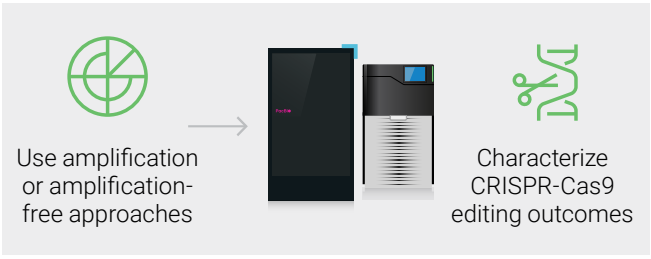
Application brief

HIGHLY ACCURATE HIFI READS FOR GENE EDITING RESEARCH

With highly accurate long reads (HiFi reads), powered by Single Molecule, Real-Time (SMRT®) sequencing technology, you can more comprehensively validate gene editing techniques such as CRISPR-Cas9 approaches.

Sequence with confidence

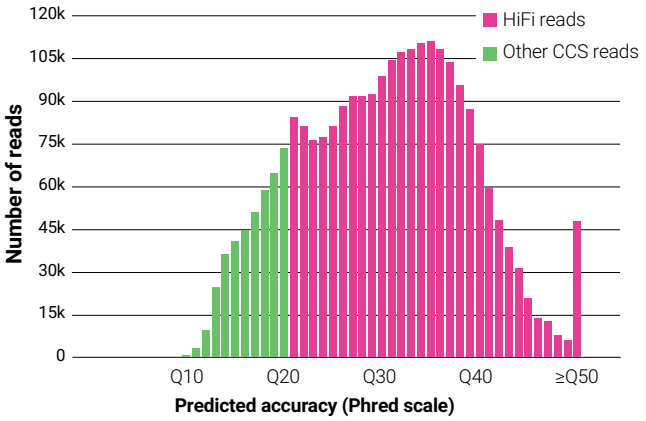
- Detect and accurately measure the efficiency of both on- and off-target effects
- Assess insertional mutagenesis in greater detail
- Help assess the potential safety of resulting constructs



The advantages of HiFi reads for gene editing research

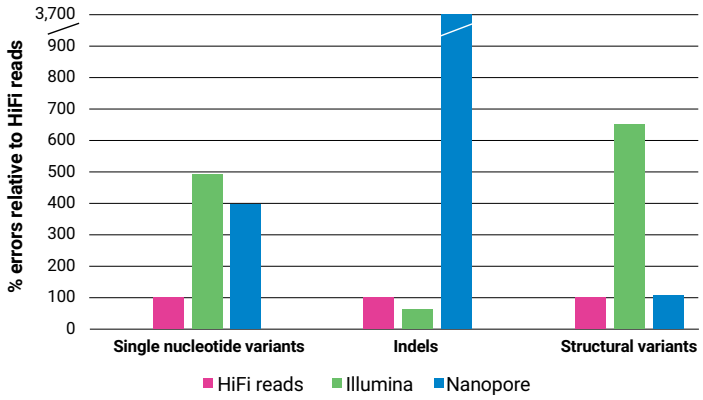
- Long read lengths up to 20 kb to span complete genes or regions of interest
- High accuracy of 99.9% (Q30) to provide Sanger-quality, base-level resolution
- More comprehensively assess variants from base-level modifications to structural rearrangements
- Uniform coverage to detect variants in repetitive 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).

Workflow

Sample prep

Use a range of starting materials that fit your project, including DNA amplicons or target capture-based methods

Library prep

Implement standardized and automatable workflows that support a range of target types and up to 96-target multiplexing

HiFi sequencing

Generate 1–3 million HiFi reads up to 20 kb in length on the Sequel IIe or Revio™ systems

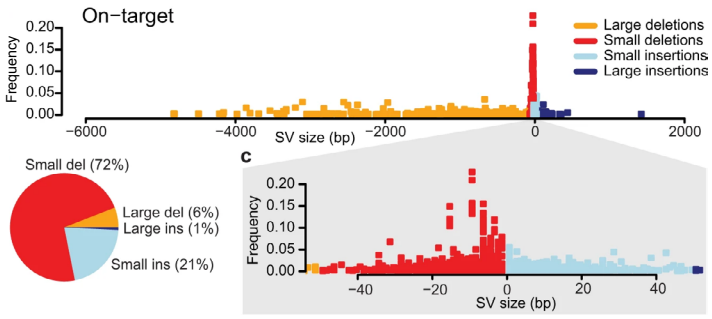
Data analysis

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

How can HiFi sequencing power your gene editing research?

