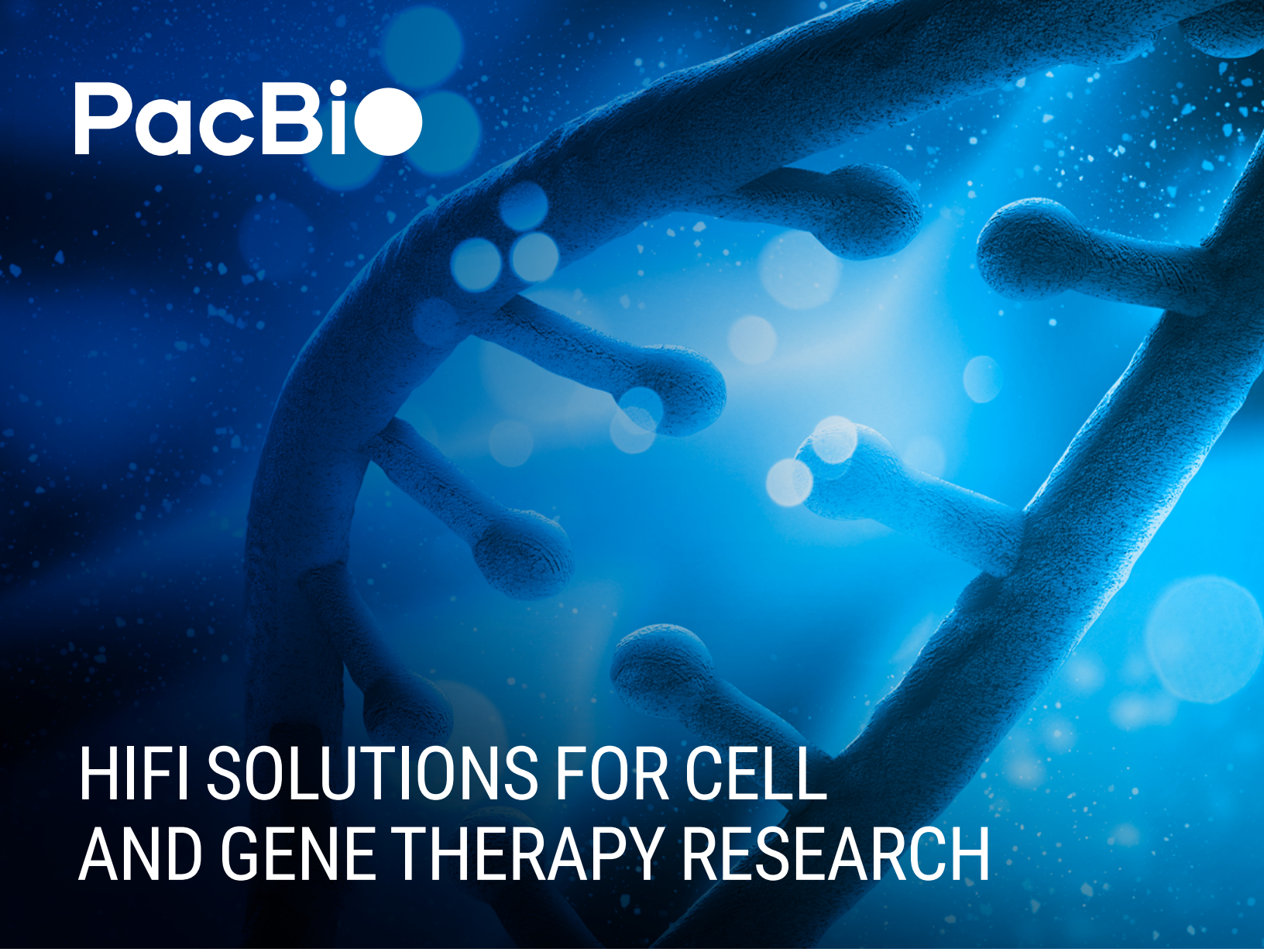


HIFI SOLUTIONS FOR CELL AND GENE THERAPY RESEARCH

Long-read sequencing at Sanger-level accuracy

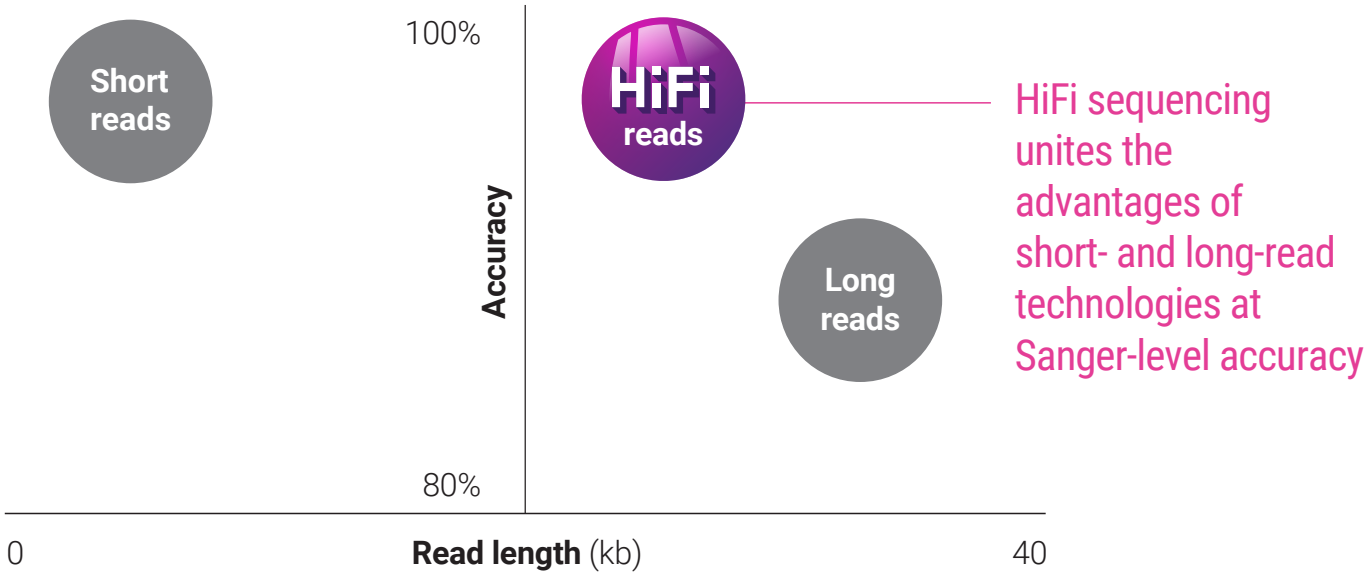
Cell and gene therapy have emerged as promising tactics to combat disease and require equally innovative methods to facilitate their development.

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
- **Plasmid sequencing:** Capture and verify the full length of your plasmid at Sanger-level accuracy
- **Gene editing research:** Fully understand the potential outcomes of gene editing approaches
- **Cell therapy research:** Assess variation and confirm genomic integrity at scale
- **mRNA vaccines:** 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?



HiFi sequencing unites the advantages of short- and long-read technologies at Sanger-level accuracy

The benefits of HiFi reads

- Long read lengths up to 20 kb
- Easy library preparation
- Low coverage requirements
- Small file sizes to minimize compute time
- Uniform coverage
- High read accuracy (99.9%)
- More comprehensively assess variants
- A single technology solution

