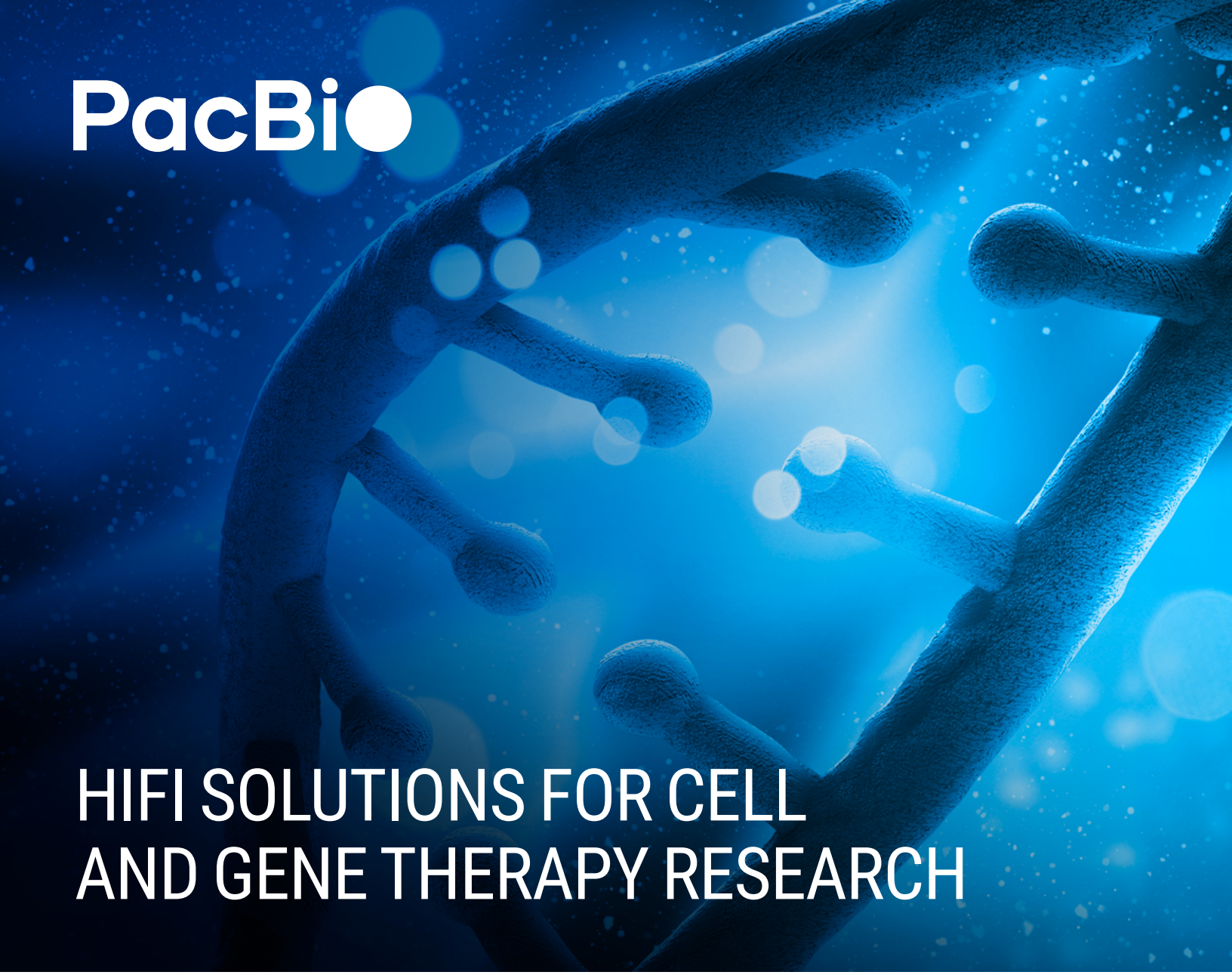


HIFI SOLUTIONS FOR CELL AND GENE THERAPY RESEARCH

Long-read sequencing at Sanger-level accuracy

Cell and gene therapy have emerged as promising tactics to combat disease and require equally innovative methods to facilitate their development.

Highly accurate long-read sequencing provides more complete and unbiased information crucial for cell and gene therapy development, ensuring the confidence you need to advance your projects.