Sequence beyond your target to fully understand the potential outcomes of gene editing approaches

Understanding the extent of CRISPR-Cas9 editing requires long read lengths and high accuracy to capture both on- and off-target effects. Accurately identifying genome modifications like large-scale deletions, insertions and structural changes is necessary to fully understand editing outcomes.[1,2]



SMRT sequencing of amplicons around CRISPR-Cas9 cleavage sites revealed large deletions and insertions around both on-target and off-target cleavage sites (marked with 0 bp).[1]

Assess insertional mutagenesis in greater detail

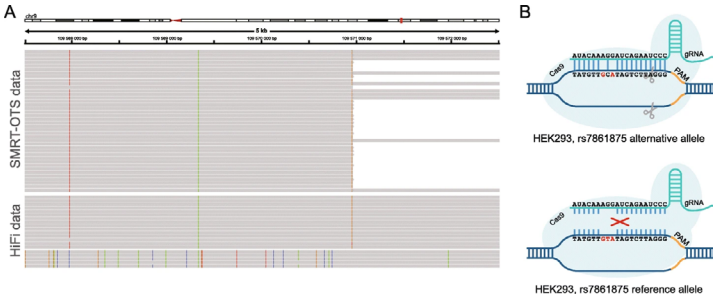
Gene insertion with homology-directed repair (HDR) at a CRISPR target locus can lead to small and large indels. HiFi sequencing can characterize these mutations as well as concatenations and other complex changes as a result of HDR-based gene insertion.[3]

Detect ultra-rare off-target mutations

HiFi sequencing can efficiently detect ultra-rare substitution mutations in whole genomes with a sensitivity of ~1×10^{-8} mutations per base pair and can efficiently detect on- and off-target base editing in *E. coli*.[4]

Understand the effects of haplotype and SNVs on gene editing through allele-specific resolution

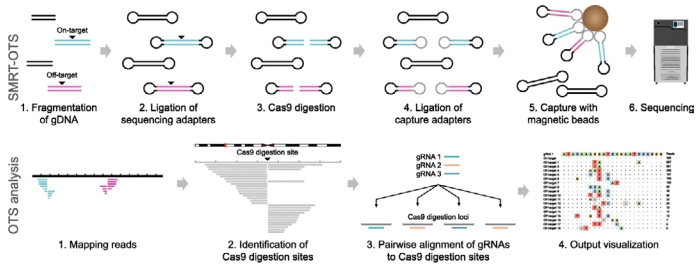
Genetic variation such as SNVs may introduce allele-specific Cas9 cleavage. HiFi sequencing can discriminate and resolve editing efficiency with an allele-specific approach if SNVs cause differential gRNA binding.



HiFi reads reveal allele-specific SNVs within a gRNA binding site (A) and demonstrate differential editing efficiency between reference and alternative alleles (B).[5]

Avoid PCR biases and limitations by using amplification-free approaches

Many approaches to detect on- and off-target sites rely on PCR amplification, which can introduce biases and can fail to capture cleavage sites in regions of the genome that are repetitive or GC-rich. Single-Molecule Real-Time Off-target-sequencing (SMRT-OTS) takes advantage of PacBio long-read technology to overcome those limitations.



Conceptual workflow of the SMRT-OTS method for Cas9 cleavage detection.[5]

Learn more about targeted sequencing with PacBio for gene editing validation: pacb.com/target

KEY REFERENCES

1. Höijer I, Emmanouilidou A, Östlund R, et al (2022) CRISPR-Cas9 induces large structural variants at on-target and off-target sites in vivo that segregate across generations. *Nat Commun* 13:627. https://doi.org/10.1038/s41467-022-28244-5
2. Kosicki M, Tomberg K, Bradley A (2018) Repair of double-strand breaks induced by CRISPR–Cas9 leads to large deletions and complex rearrangements. *Nat Biotechnol* 36:765–771. https://doi.org/10.1038/nbt.4192
3. Tei C, Hata S, Mabuchi A, et al (2023) Comparable analysis of multiple DNA double-strand break repair pathways in CRISPR-mediated endogenous tagging. https://doi.org/10.1101/2023.06.28.546861
4. Miranda JA, Fenner K, McKinzie PB, et al (2023) Unbiased whole genome detection of ultrarare off-target mutations in genome-edited cell populations by HiFi sequencing. *Environ Mol Mutagen*. https://doi.org/10.1002/em.22566
5. Höijer I, Johansson J, Gudmundsson S, et al (2020) Amplification-free long-read sequencing reveals unforeseen CRISPR-Cas9 off-target activity. *Genome Biol* 21:290. https://doi.org/10.1186/s13059-020-02206-w

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