



# A NEW PARADIGM: HiFi reads for highly accurate long-read sequencing



With PacBio® HiFi reads, you no longer need to compromise between long read lengths and high-accuracy sequencing.

#### The benefits of HiFi reads:

- Long read lengths up to 25 kb
- High read accuracy >99.9%
- Easy library preparation
- Low coverage requirements
- Small file sizes to minimize compute time
- A single technology solution for a range of applications

PacBio is the only sequencing technology to offer highly accurate long reads. **A typical 20,000 bp HiFi read has ~8 incorrect bases**

PacBio

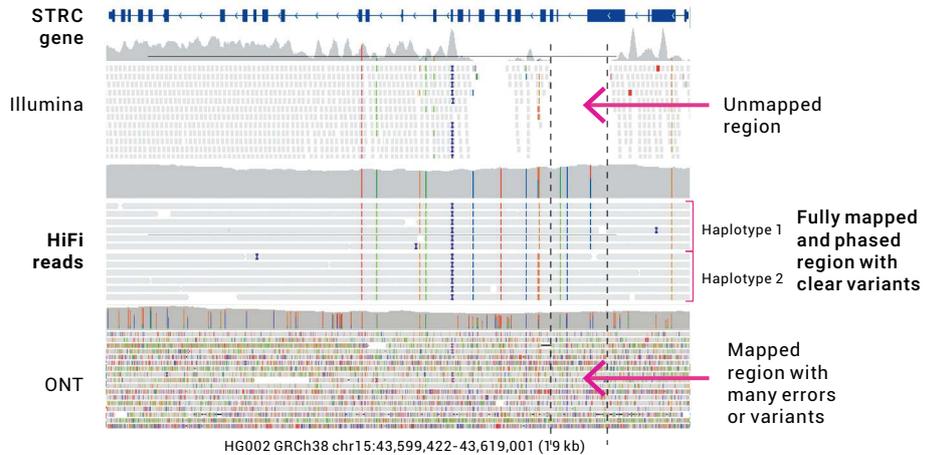
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# Achieve comprehensive variant detection

HiFi reads let you accurately detect all types of variants with high precision and recall and phase haplotypes, even in hard-to-sequence regions of the genome missed by other technologies.

“HiFi reads really allow us to call accurate structural variations and other types of variations that you can't actually see with short-read sequencing.”

Jeremy Schmutz, Faculty Investigator,  
HudsonAlpha Institute of Biotechnology



STRC gene alignments from *Genome in a Bottle* (GIAB), HG002\_NA24385\_son.

## Precision | Recall (%)

	Illumina	ONT	HiFi reads
<b>SNVs</b>	99.8   99.4	99.7   99.5	<b>99.9   99.9</b>
<b>Indels</b>	99.7   99.5	86.6   63.6	<b>99.4   99.4</b>
<b>SVs</b>	94.7   62.4	95.7   95.8	<b>96.1   96.0</b>

Detect all variant types with high precision and recall

SNVs, indels: *PrecisionFDA Truth Challenge V2* HG003 DeepVariant callsets evaluated against GIAB v4.2 benchmark using hap.py. SVs: HG002 callsets evaluated against GIAB v0.6 SV benchmark using Truvari, Illumina: DRAGEN 3.5, ONT: Sniffles at 30x, PacBio: pbsv 2.1.0 at 30x

## Produce reference-quality *de novo* assemblies

HiFi reads generate complete, contiguous, and correct *de novo* assemblies of any genome, including the large and highly complex California Redwood.

### California Redwood genome (27 Gb hexaploid)

	OONT 23-fold + short reads 122-fold	HiFi reads 22-fold
<b>Assembly size (Gb)</b>	26.5	<b>47.7</b>
<b>Contig N50 (Mb)</b>	0.11	<b>1.92</b>
<b>Average alignment of <i>A. thaliana</i> genes*</b>	80.80%	<b>87.70%</b>
<b>Assembly time</b>	5–6 months	<b>6 days</b>

“If your genome isn't HiFi, it's no longer reference grade.”