Gene therapy research: Leverage single-molecule resolution for precise discovery, design enhancement, and quality evaluation of AAV vectors

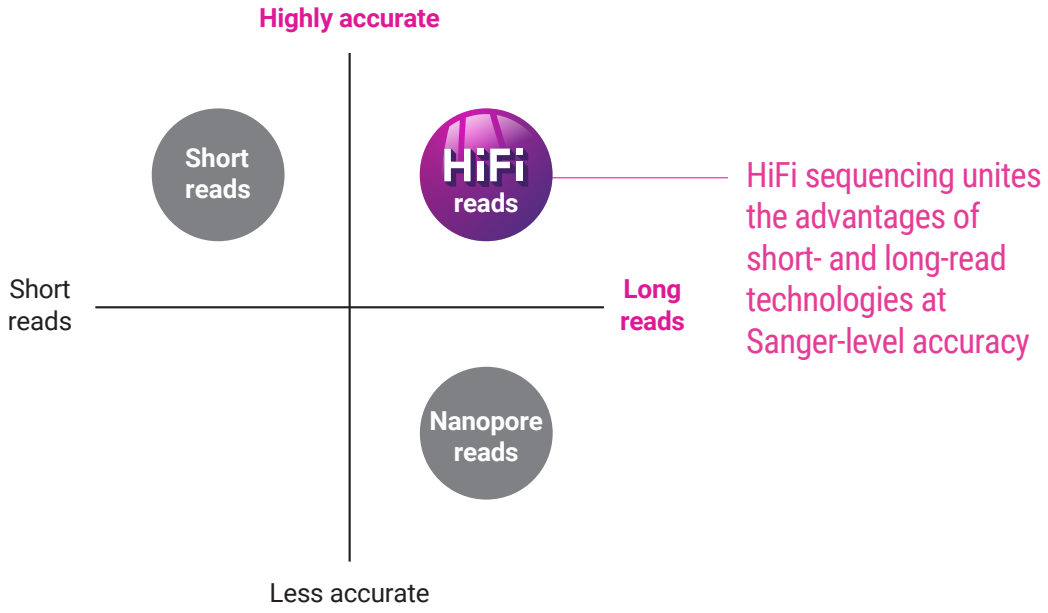
Gene editing research: Fully understand the potential outcomes of gene editing approaches

Cell therapy research: Assess variation and confirm genomic integrity at scale

mRNA vaccine research: Characterize full-length mRNA sequences to assess potential vaccine stability and efficacy

What is HiFi sequencing?

PacBio® HiFi sequencing unites long reads and accuracy, giving you the highest quality genomic data. When it comes to your cell and gene therapy research, why compromise with technologies that provide limited information?



The benefits of HiFi reads

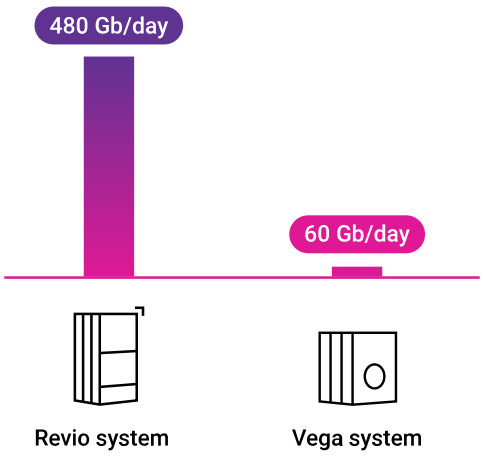
- Long read lengths
- Low DNA input requirements and easy library prep
- Low coverage requirements
- Small file sizes to minimize compute time
- Supported analysis tools consolidated into a single pipeline
- High read accuracy
- Compatible ecosystem partners to enable an end-to-end workflow
- A single technology solution

What can you do with one SMRT Cell?

	Vega™ system HiFi within reach	Revio® system with SPRQ™ chemistry HiFi at scale	
	1 SMRT® Cell	1 SMRT Cell	4 SMRT Cells
Application	Samples per run		
Whole genome sequencing			
Human genome (20x coverage)	1	2	8
Human methylation profiling (5x)	4	8	32
***De novo* assembly (1 Gb genome)**	2	4	16
Microbial de novo assembly (2 Gb total)	384	384	1,536
Targeted panels			
Amplicon sequencing	>1,000	>1,000	>4,000
Target enrichment			
20 Mb panel	12	16	64
2 Mb panel	72	96	384
100 kb panel	288	384	1,536
PureTarget™ repeat expansion panel	48	48	192
RNA sequencing			
Kinnex™ single-cell RNA sequencing	1 (3,000–6,000 cells)	1 (6,000–10,000 cells)	4 (6,000–10,000 cells)
Kinnex full-length RNA sequencing			
5M reads	6	12	48
10M reads	3	6	24

All sample throughputs are estimates for either the Vega system with 1 SMRT Cell or the Revio system using SPRQ chemistry with both 1 or 4 SMRT Cells. Coverage may vary based on sample quality, library quality, and fragment lengths. Currently available SMRTbell® adapter index plates 96A-96D contain a total of 384 SMRTbell barcoded adapters. Microbial de novo assembly assumes microbes with 2 Gb of total genome size at 30x per sample. Single-cell transcriptomics assumes ≥80 million reads per library on the Revio system and ~50-60 million reads per library on the Vega system. Full-length RNA sequencing assumes a total of 60M reads for Revio SPRQ and 30M reads for Vega, regardless of plexity. Amplicon sequencing assumes a 12-hour movie time for 1–5 kb, 24-hour movie time for 5+ kb, and >50× per sample. Target enrichment assumes >50× per sample.

