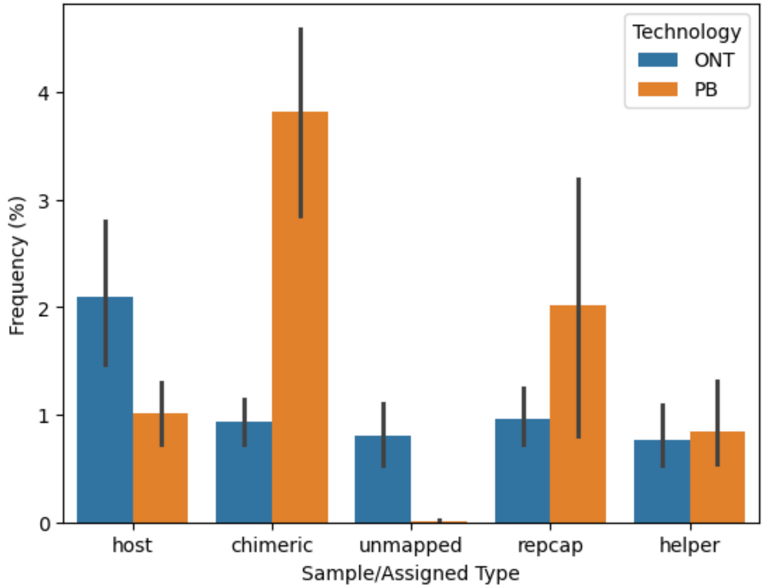
End-prep and nick repair

60 min

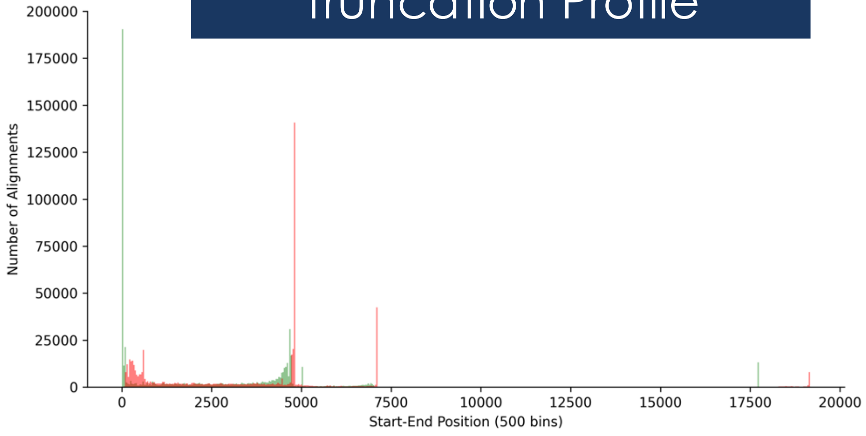
Ligation of sequencing adapters

Loading

Contaminant Profile

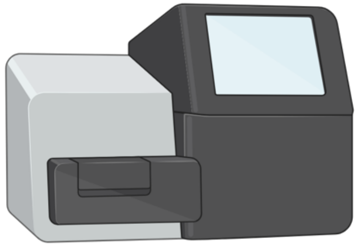


Truncation Profile



ElevateBio's Application Schema for NGS & AAV

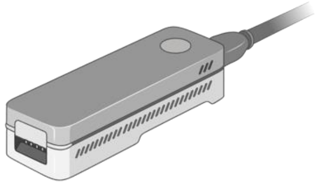
Short Read Sequencing



Use Case

- Affordable for high numbers of samples.
- Characterize identity and contaminant profiles.

ONT



Use Case

- Appropriate for fast and affordable for low-medium throughput.
- Characterize identity, truncations, and contaminant profiles.

PacBio

Use Case

- Higher cost. Targeted at near final product.
- Potential for all-in-one sequencing for identity, truncations, contamination profile, and % full length.

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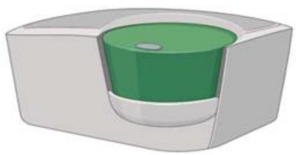
NGS Complements Existing AAV Analytical Tools

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

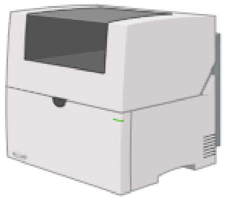


VG Titers

AUC

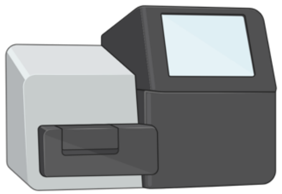
Capsid Content Mass

TapeStation/Electrophoretic



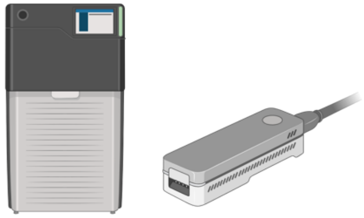
Nucleic Acid Sizing

Short Read Sequencing



Sequence Identity
Sequence Contaminants

Long Read Sequencing



Sequence Identity
Sequence Contaminants
Sequence Species

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Gary Sommerville PhD

*Presenting