HIGHLY ACCURATE HIFI READS FOR GENE EDITING VALIDATION

With highly accurate long reads (HiFi reads) from the Sequel® II and Sequel IIe system, powered by Single Molecule, Real-Time (SMRT®) sequencing technology, you can comprehensively validate gene editing techniques including adeno-associated virus (AAV) and CRISPR-Cas9 approaches.

Sequence with confidence

- Discover novel potential capsid constructs
- Conduct vector quality control experiments
- Detect and accurately measure the efficiency of both on- and off-target effects
- Assess the safety of resulting constructs

Characterize CRISPR-Cas9 editing outcomes →

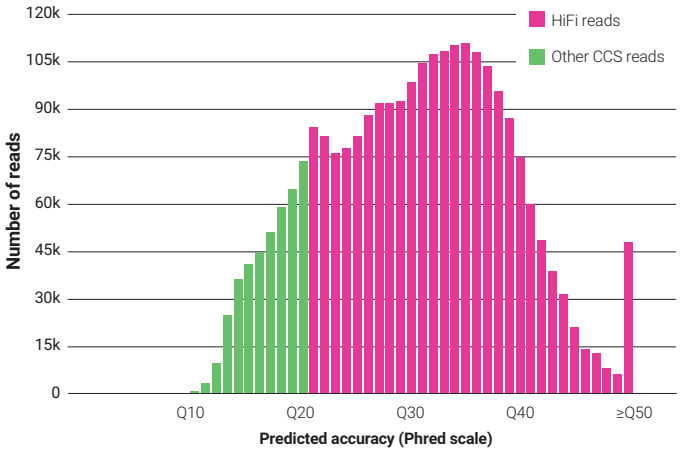
Develop and assess AAV-based therapeutic approaches →

Use amplification or amplification-free approaches

The advantages of HiFi reads for gene editing validation

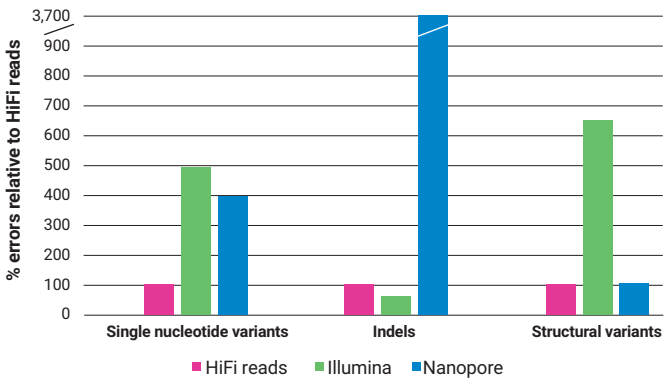
- Long read lengths up to 25 kb to span complete genes or regions of interest
- High accuracy of 99.9% (Q30) to provide Sanger-quality, base-level resolution
- Comprehensively detect all variant types to ensure both base-level modifications and structural rearrangements are captured
- Uniform coverage to detect variants in repetitive and extreme GC-regions in an unbiased manner

High accuracy



Data from a 15 kb size-selected human library using the SMRTbell® express template prep kit 2.0 on a Sequel IIe system (2.0 chemistry, Sequel IIe system software v10, 30-hour movie).

Comprehensive variant detection



Variant calling performance against *Genome in a Bottle* benchmarks for PacBio HiFi reads (35-fold, Sequel II system, 2.0 chemistry); Illumina (35-fold, NovaSeq); Oxford Nanopore (60-fold, PromethION R9.4.1).

PacBio

Workflow

With easy-to-use, high throughput sequencing, you can get the accuracy you need at an affordable cost

Sample prep

Use single-stranded DNA (ssAAV), self-complementary DNA (scDNA)[1,2,3] or DNA amplicons as starting material.

Library prep

Use standardized and automatable workflows for a range of target types with multiplexing options for up to 96 targets.

SMRT sequencing

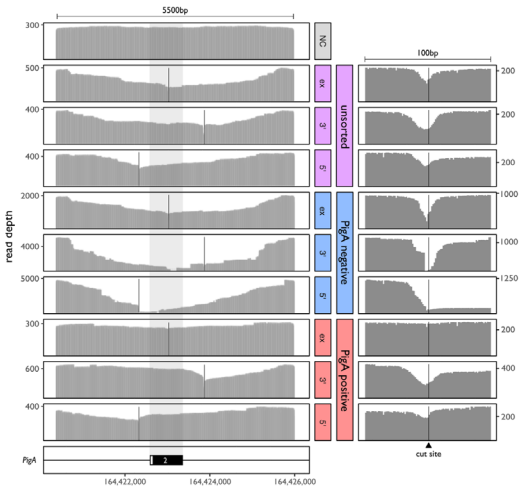
Use 10–20 hr movies to generate 1–3 million HiFi reads up to 20 kb in length per SMRT® Cell 8M on Sequel II or IIe systems.

Data analysis

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

Sequence beyond your target

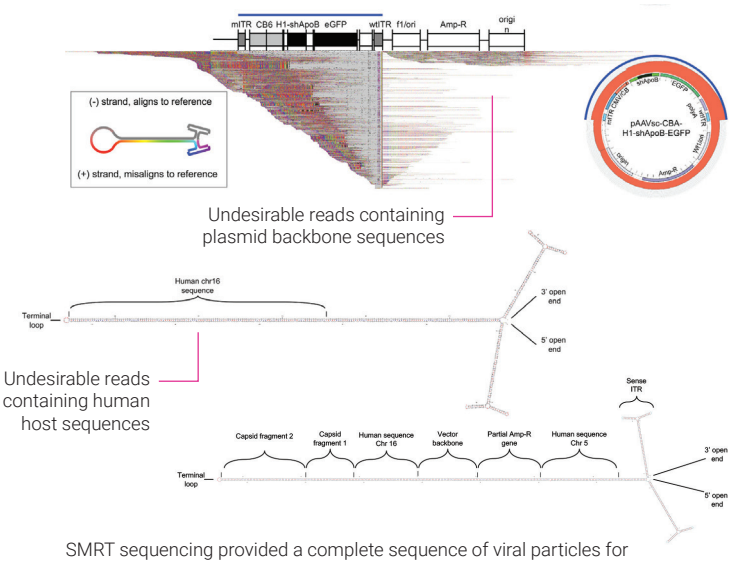
Understanding the extent of CRISPR-Cas9 editing requires long read lengths and high accuracy to capture both on- and off-target effects to fully evaluate editing outcomes.[4,5,6,7]



Analysis of the PigA locus edited with the CRISPR-Cas9 method using selected gRNAs. SMRT sequencing of a 5.5 kb amplicon around gRNA cut sites (vertical lines) enabled detection of large-scale deletions and structural changes missed by other methods.[6]

Assess gene transfer vectors

Performing quality control of AAV vectors with highly accurate long reads enables complete sequencing of viral particles to ensure correct packaging.[4,5,8]



SMRT sequencing provided a complete sequence of viral particles for heterogeneous vector populations to the pCis-plasmid reference. This allowed for unambiguous detection of plasmid and host gDNA that was encapsidated.[8]

KEY REFERENCES

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8. Tai, P. W. L. et al. (2018) **Adeno-associated virus genome population sequencing achieves full vector genome resolution and reveals human-vector chimeras.** *Molecular Therapy: Methods & Clinical Development.* Vol 9. 130-141.

Learn about highly accurate HiFi reads for gene editing validation: **pacb.com/target**

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