PacBio HiFi sequencing instruments



	Revio system	Vega system
Independent stages	4	1
Run time	24 hrs	24 hrs

Gene therapy research

The PacBio advantage

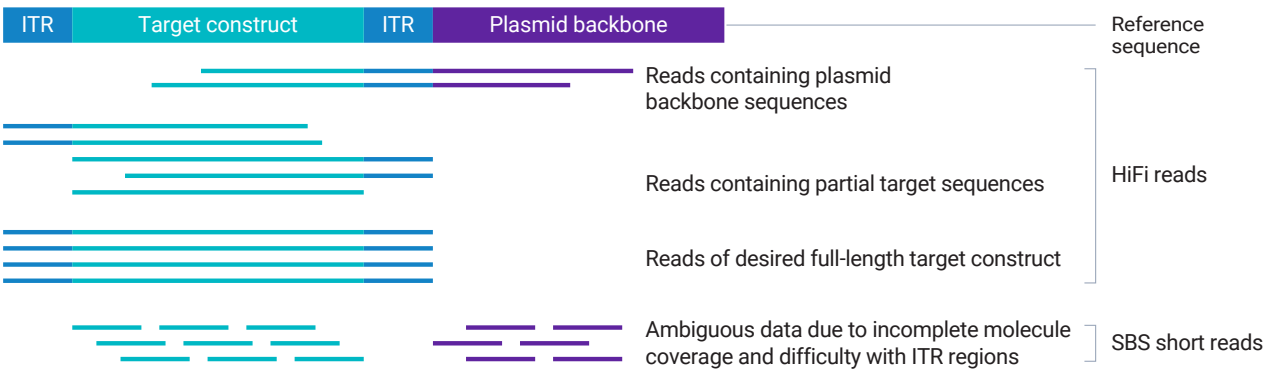
- **Rapidly accelerate the development** of novel vectors by leveraging sequencing at single full-length molecule resolution with Sanger-level accuracy
- **Monitor and improve AAV discovery** and manufacturing to reduce the risk of costly failures
- **Optimize rAAV vector discovery** and design for increased chances of success for improved vector properties
- **Easily implement end-to-end protocols** to save development time

Leverage single-molecule resolution for precise discovery, design enhancement, and quality evaluation of AAV vectors

In contrast to traditional sequencing methods that offer incomplete insights into AAV vectors, HiFi sequencing revolutionizes AAV characterization, with the ability to reveal hidden issues crucial for gene transfer efficacy and safety.

Monitor and improve AAV manufacturing

- Assess packaged impurity sequence profiles like identity, size distribution, and relative abundance.
- Profile payload sequence length and integrity.
- Identify payload sequence truncation hotspots.
- Characterize inverted terminal repeat (ITR) rearrangements and their integrity.
- Verify whether a target construct or gene of interest is correctly expressed and spliced.



Identify or engineer novel AAV capsids

Discover and engineer novel capsids with tissue-specific tropism for targeted delivery of AAV-based gene therapies to help improve the effectiveness and potential safety of your research approach. Sequencing of the entire cap gene as one molecule enables you to screen a wider range of variants to set your projects up for success.[2–4]

Assess host genome integration to avoid risk

AAV integration into the host genome could pose a safety risk. HiFi sequencing allows unambiguous identification of integration sites and resolves integrated concatemers and rearrangements that cannot be detected with short-read methods.[5]

