SCALE HUMAN DISEASE RESEARCH WITH HIFI SEQUENCING

Reveal the complexity of the human genome to power human disease research

The human genome holds a wealth of knowledge that reveals the underpinnings of human health. Despite accomplishing a sequence map of much of the genome in the early 2000s, scientists could not declare the human genome as "complete" until recent years. With the initial draft primarily restricted to coding-heavy regions, this reference lacked coverage of genes and regulatory elements that can play significant roles in health and disease. Only now, with the advancement of highly accurate long-read sequencing, could these missing pieces be resolved and fully complete the genome.

PacBio® HiFi sequencing gave rise to this achievement and has dramatically improved understanding of the human genome by:

- Resolving gaps that are unattainable by other sequencing methods
- Accurately mapping complex regions
- Enabling more contiguous genome assemblies.

Achieving a complete map of the human genome is crucial for accurate identification of disease-causing genetic variants, understanding evolutionary relationships, and developing targeted therapies. By bridging these gaps, scientists can improve the understanding of genetic drivers of disease and disease risk, ultimately leading to improved human health.

2000
The first human genome is sequenced by the Human Genome Project

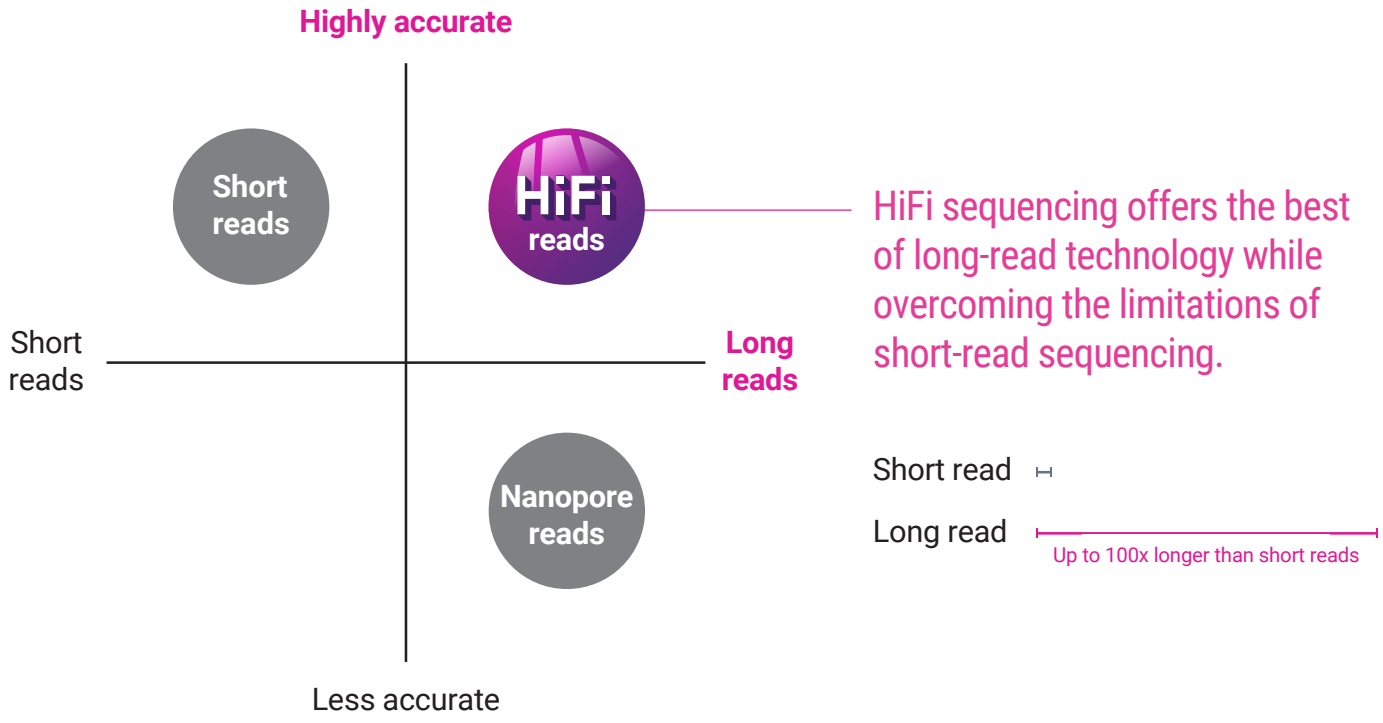
2022
This initial reference genome is made complete by the Telomere-to-Telomere Consortium with gapless chromosome assemblies

