# Powering rare disease research and drug development with PacBio long-read sequencing

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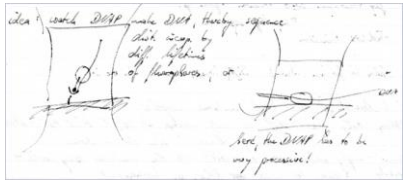
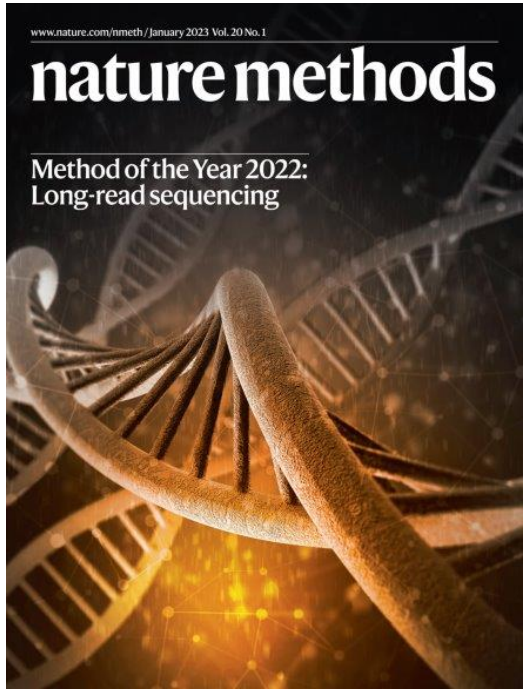
# Who is PacBio?

