#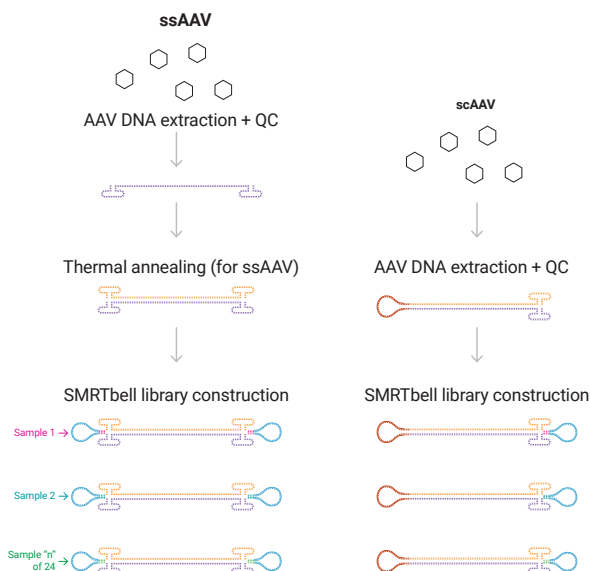# Accelerate discovery and comprehensively assess adeno-associated virus (AAV) vector quality with highly accurate long reads (HiFi reads)

With Single Molecule, Real-Time (SMRT®) sequencing on the Sequel® IIe and Revio™ systems, you can sequence AAV genome populations to identify truncation, mutation, and host integration events. The AAV workflow from PacBio® accommodates both scAAV and ssAAV constructs with easy on-instrument HiFi read generation.
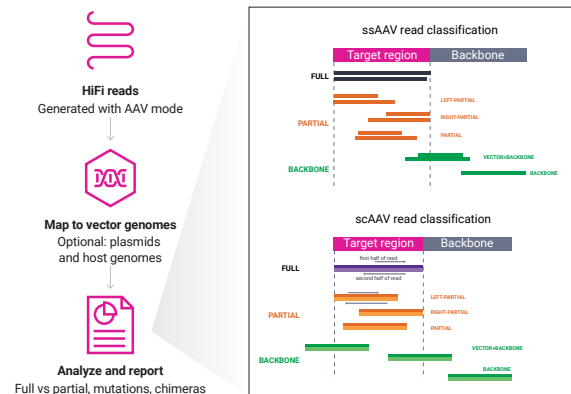
From AAV to SMRTbell® libraries with data analysis solutions

ssAAV/scAAV | Vector design and production → PacBio multiplexed AAV protocol → CCS | + − +/− | PacBio sequencing → Form Bio analysis

Use PacBio's AAV protocol[1] to:

- Convert ssAAV DNA to double-stranded DNA by thermal annealing
- Multiplex up to 24 samples using barcoded SMRTbell adapters

Use the on-instrument *AAV* mode[2] to:

- Generate HiFi reads for proper handling of scAAV and ssAAV structures

Analyze your AAV PacBio data with:

- GitHub command-line analysis,[3] or
- Form Bio's cloud-based analytical solution[4]

HiFi library workflow for AAV

ssAAV

AAV DNA extraction + QC

Thermal annealing (for ssAAV)

SMRTbell library construction

Sample 1 →
Sample 2 →
Sample "n" of 24 →

scAAV

AAV DNA extraction + QC

SMRTbell library construction

Bioinformatics workflow for AAV

HiFi reads
Generated with AAV mode

Map to vector genomes
Optional: plasmids and host genomes

Analyze and report
Full vs partial, mutations, chimeras

ssAAV read classification — Target region / Backbone — FULL, PARTIAL, LEFT-PARTIAL, RIGHT-PARTIAL, PARTIAL, BACKBONE, VECTOR+BACKBONE, BACKBONE