A typical 20,000 bp HiFi read has ~**8 incorrect bases**

Gene therapy research

The PacBio advantage

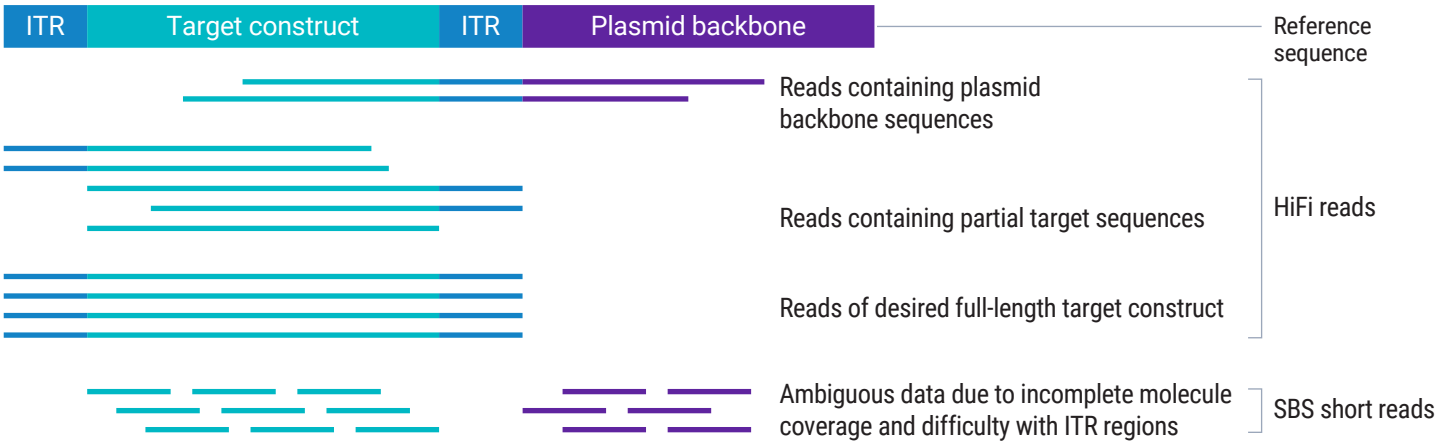
- **Rapidly accelerate the development** of novel vectors by leveraging sequencing at single full-length molecule resolution with Sanger-level accuracy
- **Monitor and improve AAV discovery** and manufacturing to reduce the risk of costly failures
- **Optimize rAAV vector discovery** and design for increased chances of success
- **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, revealing 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]

Plasmid sequencing

The PacBio advantage

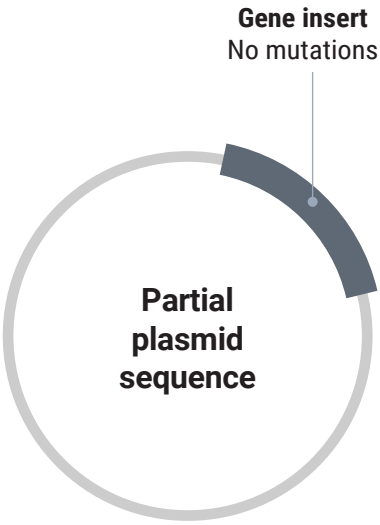
- **Sequence full plasmids** with Sanger-level accuracy and long read lengths
- **Achieve uniform coverage** across repetitive regions, homopolymers, and GC-rich regions that pose challenges for other sequencing methods
- **Accelerate turnaround times** and increase data security with in-house sequencing

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

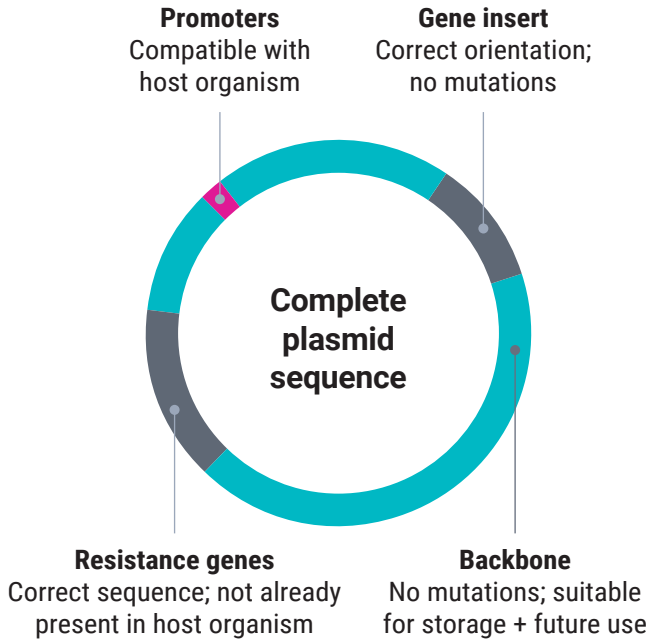
Plasmids play a vital role in cell and gene therapy development. Traditional Sanger sequencing is limited to confirming insert sequences and may involve complex primer walking. With HiFi sequencing, you can sequence full-length plasmids —including backbone, promoters, resistance genes, and gene inserts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