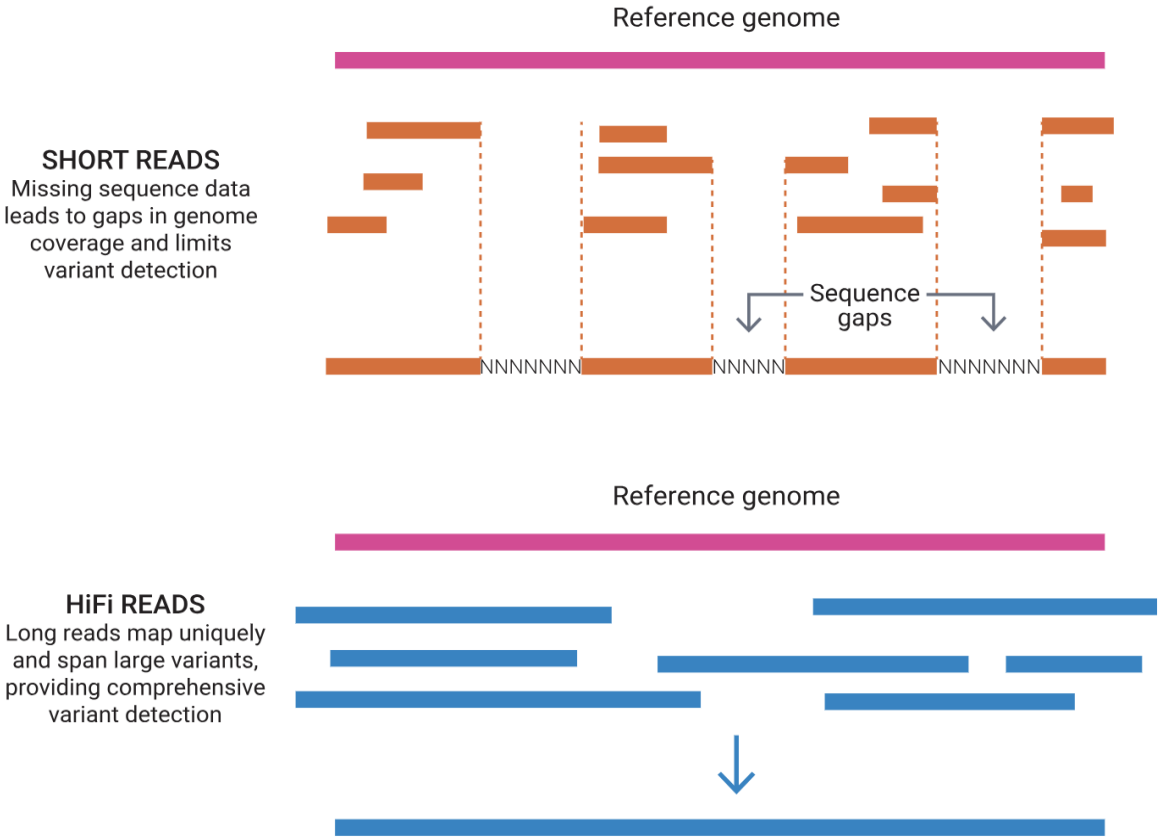



Nanofluidic design, single-molecule real-time chemistry
Cornell University, 1997

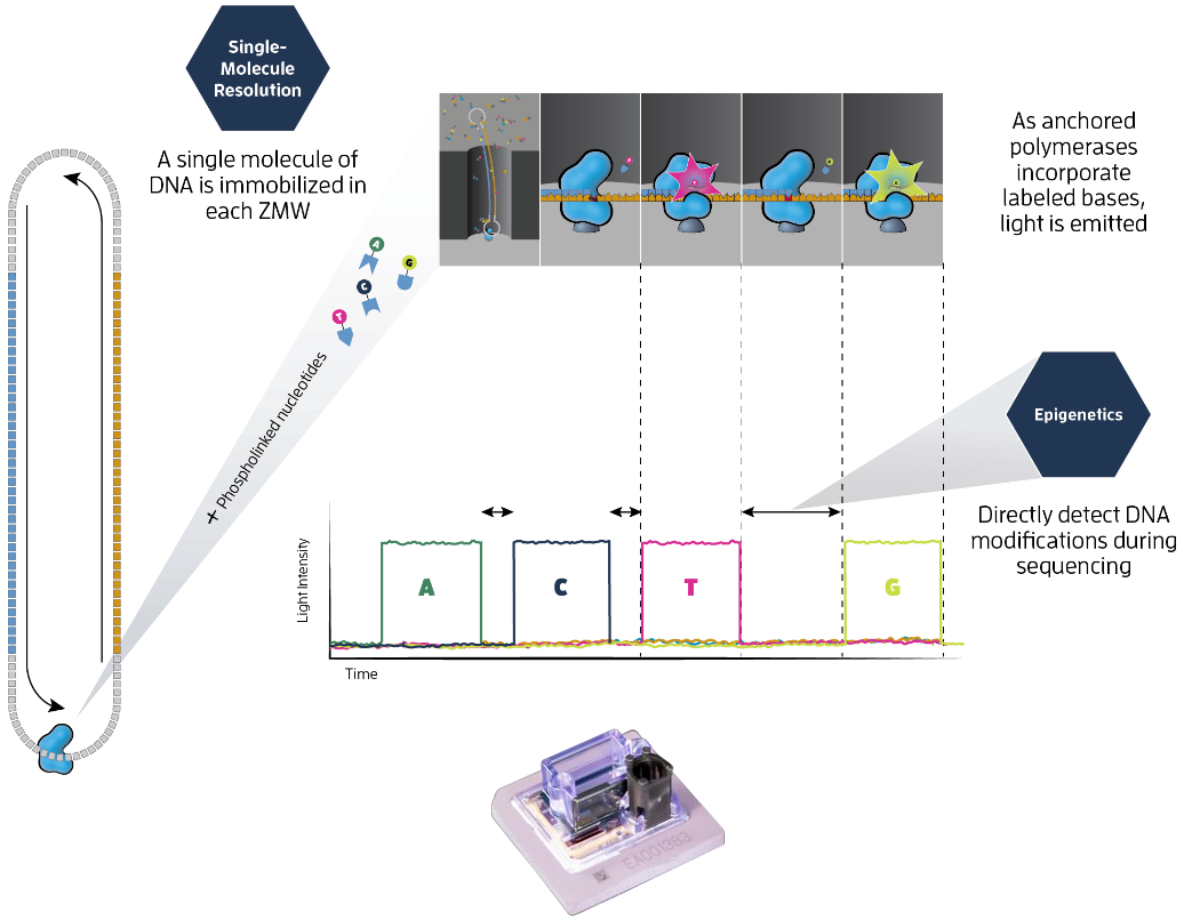
Menlo Park, CA

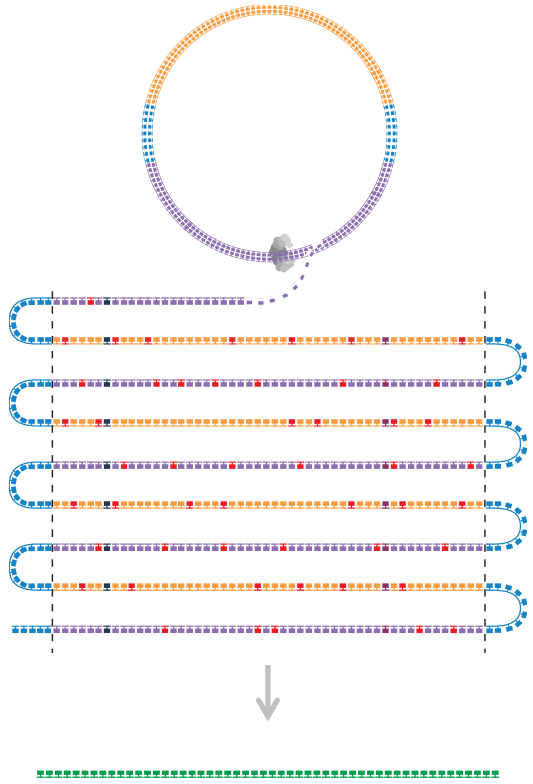
# What is long-read sequencing?



# What is HiFi sequencing?



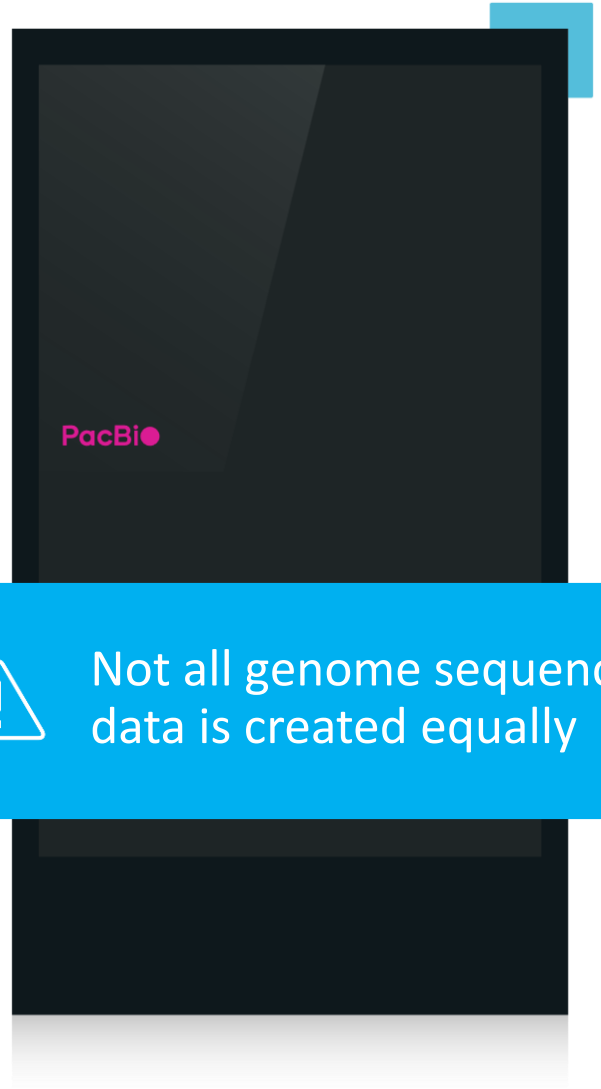



**HiFi read**

>99.9% accuracy

15-20 kb (up to 100x longer than short reads)

# Putting the “W” back in WGS




Not all genome sequence data is created equally

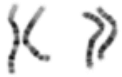
| Short-read WGS | HiFi WGS |
|---|---|
| | Structural variation |
| | Repeat expansions |
| | Segmental duplications |
| | Phasing/haplotype |
| SNPs / small indels | SNPs / small indels |

| | |
|---|---|
| ✕ Needs a reference genome | ✓ Clinical-grade, reference quality |
| ✕ Miss 100s of millions of base pairs | ✓ Resolve large, complex regions |
| ✕ Blind to ~400 medically relevant genes in dark regions | ✓ Methylation included |

