HIGHLY ACCURATE HIFI READS FOR GENE EDITING IN PLANTS + ANIMALS

With highly accurate HiFi reads, you can discover, design, and validate genome engineering approaches with confidence, ease, and sensitivity. Gene editing starts with having the confidence of WGS information and the tools to validate editing outcomes.

Sequence with confidence

Agriscientists know that to combat climate change and feed an ever-growing population, a variety of different programmable molecular techniques are needed to increase our food supply and shorten the development time to bring new crops to market.

Gene editing and HiFi sequencing provide fast, reliable solutions that can:

- Accelerate the breeding process
- Modify target genes to improve traits
- Improve plant resistances to biotic and abiotic stresses
- Increase plant architecture to better resist climate change

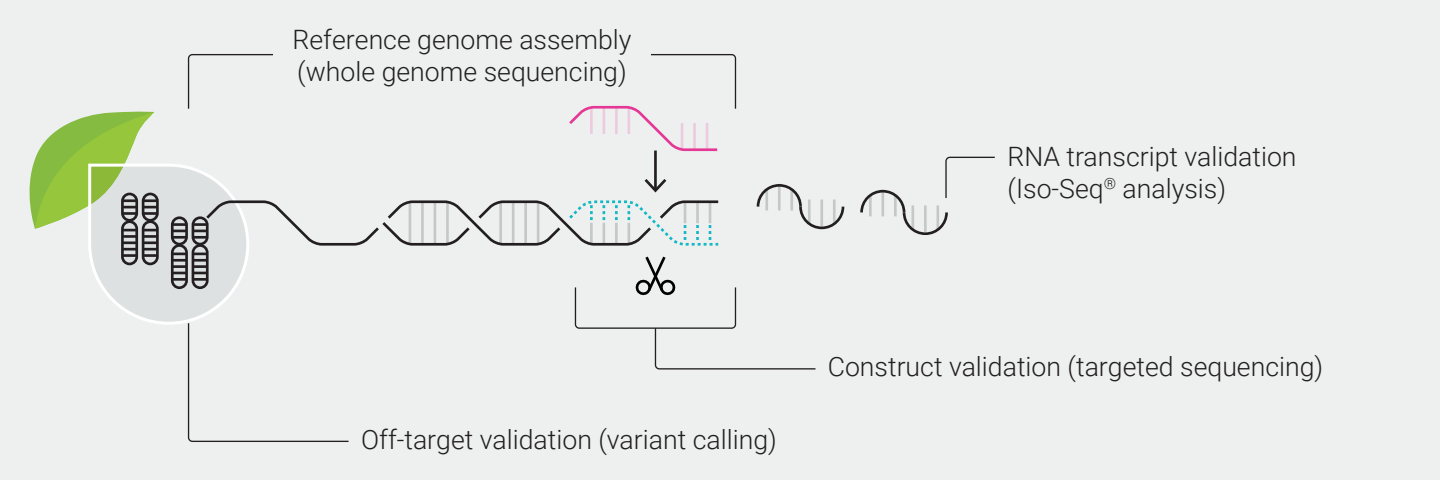
Characterize CRISPR-Cas9 editing outcomes →

Evaluate on- and off-target effects →

Use WGS or targeted-based approaches

The advantages of HiFi reads for gene editing approaches

- Generate high-quality reference genomes to design editing experiments
- Validate constructs with high accuracy
- Confirm edits even in high heterozygous or GC-rich regions
- Evaluate off-target effects to confirm no unintended effects



PacBio

Gene editing workflow at a glance

Sample prep

Flexible DNA input requirements down to 5 ng

Library prep

Adjustable multiplexing options to maximize throughput

SMRT sequencing

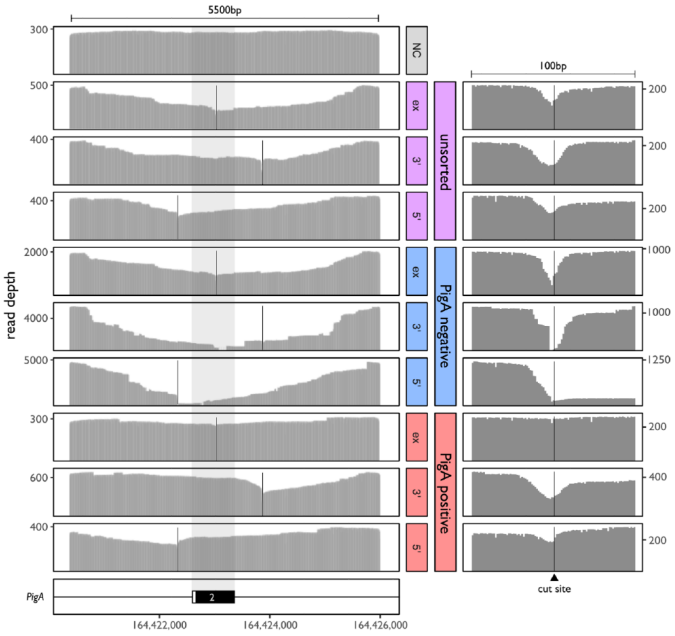
Run a single SMRT® Cell 8M at a time or several consecutively for set-it-and-forget-it sequencing

Data analysis

Use SMRT® Link or open-source tools to analyze your HiFi data

Sequence beyond your target

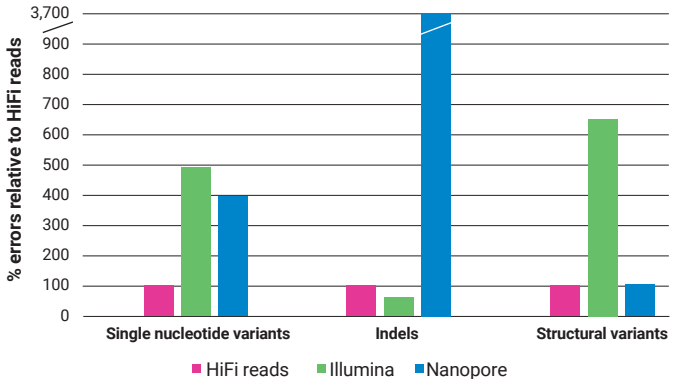
Understanding the extent of CRISPR-Cas9 editing requires long read lengths and high accuracy to capture both on- and off- target effects to fully evaluate editing outcomes on a targeted and genome-wide scale.[1–4]



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.

Comprehensive variant detection

Detect all variant types to ensure both base-level modifications and structural rearrangements are captured. HiFi long read lengths span complete genes or regions of interest with Sanger-quality, base-level resolution.



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).

Learn more about plant + animal gene editing
pacb.com/ag-blog-gene-editing

KEY REFERENCES

1. Tran, N. T. et al. (2020) AAV-genome population sequencing of vectors packaging CRISPR components reveals design-influenced heterogeneity. *Molecular Therapy. Methods & Clinical Development*, 18, 639-651
2. Larrea, A. and Tai, P.W.L. (2019) Highly accurate SMRT sequencing for gene editing applications. PacBio Webinar.
3. Kosicki, M. et al. (2018) Repair of Double-strand breaks induced by CRISPR-Cas9 leads to large deletions and complex rearrangements. *Nature Biotechnology*, 36, 765-711.
4. Höijer, I. et al. (2020) Amplification-free long read sequencing reveals unforeseen CRISPR-Cas9 off-target activity. *Genome Biology*. 21, 290.