2023
The sequence of the remaining Y chromosome is completed

2023
Long-read sequencing, the critical technology for these milestones, is named *Nature Methods* "Method of the Year"

What is HiFi sequencing?

PacBio HiFi sequencing unites long reads and accuracy, giving you the highest quality genomic data for human health research. When it comes to understanding the genetic drivers of disease, why compromise with incomplete genomes that provide limited information?



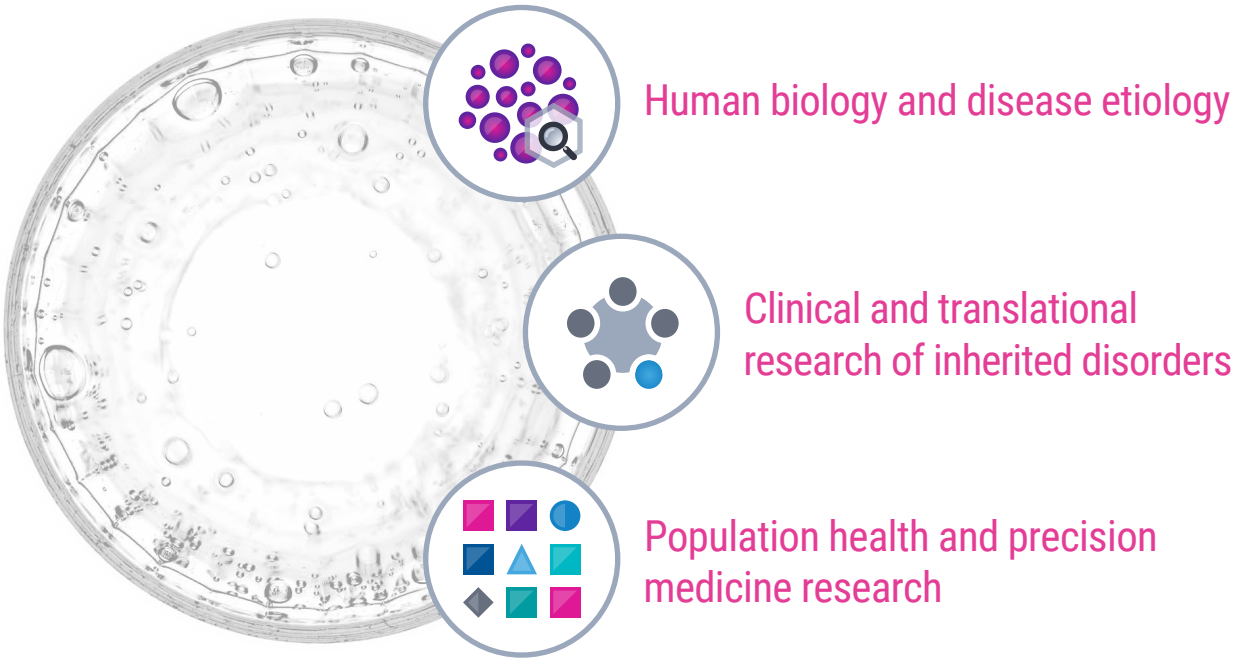
The benefits of HiFi reads

- Long read lengths up to 20 kb
- Easy library preparation
- Low coverage requirements
- Small file sizes to minimize compute time
- Supported analysis tools consolidated into a single pipeline
- High read accuracy (99.9%)
- Compatible ecosystem partners to enable an end-to-end workflow
- A single technology solution