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

SANGER SEQUENCING



PACBIO HIFI SEQUENCING



Gene editing research

The PacBio advantage

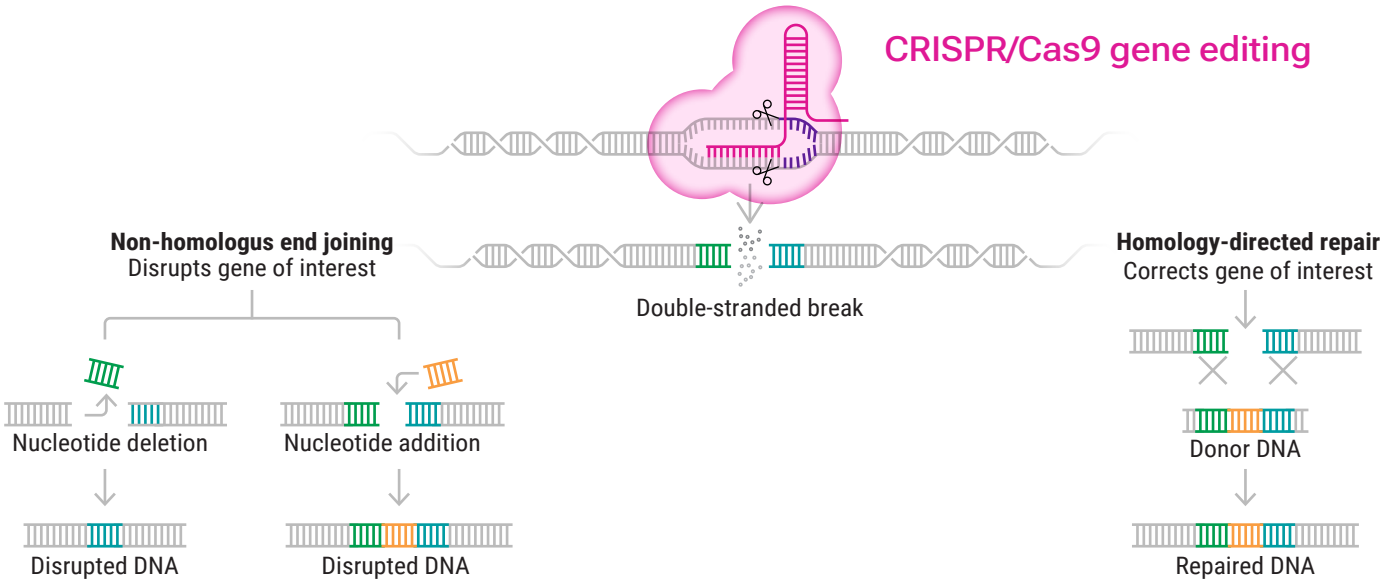
- **Detect and accurately measure** on- and off-target gene editing effects to improve the potential safety and efficacy characterization
- **Analyze insertions** in greater detail and with haplotype resolution
- **Implement a range of highly accurate targeted or whole genome sequencing approaches** (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]



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 your discovery** and development with faster, more accurate, and more comprehensive results than other technologies
- **Generate phased genomes** with 5mC 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 (Revio™ system) 30 hours (Sequel® systems)	44 hours	72 hours
Yield	90 Gb (Revio system) 20–30 Gb (Sequel systems)	2,400–3,000 Gb	50–110 Gb
Variant calling — SNVs	✓	✓	✓
Variant calling — indels	✓	✓	✗
Variant calling — SVs	✓	✗	✓
5mc methylation	✓	✗	✓
Phasing	✓	✗	✓

mRNA vaccines

The PacBio advantage

- **Sequence full-length mRNA** at high accuracy to determine size and isoform distribution to most 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