Gene editing research

The PacBio advantage

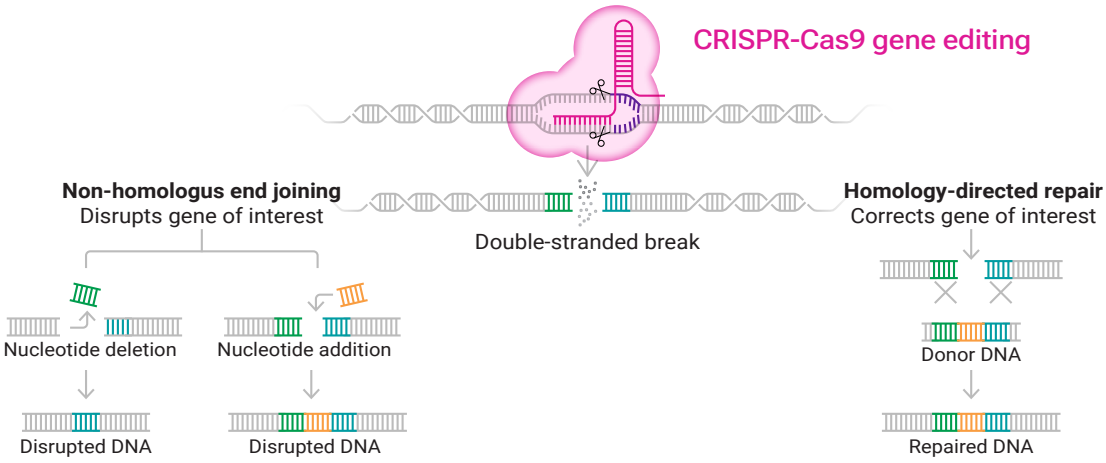
- **Detect and accurately measure** on- and off-target gene editing effects to improve potential risk to safety and efficacy
- **Analyze insertions** in greater detail and with haplotype resolution
- **Implement a range of highly accurate targeted or whole genome sequencing approaches** (including amplification-free methods) with the flexibility to fit your workflows

Assess on- and off-target gene editing effects more comprehensively

CRISPR-Cas9 and other gene editing methods are crucial for cell and gene therapy advancement. Using highly accurate long reads with HiFi sequencing technology, you can thoroughly verify gene editing and insertion strategies, whether for knock-outs or integrations.

Fully understand the potential outcomes of gene editing approaches

Fully grasping the scope of CRISPR-Cas9 editing demands long read lengths and high accuracy to detect both on- and off-target effects that may be overlooked by other methods. Precisely identifying genome modifications, like large-scale deletions, insertions, or structural changes, is crucial for a more comprehensive understanding of editing outcomes.[7,8]



Can result in long and short indels.
HiFi sequencing is one of the only known methods to accurately detect edits of different lengths ranging from single bases to several kb.

Can result in imprecise recombination, structural variants, or concatenation.
HiFi sequencing is one of the only known methods that can capture all outcomes at base-level accuracy.

Addgene. (2017) Chapter 3: Using CRISPR in Your Experiments. CRISPR 101: A Desktop Resource.

Analyze insertion sites more comprehensively

Gene insertion at a CRISPR target locus can result in both small and large indels. Similarly, evaluating the integration site of chimeric antigen receptor (CAR) into T-cell genomes is vital for CAR-T development in assessing safety and efficacy. Highly accurate long-read sequencing can catalog these insertions, including mutations, concatenations, and other complex mutations that short-read sequencing might overlook.

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

Genetic variation such as SNVs may introduce allele-specific Cas9 cleavage. HiFi sequencing can discriminate and resolve editing efficiency at haplotype resolution.[10]

Cell therapy research

The PacBio advantage

- **Accelerate discovery and development** with faster, more accurate, and comprehensive results than other technologies
- **Generate phased genomes** with 5mC and 6mA methylation information and structural variant calling to ensure high-quality starting materials and to avoid costly failures

Confirm genomic integrity at scale

Master cell banks and cell lines are prevalent in cell therapy research and development, where ensuring genomic integrity is paramount for quality and safety.

HiFi sequencing offers exceptional accuracy and turnaround time, enabling precise assessment of genomic integrity and variation, instilling the utmost confidence in your results.

	HiFi sequencing	SBS sequencing	Nanopore sequencing
Read length	15–20 kb	2×150 bp	10–100 kb
Read accuracy	99.95% (Q33)	99.92 (Q31)	99.26 (Q21)
Run time	24 hours	44 hours	72 hours
Yield	480 Gb (Revio system) 60 Gb (Vega system)	2,400–3,000 Gb	50–110 Gb
Variant calling — SNVs	✓	✓	✓
Variant calling — indels	✓	✓	✗
Variant calling — SVs	✓	✗	✓
5mc + 6mA methylation	✓	✗	✓
Phasing	✓	✗	✓

mRNA vaccine research

The PacBio advantage

- **Sequence full-length mRNA** at high accuracy to determine size and isoform distribution to more confidently assess your research products
- **Characterize homopolymers** such as poly(A) regions more accurately than with other sequencing methods to optimize your construct