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

HiFi data in action

PacBio HiFi sequencing is uniquely suited to help solve problems in fields of human disease research:



Applications to power translational and human disease research

Whole genome sequencing

Generate highly accurate whole genomes to identify potential causative variants of disease

RNA sequencing

Capture the full transcriptome with isoform-level resolution using single-cell and full-length Kinnex™ kits

Structural variant calling

Characterize large structural variants that cause rare or inherited diseases

Repeat expansion detection

Comprehensively genotype tandem repeats with the PureTarget™ repeat expansion panel and TRGT/TRVZ computational tools

Epigenetics

Simultaneously detect atypical methylation patterns that contribute to rare diseases with on-board 5mC calling, no additional workflow required

Targeted sequencing

Help detect medically relevant gene variants missed by traditional short-read sequencing with the PureTarget repeat expansion panel and Twist Alliance *Dark Genes* panel

Research in human biology and disease etiology

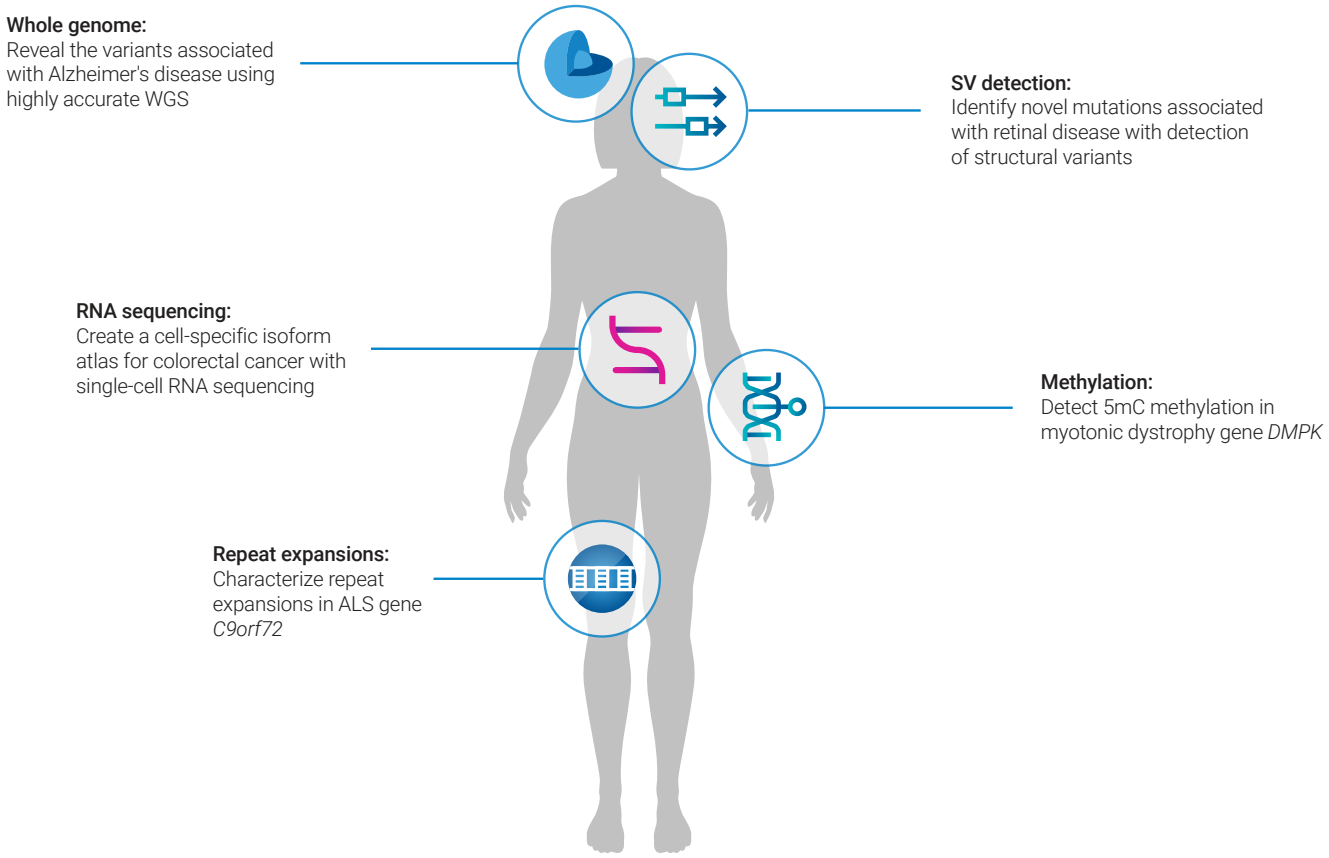
The HiFi advantage

- **Enable** cutting-edge discovery with the extraordinary accuracy and genome quality of HiFi sequencing
- **Characterize** complex causative variants of disease with accurate long read lengths
- **Accelerate** research with tools at the forefront of genomics

Reveal the complexity of the human genome to better understand human biology and the development of disease

The power of a complete reference genome

Since its original release in 2000, the human reference genome has enabled important insights into human molecular biology but has remained incomplete by only covering coding-heavy regions of the chromosomes (International Human Genome Sequencing Consortium, 2001). Now, with the advent of highly accurate long-read sequencing, the Telomere-to-telomere (T2T) consortium has finally achieved a complete reference genome using HiFi sequencing to generate gapless assemblies for the full length of all chromosomes (Nurk et al., 2022), including the Y chromosome (Rhie et al., 2023). This historic milestone marks a turning point for research into human health, as all progress in identifying genetic drivers of disease and potential therapy targets rests on the strength of the reference genome. With the extraordinary accuracy and read lengths afforded by HiFi sequencing, the quality of this newly complete reference genome assures the excellence of these discoveries.