PacBio HiFi delivers a new class of WGS

# HiFi sequencing drives rare disease solves

*What is the causal variant underlying disease? What is the disease mechanism of action?*




| Karyotype | Microarrays | Short reads | | Long reads |
|---|---|---|---|---|
| | | Exome | Genome | HiFi Genome |
| Chromosomal abnormalities | Copy-number variants >50kb | SNVs & indels, some large exonic variants | SNVs, indels, some large variants | **SNVs, indels, SVs, CNVs, phasing, translocations, inversions, repeat expansions** |
| ~5% rare disease explanation rate | ~10% | ~30% | ~40% | **>50%** |

Other HiFi tools: Kinnex full-length isoform seq, methylation, chromatin accessibility

**Familial long-read sequencing increases yield of de novo mutations**

Michelle D Noyes [1], William T Harvey [1], David Porubsky [1], Arvis Sulovari [1], Ruiyang Li [1], Nicholas R Rose [1], Peter A Audano [1], Katherine M Munson [1], Alexandra P Lewis [1], Kendra Hoekzema [1], Tuomo Mantere [2], Tina A Graves-Lindsay [3], Ashley D Sanders [4], Sara Goodwin [5], Melissa Kramer [5], Younes Mokrab [6], Michael C Zody [7], Alexander Hoischen [8], Jan O Korbel [4], W Richard McCombie [5], Evan E Eichler [9]

>25% more de novo SNPs and indels discovered with HiFi compared to short read WGS

ARTICLE · Volume 24, Issue 6, P1336-1348, June 2022 · *Open Access* · Download Full Issue

**Genomic answers for children: Dynamic analyses of >1000 pediatric rare disease genomes**

Ana S.A. Cohen [1,2,3] · Emily G. Farrow [1,3,4] · Ahmed T. Abdelmoity [4] · Joseph T. Alaimo [2,3] · Shivarajan M. Amudhavalli [3,5] · John T. Anderson [6] · Lalit Bansal [4] · Lauren Bartik [3,5] · Primo Baybayan [7] · Bradley Belden [1] · Courtney D. Berrios [1] · Rebecca L. Biswell [1] · Pawel Buczkowicz [8] · Orion Buske [8] · Shreyasee Chakraborty [7] · Warren A. Cheung [1] · Keith A. Coffman [4] · Ashley M. Cooper [4] · Laura A. Cross [5] · Tom Curran [9] · Thuy Tien T. Dang [4] · Mary M. Elfrink [1] · Kendra L. Engleman [5] · Erin D. Fecske [4] · Cynthia Fieser [4] · Keely Fitzgerald [4] · Emily A. Fleming [5] · Brandi N. Gaden [5] · Jennifer L. Gannon [1] · Rose N. Gelineau-Morel [1,4] · Margaret Gibson [1] ·

HiFi found 2x more structural variants, 4x more rare transmitted SVs compared to short read WGS

# HiFi sequencing drives rare disease solves

**NIH funds new *All of Us* Research Program genome center to test advanced sequencing tools**

JUNE 14, 2023

**PacBio and Radboud University Medical Center Team Up for New Discoveries in Rare Disease Research**

FEBRUARY 29, 2024 | HUMAN GENETICS RESEARCH

**Progress report: HiFi sequencing enables Care4Rare Consortium to advance rare disease research**

**Invitae and Pacific Biosciences Collaborate to Develop Whole Genome Sequencing-Based Assays for Pediatric Epilepsy Diagnostics**

Kansas City, October 06, 2023

**Children's Mercy Kansas City First to Use 5-Base HiFi Genomic Sequencing in the Clinical Setting**

AUGUST 7, 2023

**PacBio and GeneDx Launch Research Collaboration with the University of Washington to Study Long-Read Whole Genome Sequencing for Increased Diagnostic Yield in Neonatal Care**

HudsonAlpha researchers use highly accurate long-read sequencing technology to help diagnose rare disease