Overcome common research challenges with characterizing mRNA vaccines

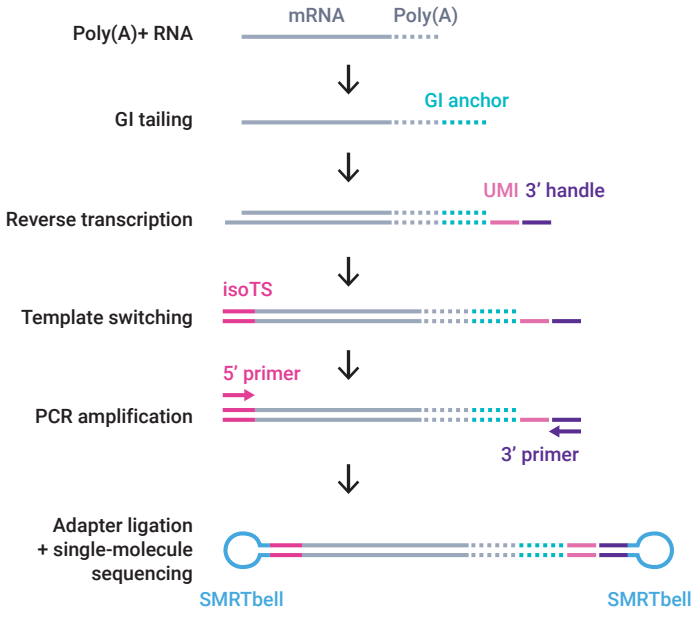
Following the COVID-19 pandemic, mRNA vaccines have emerged as effective tools against infectious disease and show potential as cancer treatments. Vital considerations for mRNA-based vaccine stability and efficacy include mRNA integrity and the length of its poly(A) tail.

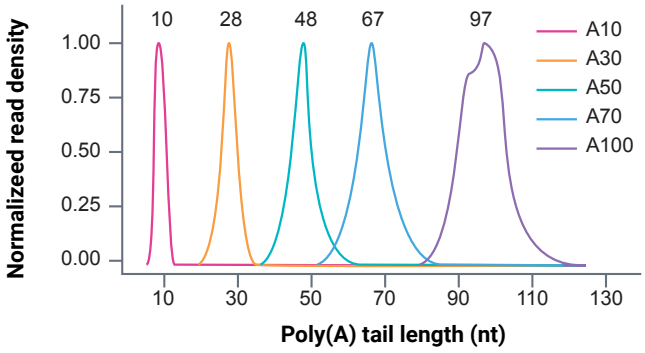
Characterize your mRNA at full length

PacBio long-read sequencing allows you to capture the full length of your transcript at single-molecule resolution, giving you isoform-level information at base-level accuracy.[11, 12]

Confidently sequence homopolymers such as poly(A)

In contrast to other methods, HiFi sequencing has the ability to accurately sequence long homopolymers such as full-length poly(A) tails. This allows you to determine the length distribution of mRNA molecules as an important determinant for vaccine efficacy.





Liu Y, Nie H, Liu H, Lu F. Poly(A) inclusive RNA isoform sequencing (PAIso-seq) reveals wide-spread non-adenosine residues within RNA poly(A) tails. Nat Commun. 2019;10:5292

A comprehensive toolbox of sequencing methods to support your project

PacBio equips you with multiple sequencing approaches to enable your research no matter the focus. This toolbox includes whole genome sequencing (WGS), plasmid sequencing, targeted sequencing, and RNA sequencing.

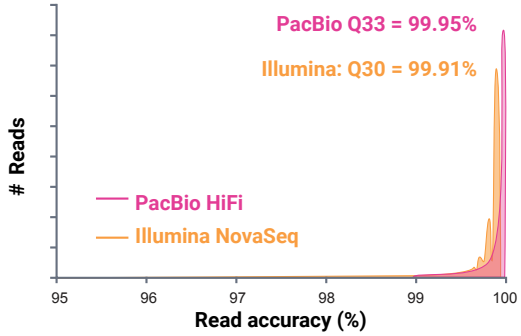
Whole genome sequencing (WGS)

The PacBio advantage

- **Achieve extraordinary accuracy**, even coverage, and genome completeness
- **Take advantage of allele resolution** (long-range phasing)
- **Enhance your R&D** with industry-leading performance for the range of variant detection needs and methylation detection

Obtain highly accurate and more complete whole genome data

As the flagship application of HiFi sequencing, WGS allows you to capture full genomes from telomere to telomere and automatically includes DNA methylation information. Highly accurate long-read sequencing allows you to confidently sequence these difficult areas with high GC content or repetitive regions. This extraordinary quality of sequencing data empowers you to discover what you might otherwise miss.



Read accuracy for whole genome sequencing.

Plasmid sequencing

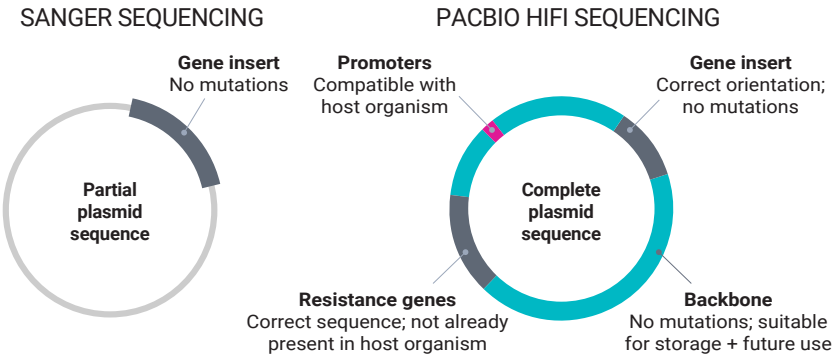
The PacBio advantage

- **Sequence full plasmids** with Sanger-level accuracy and long read lengths
- **Achieve uniform coverage** across repetitive regions, homopolymers, and GC-rich regions that pose challenges for other sequencing methods
- **Accelerate turnaround times** and increase data security with in-house sequencing