The impact of highly accurate WGS and detection of complex variants

Now with the completion of the human reference genome, applications like whole genome sequencing (WGS) and the detection of complex variants can be employed to their full potential to reveal important insights into human disease. Where traditional technologies were limited to the use of whole exome sequencing for human disease research, HiFi WGS is now leading the way for a more complete picture of causative variants for various diseases including Alzheimer's disease (Tesi, Salazar, et al., 2024), rare pediatric disease (Cohen et al., 2022), and structural variants in Mendelian disease (AlAbdi et al., 2023; Merker et al., 2018).

Not limited to only the genome, HiFi RNA sequencing technology is also making significant strides in its contribution to cell atlases, which are important for understanding developmental biology (Joglekar et al., 2024), as well as linking molecular mechanisms of disease to possible therapies (Rood et al., 2022). Using the PacBio Iso-Seq® method, full-length single-cell isoform sequencing has contributed towards a resolved atlas of cell-type isoforms in the human cortex and for colorectal cancer, the latter of which is being used for research in identifying recurring neoepitopes as potential cancer vaccine candidates.

Advances in genomics tools

These advances are only accelerated by the continued development of genomic tools that help detect complex causative variants. This includes a recent groundbreaking multiomics study that combines the genome, CpG methylome, chromatin epigenome, and transcriptome on a single Revio™ sequencing run (Vollger et al., 2023). Additionally, the development of advanced variant calling tools allows for the identification of complex disease-causing variants, like tandem repeats, using the Tandem Repeat Genotyping Tool (TRGT, Dolzhenko et al., 2024). These methods are complemented by PacBio computational tools to extract the most value out of long reads, including the WGS Variant Pipeline that consolidates these analysis tools into a single workflow for alignment, comprehensive variant calling, cohort analysis, and annotation.