scAAV read classification — Target region / Backbone — FULL (first half of read / second half of read), PARTIAL, LEFT-PARTIAL, RIGHT-PARTIAL, PARTIAL, BACKBONE, VECTOR+BACKBONE, BACKBONE
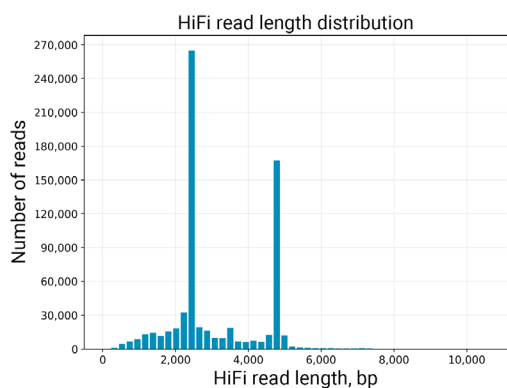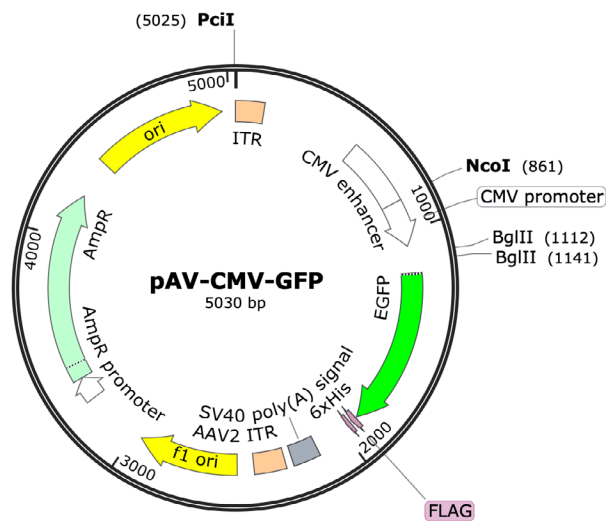
HiFi reads generated with on-instrument AAV mode are aligned to a reference vector genome and optionally other helper plasmids and host genomes. Reads are characterized as ssAAV or scAAV based on the presence or absence of self-complementary alignments, with further characterization as full or partial vectors, backbone or other contaminations, etc. For more information, visit the AAV wiki[3] or the AAV bioinformatics webinar.[5]

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AAV sample characterization

**Example 1.** HiFi sequencing identifies unexpected self-complementary structures in ssAAV

pAV-CMV-GFP
5030 bp

Example 2. HiFi sequencing confirms expected scAAV vector genomes

scAAV-CBA-eGFP
4412 bp

HiFi read length distribution

ssAAV pAV-CMV-GFP

ssAAV	**Full**	**35.8%**
	Partial	10.4%
	Backbone	0.0%
scAAV	Full	29.0%
	Partial	19.4%
	Backbone	0.8%

Table 1. Characterization of ssAAV using HiFi sequencing. Expected full-length ssAAV reads (~2.5 kb) make up only 35.8% of reads. Unexpected self-complementary structures (scAAV) of the full vector (~5 kb) make up 29% of the reads.
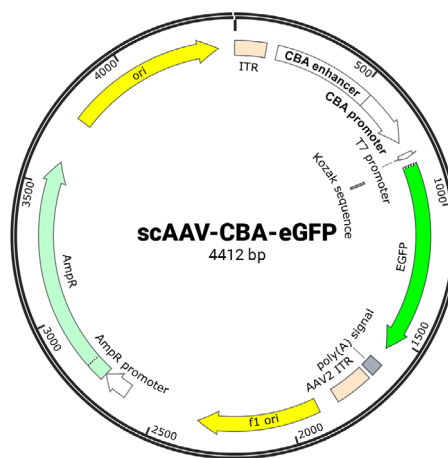
scAAV CBA-eGFP

ssAAV	Full	0.4%
	Partial	0.5%
	Backbone	0.0%
scAAV	**Full**	**95.1%**
	Partial	3.6%
	Backbone	0.1%

Table 2. Characterization of scAAV using HiFi sequencing. Percentage of reads characterized as full, partial, or backbone, based on alignment to reference vector sequences. Reads mapping with complementary structures will be characterized as scAAV.

Learn about AAV gene therapy:
pacb.com/gene-therapy

KEY REFERENCES

1. Procedure & checklist — Preparing multiplexed AAV SMRTbell libraries using SMRTbell prep kit 3.0
2. SMRT Link user guide (v11.0)
3. https://github.com/Magdoll/AAV
4. https://info.formbio.com/pacbio
5. Bioinformatics webinar series on-demand: Optimizing and confirming AAV designs with PacBio sequencing

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