Application brief

HIGHLY ACCURATE HIFI READS FOR AAV RESEARCH

PacBio® long-read HiFi sequencing allows you to sequence the entire AAV genome with extraordinary accuracy and completeness so that you can quickly discover and optimize gene therapy vectors.

Your moment of discovery in AAV research is waiting.

What can you do with highly accurate AAV sequencing?

- Rapidly accelerate the engineering and discovery of novel capsids with desired characteristics such as tissue specificity through full-length AAV genome or cap gene sequencing.
- Monitor and improve AAV manufacturing by characterizing AAV vectors and their impurities with high accuracy.
  - Assess packaged impurity sequence profiles like identity, size distribution, and relative abundance.
  - Profile payload sequence length and integrity.
  - Characterize ITR rearrangements and integrity.
- Confidently evaluate and optimize your AAV design.
  - Identify payload sequence truncation hotspots.
  - Verify whether a target construct or gene of interest is correctly expressed and spliced.
- Explore potential risks by assessing host genome integration events.

PacBio highly accurate long-read sequencing offers advantages over short reads with these features and is recommended for rAAV characterization.[1]

- Long reads span the entire genes, regions of interest, or AAV genomes, including inverted terminal repeats (ITRs).
- Q30 accuracy (99.9%) provides Sanger-quality base-level resolution.
- Detect variants beyond small insertions and deletions to ensure both base-level modifications and structural rearrangements are captured.
- Uniform coverage to detect variants in repetitive and extreme GC-rich regions in an unbiased manner.
- Sequence transcripts and evaluate splicing events with full-length cDNA sequencing.
- Easy-to-implement protocol for AAV sequencing with end-to-end workflow suitable for both scAAV and ssAAV.

Workflow




Sample prep

Use a range of starting materials that fit your project:

- Single-stranded ssAAV
- Self-complementary scAAV
- Target capture-enriched DNA
- DNA amplicons
- RNA

Library prep

Use standardized and automatable workflows with PacBio Compatible partners.

Supports a range of target types and multiplexing options for up to 96 targets

HiFi sequencing

Generate 1–3 million HiFi reads up to 25 kb in length per SMRT® Cell 8M on the Sequel® IIe system.

AAV sequencing is supported on both the Sequel IIe and Revio™ systems through on-instrument control software.

Data analysis

Use SMRT® Link, a web-based end-to-end workflow to demultiplex, analyze, and visualize your sequencing data.

Further analyze your data using publicly available bioinformatic tools,[2] or the Form Bio[3] platform.

How can HiFi sequencing empower your AAV research?

Help identify novel AAV capsids

Identifying novel capsids with tissue-specific tropism is critical for targeted delivery of AAV-based gene therapies.[4,5]

In one study, SMRT® sequencing of intact full-length AAV genomes led to the discovery of novel capsid variants.[6]

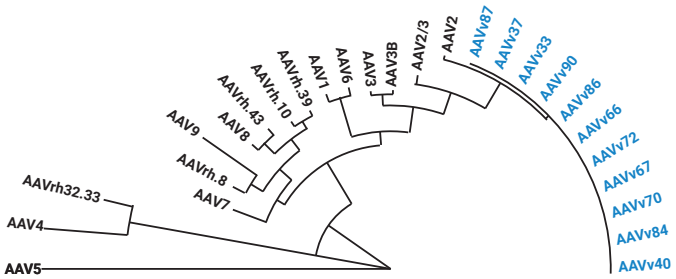

Figure 1. (from Hsu et al. 2020) Phylogenetic tree of AAV2 variants reported in this study (blue) and contemporary serotypes.

Assess gene transfer vectors

Performing highly accurate long-read sequencing enables a more complete characterization of the viral genome to ensure correct packaging.[7,8]

SMRT sequencing provides more complete viral sequences for heterogeneous vector populations. This allows for unambiguous detection and classification of plasmid and host genomic encapsidated DNA.

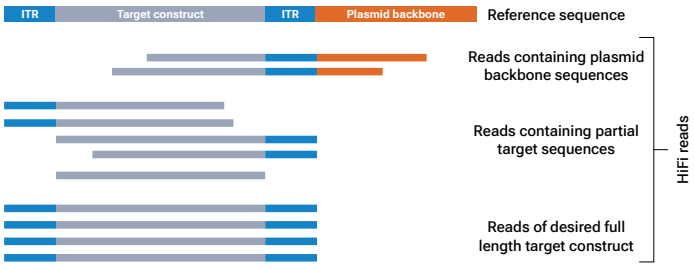

Figure 2. HiFi long reads span the full length of a viral genome including ITRs, target sequences, and plasmid backbone sequences.

Characterize ITR integrity and configuration

ITR integrity is correlated to vector genome heterogeneity. SMRT sequencing allows for full ITR sequencing and resolution of configurations and mutations that can inform understanding of AAV genome heterogeneity.[7]

Identify payload truncation hotspots

Secondary structure elements in AAV payloads such as in shRNA sequences (small hairpin RNAs) can lead to rAAV truncation hotspots.[9]

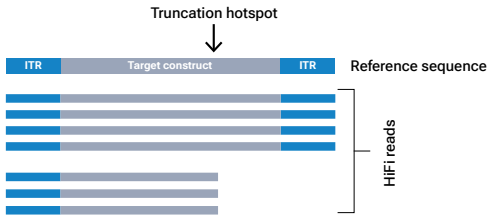

Figure 3. HiFi long reads reveal truncation hotspots within AAV payloads.

Ensure a transcript is correctly expressed and spliced using the Iso-Seq® method

Accurate splicing can be a critical factor to consider in gene therapy research. The Iso-Seq method can capture full-length transcripts and reveal isoforms not distinguishable by short-read sequencing.[10]

Help enable the assessment of AAV integration into the host cell genome, which is critical for evaluating the safety profile of the AAV vector.

SMRT sequencing allows unambiguous identification of integration sites and resolves integrated concatemers and rearrangements that cannot be detected with short-read methods.[11]

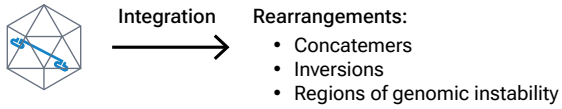

Figure 4. HiFi sequencing allows for the assessment of potential genomic rearrangements due to AAV integration.

Learn about highly accurate HiFi reads for AAV gene therapy research: pacb.com/gene-therapy

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2. https://github.com/Magdoll/AAV
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