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Clinical and translational research to combat inherited disorders

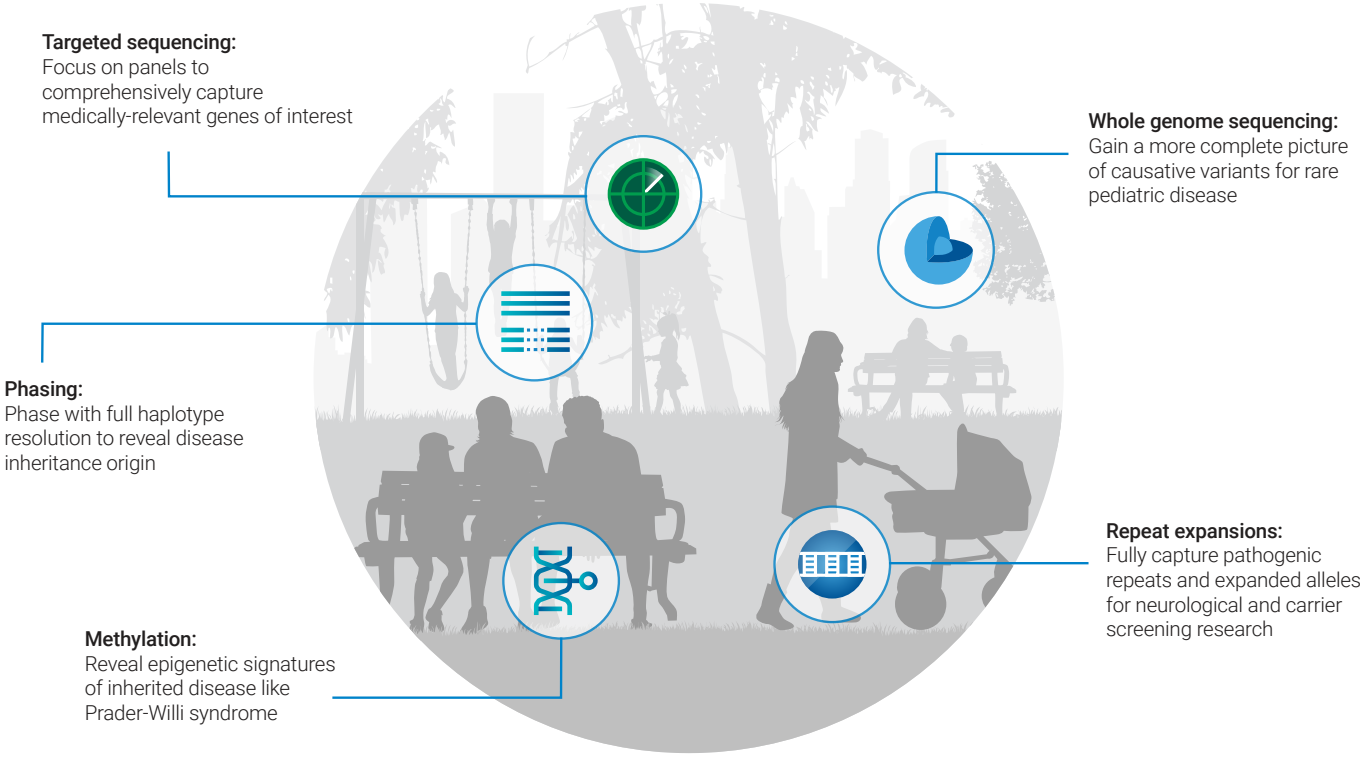
The HiFi advantage

- **Generate** highly accurate, ancestry-agnostic genomes to target genes that may cause rare and inherited disease
- **Resolve** more variants in complex medically relevant genes relative to short-read sequencing
- **Harness** computational tools and analysis partners to better solve rare disease cases
- **Consolidate** multiple assays on one platform, with end-to-end workflows to drive operational efficiency