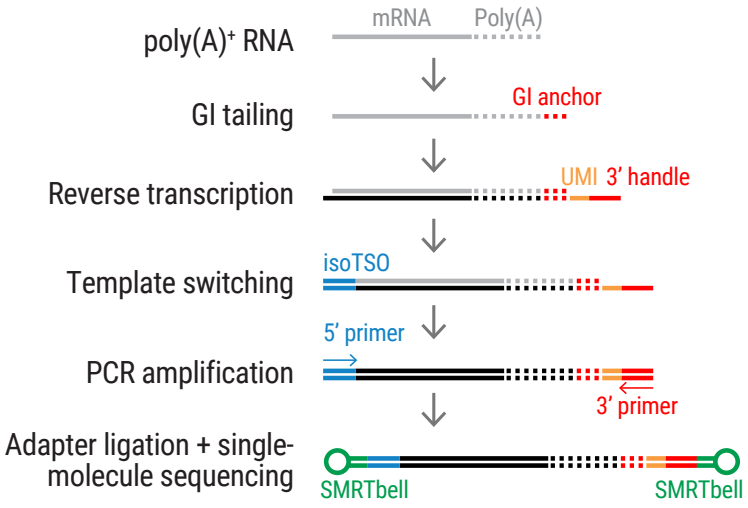
Following the COVID-19 pandemic, mRNA vaccines have emerged as effective tools against infectious disease and show potential as cancer treatments. Vital considerations for mRNA-based vaccine stability and efficacy include mRNA integrity and the length of its poly(A) tail.

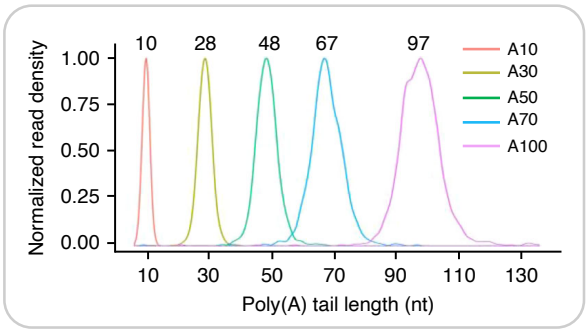
Characterize your mRNA at full length

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

PacBio

Ready to get started with HiFi sequencing?

Learn more about HiFi sequencing:
pacb.com/hifi

Learn more about plant + animal genetics:
pacb.com/gene-therapy

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

1. Xie J, Mao Q, Tai PWL, et al (2017) Short DNA hairpins compromise recombinant adeno-associated virus genome homogeneity. *Mol Ther* 25:1363–1374. https://doi.org/10.1016/j.ymthe.2017.03.028
2. Paulk NK, Pekrun K, Zhu E, et al (2018) Bioengineered AAV capsids with combined high human liver transduction in vivo and unique humoral seroreactivity. *Mol Ther* 26:289–303. https://doi.org/10.1016/j.ymthe.2017.09.021
3. Casy W, Garza IT, Chen X, et al (2023) SMRT sequencing enables high-throughput identification of novel AAVs from capsid shuffling and directed evolution. *Genes* 14:1660. https://doi.org/10.3390/genes14081660
4. Hsu H-L, Brown A, Loveland AB, et al (2020) Structural characterization of a novel human adeno-associated virus capsid with neurotropic properties. *Nat Commun* 11:3279. https://doi.org/10.1038/s41467-020-17047-1
5. Dalwadi DA, Calabria A, Tiyaboonchai A, et al (2021) AAV integration in human hepatocytes. *Mol Ther* 29:2898–2909. https://doi.org/10.1016/j.ymthe.2021.08.031
6. Wang Y, Ma X, Yang L, Ye H, Jia R. (2023) Direct Pacbio sequencing methods and applications for different types of DNA sequences. doi: https://doi.org/10.1101/2023.12.12.571020
7. 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
8. 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
9. 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
10. 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
11. Legnini I, Alles J, Karaiskos N, Ayoub S, Rajewsky N. FLAM-seq: full-length mRNA sequencing reveals principles of poly(A) tail length control. *Nat Methods.* 2019;16:879–86
12. 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

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