Capture and verify the full length of your plasmid at Sanger-level accuracy

Plasmids play a vital role in cell and gene therapy development. Traditional Sanger sequencing is limited to confirming insert sequences or may involve complex primer walking. With HiFi sequencing, you can sequence full-length plasmids —including backbone, promoters, resistance genes, and gene inserts — without relying on a reference sequence.

Owning a PacBio instrument in-house reduces turnaround time and enhances data and IP security.



Targeted sequencing

The PacBio advantage

- **Enable screening approaches** with long-range amplicon sequencing
- **Assess construct or vector integration sites** using target capture enrichment or amplification-free enrichment
- **Profile tandem repeats** using amplification-free enrichment
- **Sharpen your insights** with haplotype resolution

Targeted sequencing with HiFi technology takes your assays to the next level

Increase the utility of your targeted sequencing approaches and assays by removing the read length and accuracy limitations posed by other technologies. HiFi technology allows you to sequence genes, inserts, or large libraries at full length. Where other long-read technologies may lose reads due to an inability to align barcodes, HiFi sequencing comparatively aligns 2–3 times more barcodes.

Amplification-free target enrichment using PureTarget™ kits

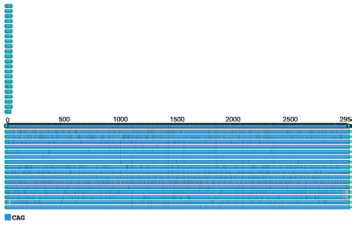
The new amplification-free PureTarget kit allows you to enrich for your target without PCR errors and less size bias. Assess repeat regions or gene editing/insertion sites at allele-specific resolution.*

In contrast to other technologies, HiFi sequencing allows you to interrogate regions that are difficult to amplify such as those with high GC content or repeats.

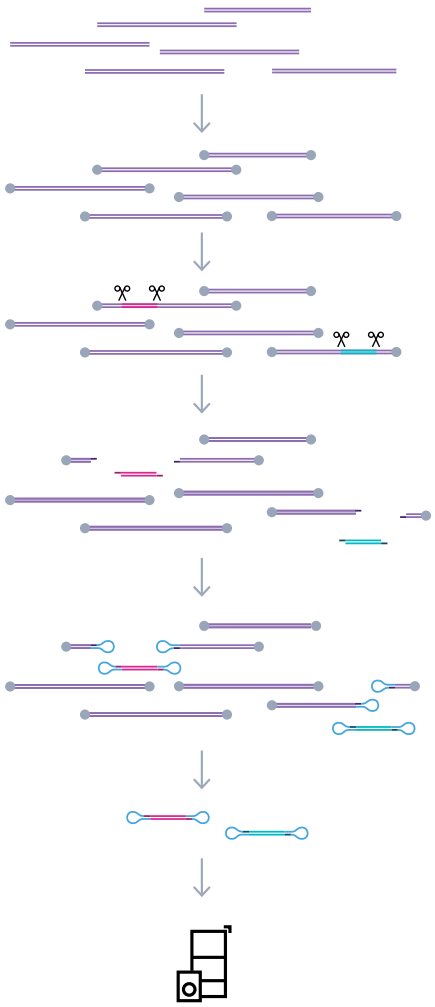
HiFi technology overcomes the challenges of short-read sequencing with the ability to identify difficult regions like long indels, concatemers, and inversions.

This allows you to accurately measure the length of tandem repeats or analyze on- and off-target editing outcomes and construct insertions (e.g., chimeric antigen receptors - CAR).

* PureTarget repeat expansion panel available to target 20 repeat expansions associated with neurological disease. Other panels require custom guide RNA design and purchase from 3rd party vendor. Contact a PacBio scientist (pacb.com/scientist) for more information.



PureTarget libraries have less size bias than other targeted sequencing methods. Using the PureTarget repeat expansion panel, a sample was sequenced at the repeat expansion in the *DMPK* gene which causes Myotonic Dystrophy 1 (https://pubmed.ncbi.nlm.nih.gov/38594146/). The expected genotype is a wild type allele less than 102 bp (< 34 trinucleotide repeat units) and an expanded allele > 6000 bp (> 2000 repeats). The wild type allele is estimated to be 13 repeats long and is spanned by 284 reads while the long allele is estimated to be 3,039 repeats long and is fully spanned by 64 reads. The sample was sequenced in an 8-plex on the Sequel IIe system and each sample was prepared with 2 µg of genomic DNA.