Drive research on clinically-relevant genes with the most complete human genome

Shining a light on dark genes

Innovations in genome sequencing have led to the flourishing of sequencing for medically relevant genes, but many of these genes still prove to be difficult to characterize in clinical research. The Genome in a Bottle Consortium provides benchmark sets for medically relevant genes, but data for nearly 400 genes was excluded from this original release due to the repetitive and polymorphic complexity of these genes. Recent work by Wagner et al., (2024) however, rectifies this missing data with the characterization of an additional 273 challenging autosomal genes using a HiFi haplotype-resolved whole-genome assembly. This work suggests that assemblies at this resolution may serve as the blueprint for future benchmarks that span the whole genome.



A recent study confirms that not all sequencing methods are created equally when it comes to assessing and understanding clinically relevant genes. Research from the All of Us initiative (Mahmoud et al., 2024) conducts a head-to-head comparison between long- and short-read technologies for sequencing complex medically relevant genes and determines that HiFi sequencing captures the most accurate picture of these genes. This study indicates that HiFi sequencing allows for the identification of more variants at lower depth than legacy methods, as well as the discovery of previously hidden variations – something that has not been achievable with current short-read sequencing. This conclusion signals that HiFi sequencing is surpassing traditional short-read technology in the ability to characterize and understand a wide array of causal variants for rare or inherited disease. This superiority has been borne out in recent research across a broad spectrum of rare diseases (AlAbdi et al., 2023; Cohen et al., 2022; Steyaert, Sagath et al., 2024) and neurodevelopmental disorders (Chen et al., 2023; Hiatt et al., 2024).

Innovation for rare and inherited disorders

For many rare disorders with a lack of functional evidence or where DNA sequencing is uninformative, a growing body of research suggests that RNA sequencing may have diagnostic utility. With the Iso-Seq method, researchers can discover aberrant expression or splicing, prioritize candidate variants, and identify allele-specific expression patterns. The addition of Kinnex RNA sequencing to the sequencing toolbox furthers the understanding of the role of isoforms in disease pathogenesis.

To enable clinical researchers to overcome the challenges posed by traditional sequencing technology, targeted sequencing panels like the Twist Alliance Dark Genes and Twist Alliance Pharmacogenomics panels were designed by collaborators to phase and more comprehensively detect variants in medically relevant genes. Targeted sequencing offers cost-effective scalability on the Revio system, and allows clinical researchers to focus on genes of interest, while still benefiting from all advantages of HiFi sequencing. These panels are now joined by the PacBio PureTarget repeat expansion panel, which captures some of the most challenging repetitive regions of the genome while preserving methylation status. These technologies speak to a growing appreciation of the role of HiFi long-read sequencing in its potential as a single platform for clinical research applications like rare disease, pharmacogenomics, neurology and cardiology research, and carrier screening.

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Population health and precision medicine research

The HiFi advantage

- **Sequence** high-volume whole genomes cost effectively at extraordinary accuracy
- **Assess** population-specific variants at a large scale with 15× higher throughput than previous technology
- **Characterize** complex genes with scalable workflows

Capture the full range of genetic diversity to drive individual- and population-level initiatives

Pangenomes to establish population-specific health outcomes

Advances in genomic technology have proven especially important when building a pangenome, which is meant to represent the entire set of possible genes in a genome rather than select genes derived from a reference genome from a few individuals. Human pangenomes are crucial for capturing variation that may be specific to a certain population and can have significant impacts to human health (Ji et al., 2022) like the gene that causes Thalassemia, an inherited blood disorder disproportionally concentrated in the Eastern Hemisphere (Luo et al., 2021). With accurate detection of large structural variants, HiFi sequencing has been used to construct a pangenome reference of Chinese (Gao et al., 2023) and Arab (Uddin et al., 2023) populations, yielding millions of novel variants unique to these populations and not previously included in the existing HPRC draft human pangenome reference (Liao et al., 2022). These groundbreaking new references now enable genomic analyses that better reflect the full extent of genetic diversity, including populations that have been underrepresented in existing genomes, and allow for actionable population health initiatives. This advance has been made possible by the comprehensive data and remarkable scalability afforded by the high-throughput long-read sequencing technology available on the Revio system.