Children's Mercy KANSAS CITY

INVITAE

KK Women's and Children's Hospital SingHealth

HUDSONALPHA INSTITUTE FOR BIOTECHNOLOGY

Stanford University

Genomic ANSWERS for KIDS

横浜市立大学 YOKOHAMA CITY UNIVERSITY

Radboud Universiteit

All of Us RESEARCH PROGRAM

Gabriella Miller Kids First PEDIATRIC RESEARCH PROGRAM Data Resource Center

UNIVERSITY of WASHINGTON

Cincinnati Children's® Hospital Medical Center

DISCOVERY LIFE SCIENCES

# HiFi sequencing for drug development: ASO design

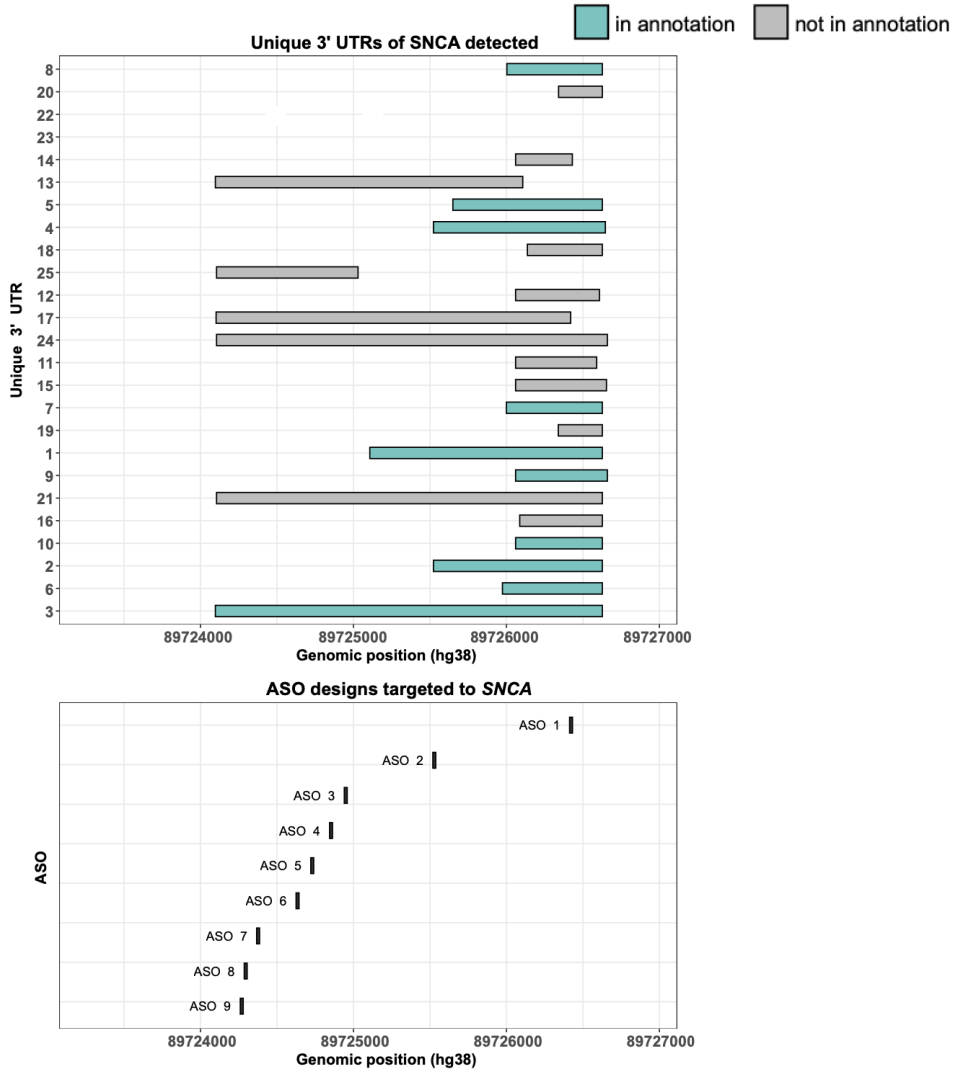
## Long read sequencing enables:

- Precise mapping of disease-causing mutations with base-pair level resolution
- Accurate identification of splice sites and regulatory elements
- Better understanding of local sequence context and target sites
- Improved detection of off-target effects, potentially improving safety profile