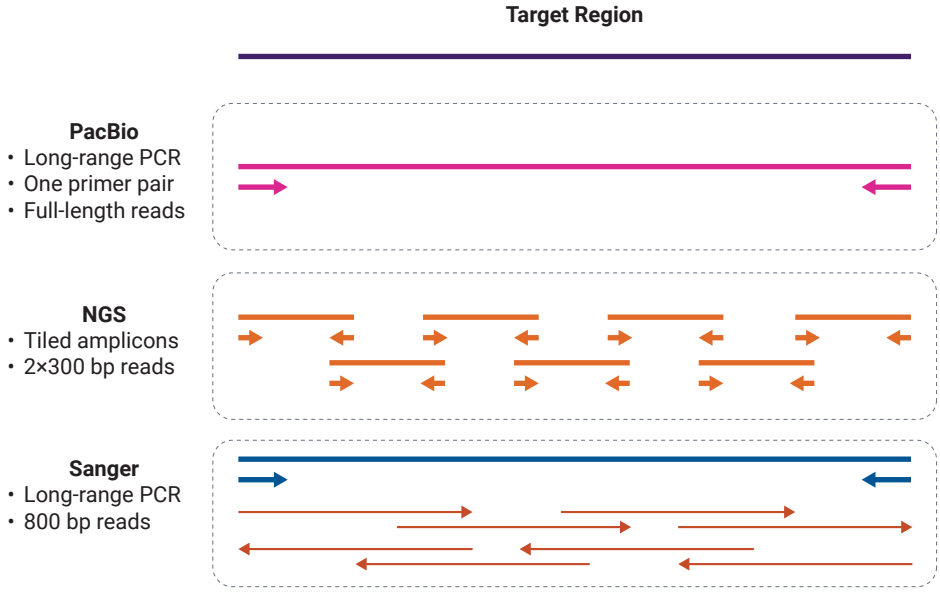


Amplicon sequencing

HiFi amplicon sequencing offers a simplified workflow compared to other sequencing technologies. Easily sequence single genes/ target regions or small panels at low cost using supported barcoding and library prep products. If you can amplify it, you can sequence it at full length.

Amplicon sequencing with NGS or Sanger is complex

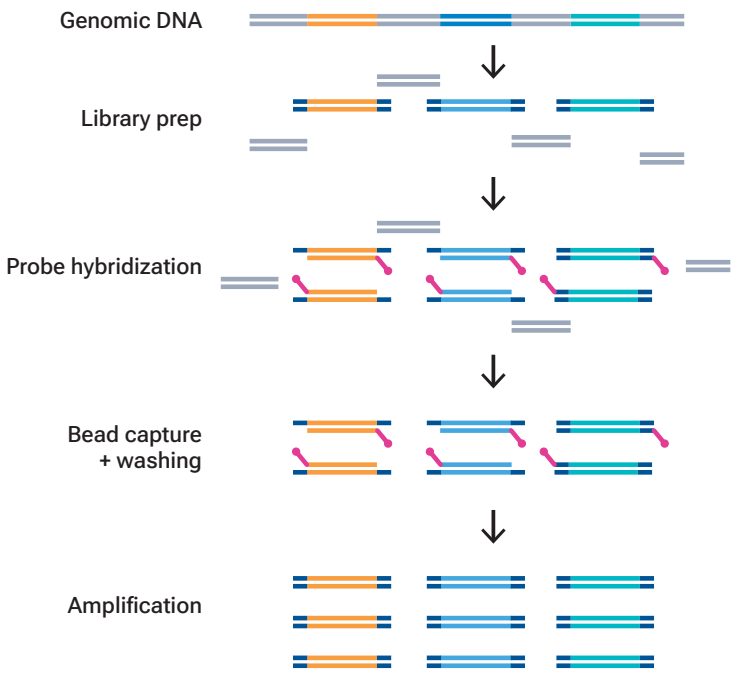
	HiFi reads	NGS	Sanger
Primer pairs	1	Multiple	Multiple
Assay optimization	Easy	Complex	Complex
Amplicon pools	1	Multiple	1 or multiple
Primer design	Robust to variants	Complex	Complex
Structural variants	Yes	No	Limited
Phasing	Complete	Very limited	Limited



Hybrid capture

HiFi target enrichment can help evaluate vector integration and reveal concatemers, inversions, and regions of genomic instability

Hybridization capture allows you to sequence small to large panels at low cost, high throughput, and with a standard workflow. Supported products from PacBio + Twist Biosciences easily allow for customization and sequencing of difficult-to-assess genes at high accuracy and haplotype resolution.