Population health initiatives for precision medicine and population screening

A complementary advantage in expanding population representation in genome reference sets is the potential to implement population health programs at scale. These population health efforts, like precision medicine, and carrier and risk screening, aim to understand the impact of genetic variation, environment, and lifestyle on the health of individuals, as well as the role of genetics in the development of complex traits, such as susceptibility to certain diseases. This impact is best understood through analyses of the *CYP2D6* gene (Qiao, Yang, et al., 2015; Buermans et al., 2017), which is responsible for the metabolism of a large portion of pharmaceutical drugs. HiFi sequencing has enabled the characterization of several functional rare and novel *CYP2D6* alleles that affect metabolic traits that are unique to the previously unrepresented Solomon Island population (Charnaud, Munro, et al., 2022).

PacBio helps support this type of pharmacogenomic research through a collaboration with Twist Bioscience. The Twist Alliance Long-Read PGx Panel enables the characterization of haplotypes and star allele architecture of up to 50 clinically relevant PGx genes, with high accuracy and high coverage (van der Lee et al., 2022). Similarly, the PureTarget panel, which supports research in screening of ALS, fragile X syndrome, and similar ataxias, can also be used for large scale screening of populations. Altogether, the ability of HiFi sequencing to generate extraordinarily accurate genomes at scale holds the promise of revolutionizing the future of population health.

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Sequence the human genome at scale with the Revio system

HiFi sequencing on the Revio system brings higher throughput to human disease research

Multiomics in every run

SMRT Cell 1	SMRT Cell 2	SMRT Cell 3	SMRT Cell 4
1 whole human genome with 5mC calling	24 samples multiplexed for carrier screening of *FMR1* with PureTarget	1 sample for Kinnex single-cell isoform sequencing for a human cell atlas project	72 samples multiplexed Twist Alliance Pharmacogenomic panel

$1,000* USD complete, phased genome per SMRT® Cell

360 Gb of HiFi reads per day, 1,300 human whole genomes per year

Scalable workflows with high-throughput library prep and automation partners

A simplified user experience, including adaptive loading, fewer consumables, rapid run setup, and real-time performance run previews

On-board 5mC methylation calling with every sequencing run and no additional library prep

Configurable sequencing run times and four independent sequencing stages for workflow flexibility

* U.S. list price is $995 for sequencing reagents for one Revio SMRT Cell, which has an expected yield of 90 Gb, equivalent to a 30X human genome. Your local sales representative can provide detailed pricing in your currency.

Application	Samples per SMRT Cell*	Samples per Revio run using 4 SMRT Cells*	Estimated samples per year†
Whole genome sequencing			
***De novo* assembly**	1	4	~1,300
Variant detection	Structural variants: 3 All variants: 1	Structural variants: 12 All variants: 4	Structural variants: ~3,900 All variants: ~1,300
RNA sequencing			
Kinnex single-cell RNA	1	4	~1,300
Kinnex full-length RNA	4	16	~5,200
Targeted sequencing			
Amplicon sequencing	≥1,000	≥4,000	~2.6M for 1–5 kb ~1.3M for 5+ kb
Target enrichment	20 Mb panel: 12 2 Mb panel: 72 100 kb panel: 288	20 Mb panel: 48 2 Mb panel: 288 100 kb panel: 1,152	20 Mb panel: ~15,600 2 Mb panel: ~93,600 100 kb panel: ~374,400
PureTarget repeat expansion panel	48	192	~62,400

* All sample throughputs are estimates per Revio run using 1 or 4 SMRT Cells. Coverage may vary based on sample quality, library quality, and fragment lengths. Currently available SMRTbell adapter index plates (96A-D) each contain 96 indexed adapters. Whole genome sequencing for a 3 Gb human-like genome at >15× per haplotype for de novo assembly, >10× coverage for structural variants, and >30× coverage to detect more variants. Single-cell transcriptomics assumes ≥80 million reads per library. Full-length RNA assumes a total of 40M reads regardless of plexity. Amplicon sequencing assumes 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.