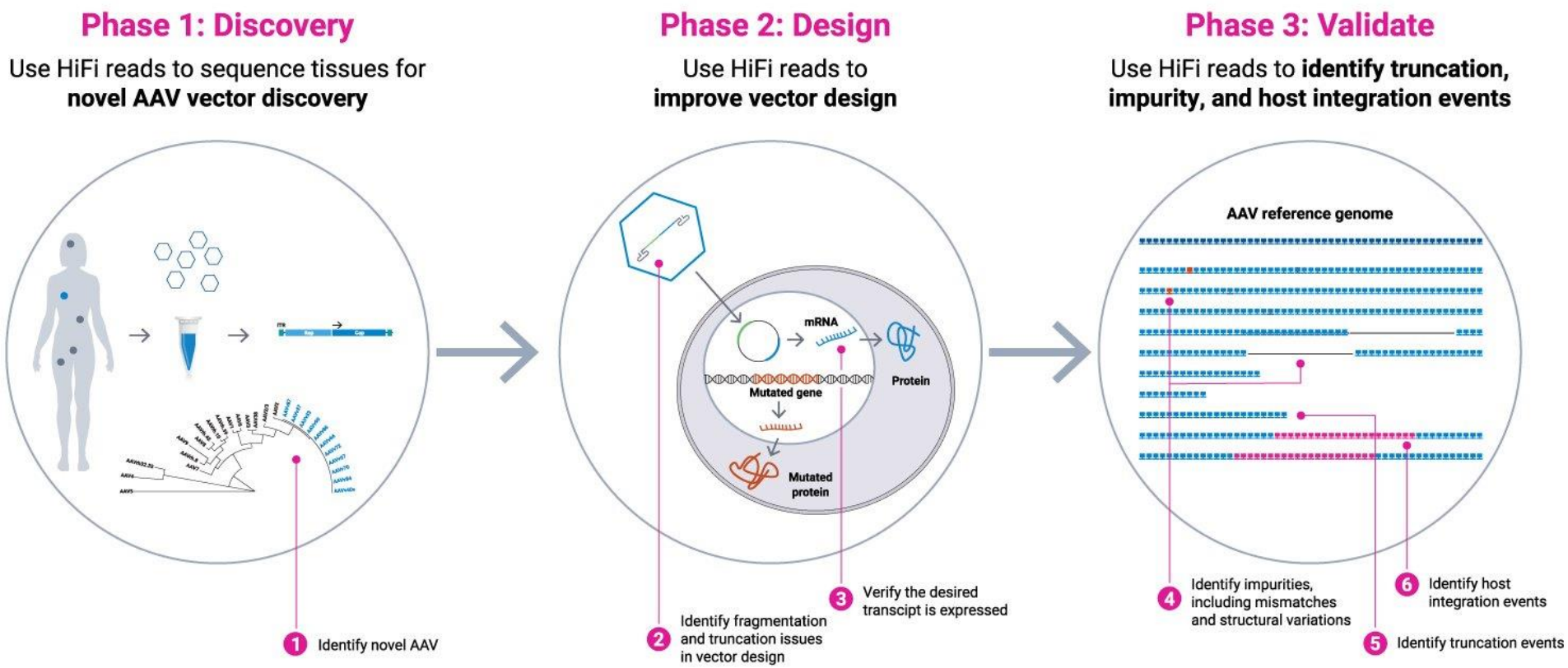
**The diversity of *SNCA* transcripts in neurons, and its impact on antisense oligonucleotide therapeutics**

James R. Evans, Emil K. Gustavsson, Ivan Doykov, David Murphy, Gurvir S.Virdi, Joanne Lachica, Alexander Röntgen, Mhd Hussein Murtada, Chun Wei Pang, Hannah Macpherson, Anna I.Wernick, Christina E.Toomey, Dilan Athauda, Minee L. Choi, John Hardy, Nicholas W.Wood, Michele Vendruscolo, Kevin Mills, Wendy Heywood, Mina Ryten, Sonia Gandhi

**doi:** https://doi.org/10.1101/2024.05.30.596437



# HiFi sequencing for drug development: gene editing



# HiFi sequencing for drug development: gene editing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scientific reports

