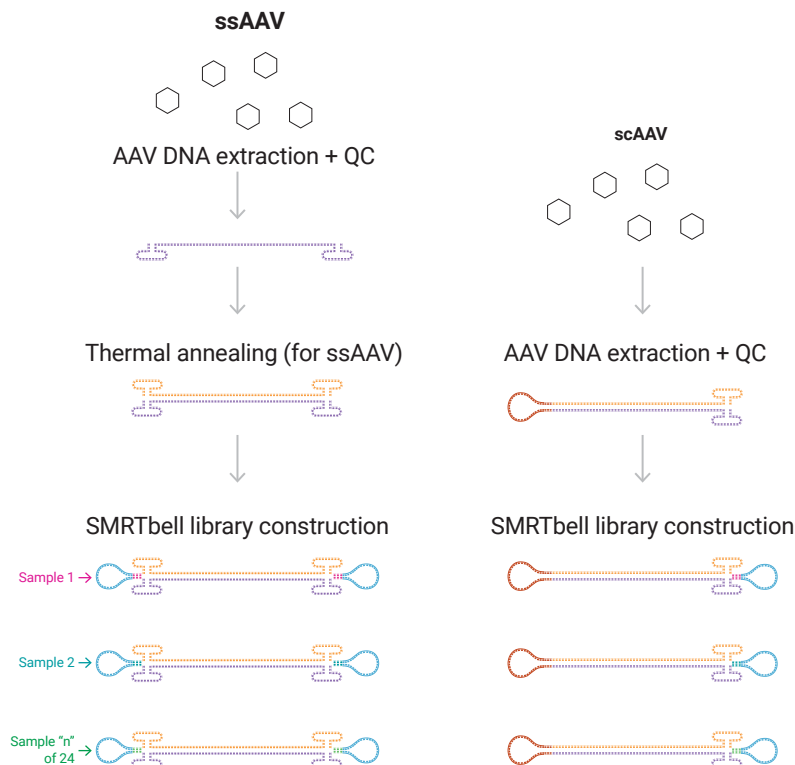


AAV SEQUENCING – BEST PRACTICES

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 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



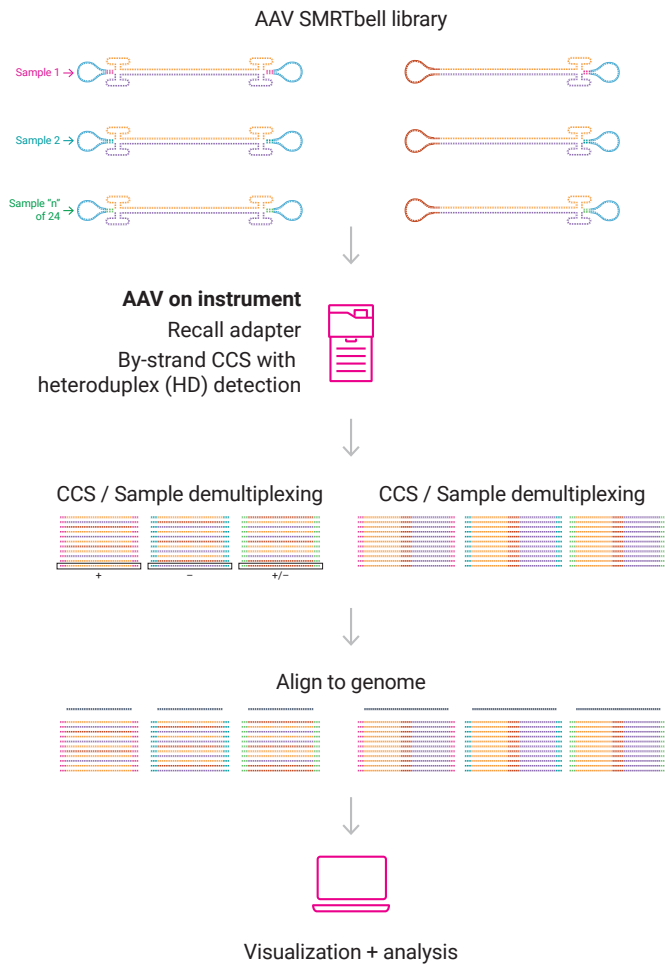
Structures of ss (single-stranded) and sc (self-complementary) AAV DNA molecules. The ssDNA structure at left requires a second strand (as shown in yellow) for SMRTbell formation.



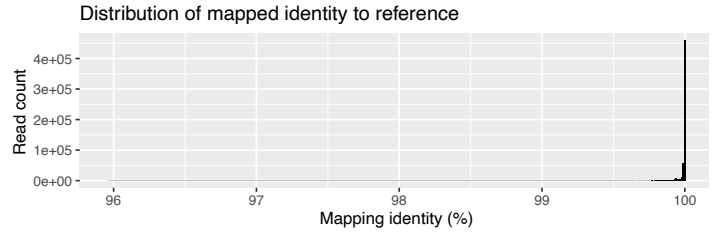
Workflow recommendations

- Convert ssAAV DNA to double-stranded DNA by thermal annealing or other validated methods¹
- Multiplex up to 24 samples using SMRTbell barcoded adapters¹
- Use AAV Run Design² to generate HiFi reads for proper handling of scAAV and ssAAV structures
- AAV bioinformatics analysis³ generate files ready for visualization and QC reporting

Data analysis workflow



Figures: ssAAV_scAAV (1:10) report



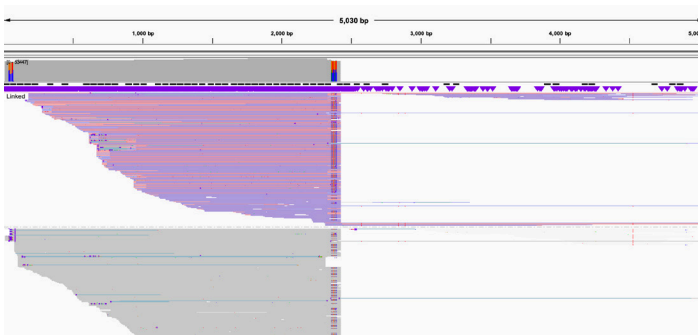
Assigned type	Assigned subtype	Count	Frequency (%)
scAAV	full-full	3822	10.60
scAAV	full	31453	87.27
Unknown	full	768	2.13



"[PacBio SMRT sequencing] has the capacity to sequence vectors from ITR to ITR without the need for bioinformatic reconstruction of the full genome."

Tran et al. *Human Gene Therapy*⁴

Data release and sample report



IGV screenshot for ssAAV in a 1:10 mixed ssAAV:scAAV sequenced with binding kit 3.1, 2 hr pre-extension and 24 hr movie time with on-instrument AAV Run Design mode. **Dataset available for download.**

Table: AAV run read summary

HiFi reads	HiFi read length	HiFi read quality	Aligned reads
Total 301,033			ssAAV: 43,048 (14%)
Single-stranded 128,419	3,785 bp	Q32	scAAV: 258,840 (85%)
Heteroduplex 172,614			Unaligned: 1465 (<1%)

KEY REFERENCES

1. Procedure & checklist – Preparing multiplexed AAV SMRTbell libraries using SMRTbell prep kit 3.0
2. SMRT Link user guide (v11.0) – PacBio website
3. <https://github.com/Magdoll/AAV>
4. Tran et al., Human and insect cell-produced recombinant adeno-associated viruses show differences in genome heterogeneity. *Human Gene Therapy* (2022)



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

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