† Estimated samples per year calculated by assuming 1,300 samples per year for each Revio system run using 4 SMRT Cells, 365 days in a year, and 90% utilization.

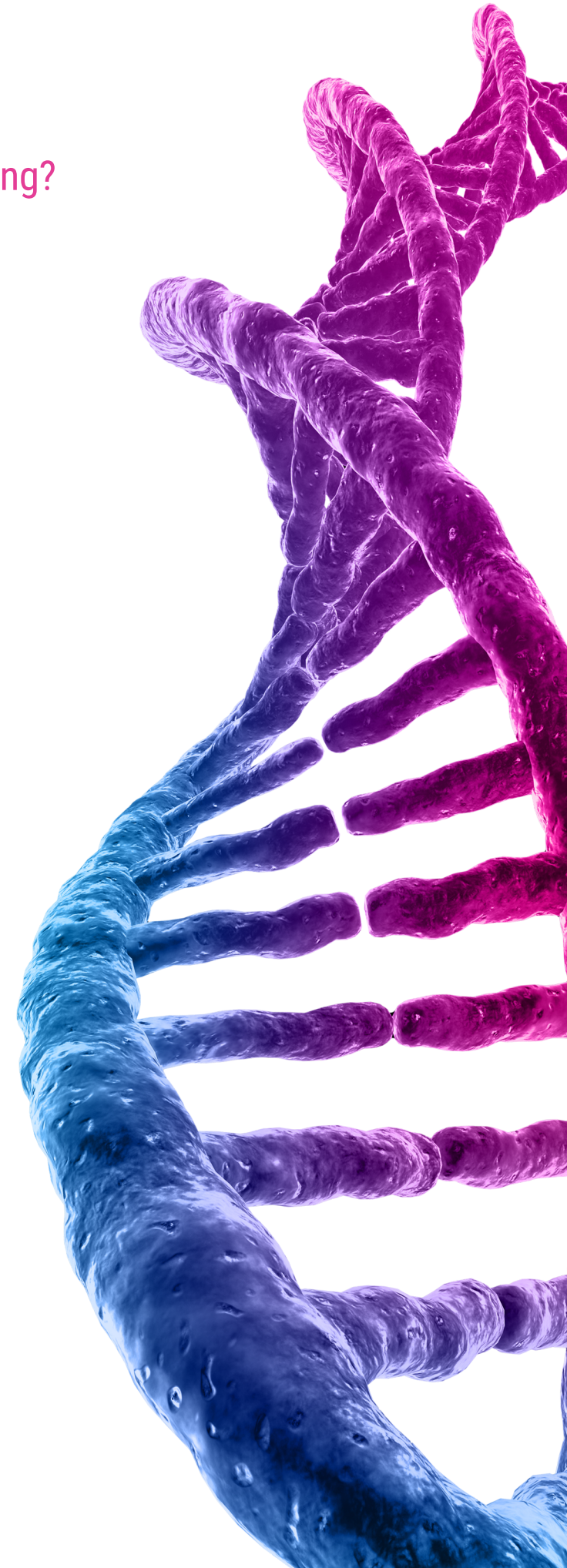
PacBio

Ready to get started with HiFi sequencing?

Learn more about HiFi sequencing:
pacb.com/hifi

Learn more about human genomics:
pacb.com/human-genomics

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



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