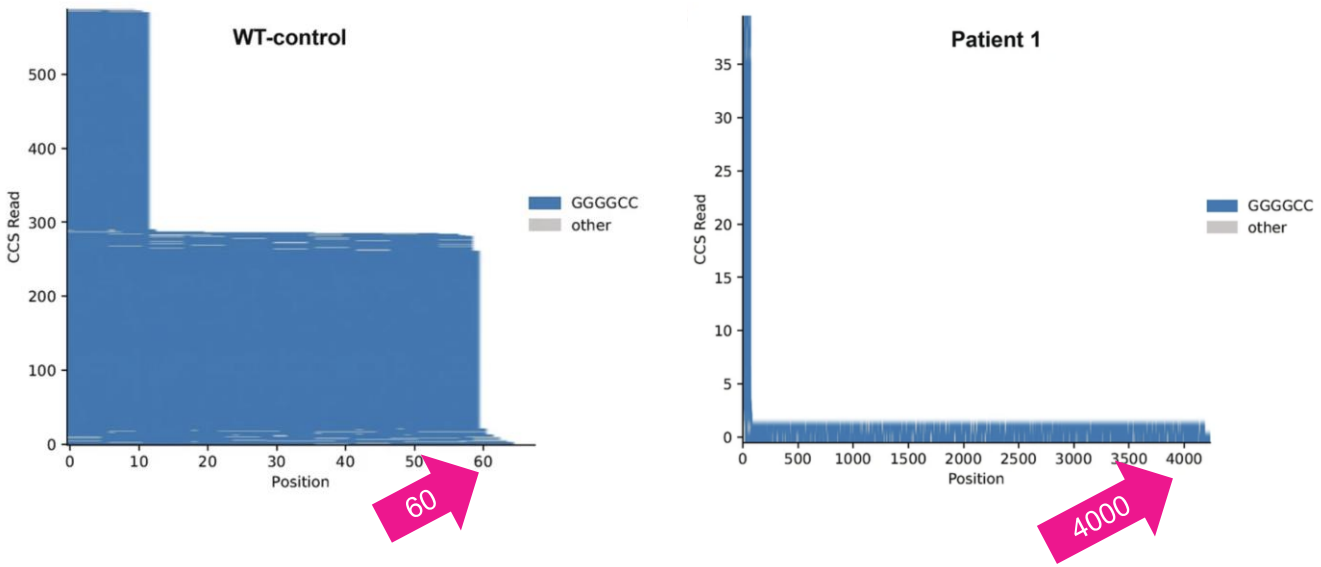
OPEN

Validated assays for the quantification of C9orf72 human pathology

S. E. Salomonsson[1,2,8], A. M. Maltos[3,8], K. Gill[1,2,3], O. Aladesuyi Arogundade[1,2], K. A. Brown[1,2], A. Sachdev[3], M. Sckaff[1,2,3], K. J. K. Lam[1,2], I. J. Fisher[1,2], R. S. Chouhan[1,2], V. S. Van Laar[4,5], C. B. Marley[3], I. McLaughlin[6], K. S. Bankiewicz[4,5], Y.-C. Tsai[6], B. R. Conklin[1,7] & C. D. Clelland[1,2]

*"**SMRT sequencing can accurately measure the repeat expansion** […] to the C9orf72 locus in what has been a traditionally hard to sequence genomic region."*

*"This is of particular value to sizing and phasing the repeat expansion and determining changes to the gene locus after gene editing"*




Salomonsson SE, Maltos AM, Gill K, Arogundade OA, Brown KA, Sachdev A, et al. Validated assays for the quantification of C9orf72 human pathology. Sci Rep. 2024;14:828.

# HiFi sequencing for drug development

Long-read sequencing enhances the discovery and development processes with high-quality data for confidence in results


**PacBio HiFi sequencing supports:**

- Target identification
- Biomarker discovery
- Library screening
- Directed evolution
- Cell line characterization
- Plasmid sequencing
- Animal model research
- Genotoxicity assessment

"PacBio long-read sequencing was and is fundamental to discovering Rose's drug, as well as other potential treatments for kiddos with diseases like hers. It provides a map for us early on that allows us to design the most impactful drug that we can at unprecedented speeds."

- Casey McPherson, To Cure a Rose Foundation / Chrysalis Genetics

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# Thank You

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