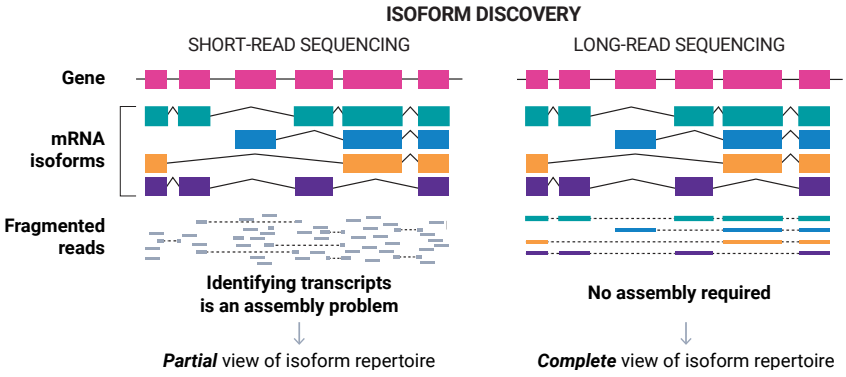
RNA sequencing

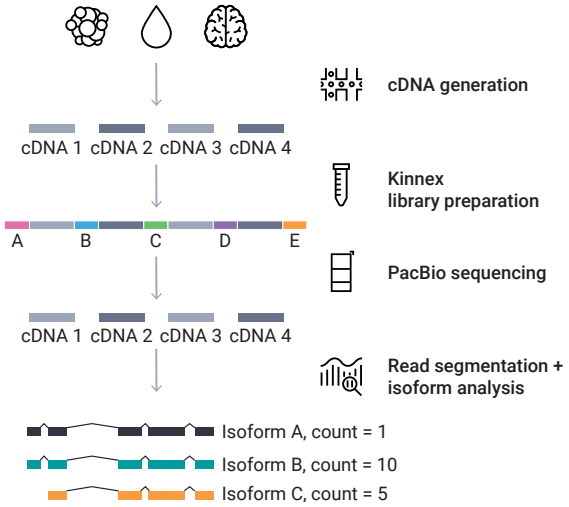
The PacBio advantage

- **Exceptional data** providing answers short reads cannot
- **Isoform resolution** to help identify potential targets you otherwise could not
- **Increased throughput** through concatenation using Kinnex™ kits helps to understand transcriptional changes through differentiation, drug treatment, etc.
- **Scalable, cost-effective RNA sequencing** with Kinnnex kits

RNA expression and isoform-specific expression are critical to the understanding of cells and disease pathologies.

Long-read RNA sequencing eliminates the need for transcript assembly, which cannot accurately resolve isoform structure. Long-read RNA sequencing using PacBio (the Iso-Seq method) sequences the full-length cDNA to provide an unambiguous view of the transcriptome.





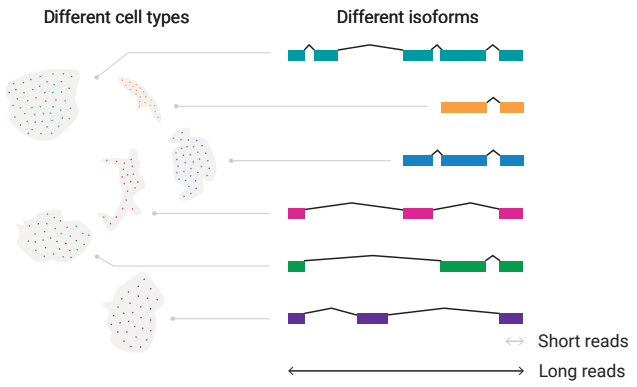
Bulk RNA sequencing

Obtain full-length isoform quantification with flexible sample multiplexing. Increase the sequencing depth by concatenation using Kinnex kits. Bulk RNA sequencing is ideal for relative gene expression characterization and detection of novel isoforms or splice sites. Increase your ability to understand biological pathways and disease mechanisms using exceptional data with more reads in less time.

Single-cell RNA sequencing

Identify and characterize cell types as well as profile immune cells by taking advantage of highly accurate cell type-specific isoform sequencing with Kinnex kits. Concatenation through Kinnex allows you to capture more full-length cDNAs per cell.

Kinnex scRNA kits are compatible with *10x Single Cell 3'* or *5' kits* and result in a higher percentage of usable reads than other technologies. This increases your ability to recover/ assign up to 5-fold more cells than using other long-read sequencing methods.



PacBio

Ready to get started with HiFi sequencing?

Learn more about HiFi sequencing:
pacb.com/hifi

Learn more about gene therapy solutions:
pacb.com/gene-therapy

Connect with a PacBio scientist to get started:
pacb.com/scientist

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