Kevin McKernan,  
Medicinal Genomics

— Haplotype resolution

— More complete genes

— HiFi sequencing produces high-quality results quickly and affordably

\*Completeness of alignments of 458 *Arabidopsis thaliana* genes, measured by how much of each gene was covered in the alignment averaged over all the genes. Learn more at [pacb.com/redwood](http://pacb.com/redwood). ONT data: Sequencing and assembling mega-genomes of mega-trees.

## Explore metagenomes in high resolution

HiFi reads enable direct error-free gene discovery in heterogeneous samples, even in low-abundance species with too little coverage for assembly.

Human fecal sample	Number of predicted genes	Mean length (bp)	Clustered genes (99% id)	Mean predicted genes / read
HF 1	19,639,322	1,005	1,012,982	7.9
HF 2	22,064,417	1,001	1,141,123	8.4
HF 3	18,059,181	1,024	1,154,341	7.6
HF 4	19,844,033	978	1,250,711	9.3
HF 5	18,396,237	970	1,087,015	9

Human fecal samples sequenced on the Sequel<sup>II</sup> system. Learn more at [pacb.com/microbial-genomics](https://pacb.com/microbial-genomics).

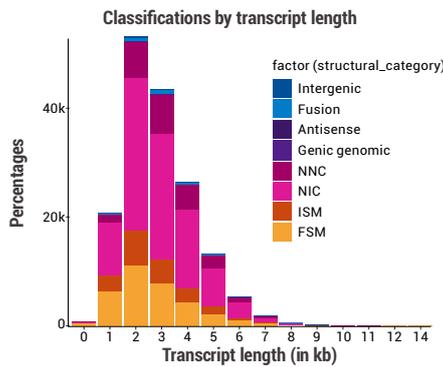
### Did You Know?

30–70% of short-read data do not map to a metagenome assembly and are not useful for identifying genes

Each individual HiFi read contains complete genes even for low-abundance species

## Characterize and annotate whole transcriptomes

Access full-length transcripts to identify novel genes and complex alternative splicing events with HiFi reads.



- **162,290 transcripts**
- **Min: 80 bp**
- **Max: 14,288 bp**
- **Mean: 3,347 bp**

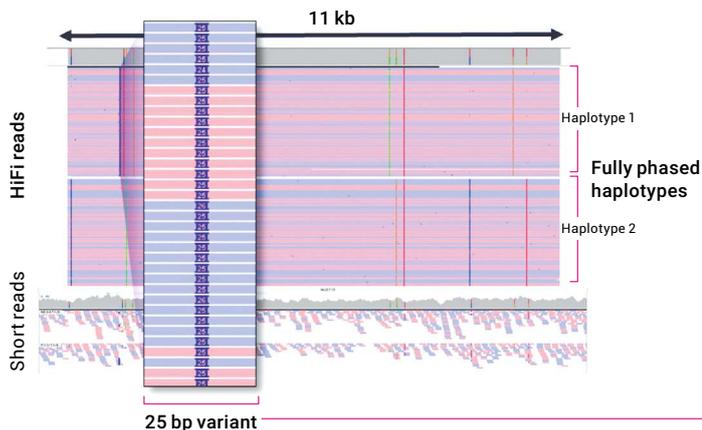
Detect full-length transcripts up to 14 kb

### Alzheimer brain sample

	Known	Novel	Total
<b>Genes</b>	17,051	619	17,670
<b>Isoforms</b>	51,660	110,630	162,290

Sequencing of an Alzheimer brain sample using the Iso-Seq<sup>®</sup> method on the Sequel II system, learn more at [pacb.com/alzheimer](https://pacb.com/alzheimer).

## Target even difficult-to-sequence genes or regions



HiFi reads let you obtain Sanger-quality accuracy and detect novel variants missed by other technologies.

An 11 kb amplicon of a clinically-actionable gene sequenced on the Sequel system. Learn more at [pacb.com/ASHGworkshop](https://pacb.com/ASHGworkshop).

Detect variants missed by short reads



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## SEQUENCE WITH CONFIDENCE

Achieve comprehensive variant detection



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