SEQUENCE CANCER VARIANTS **WITH CONFIDENCE**

PACBIO®

CANCER RESEARCH

To bring personalized medicine to all patients, cancer researchers need more reliable and comprehensive views of somatic variants of all sizes that drive cancer biology, so they can:

- Reveal patterns of structural variants to better stratify patients
- Identify fusion genes and other cancer-specific gene isoforms that may serve as biomarkers
- Characterize genotypic differences between cohorts that respond differently to treatment
- Look beyond SNVs and robustly detect all structural variants to reveal novel insights not possible with short-read sequencing

THE ADVANTAGES OF SMRT SEQUENCING FOR CANCER RESEARCH

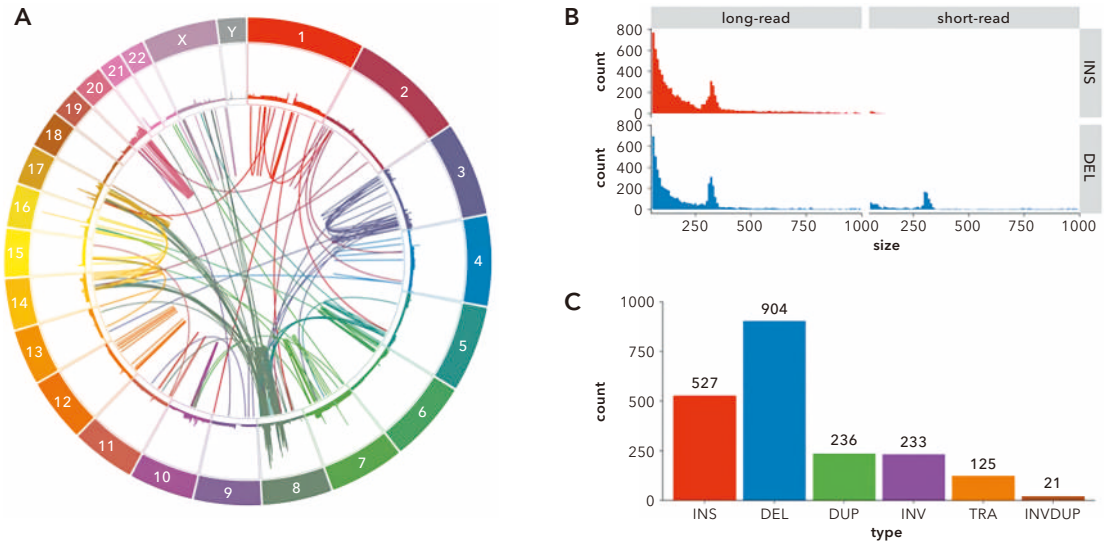
Single Molecule, Real-Time (SMRT®) Sequencing delivers the long reads, high accuracy, and uniform coverage needed to access the complete size spectrum of cancer mutations. With the highest precision and recall for detection of genomic variants >20 bp, SMRT Sequencing allows you to draw conclusions from your data with confidence[1].

EXPLORE THE RANGE OF APPLICATIONS

WHOLE GENOME SEQUENCING	Sequence and assemble complete genomes without the need for a reference to reveal patterns of variants larger and more impactful than SNVs
STRUCTURAL VARIANT CALLING	Use low-coverage whole genome sequencing to identify variants >20 bp that cannot be robustly detected with short-read sequencing, uncovering structural variant hotspots and hidden driver mutations within cohorts at lower cost per sample[1] 
TARGETED SEQUENCING	Target genomic regions that impact cancer biology in a cost-effective way to further explore variants and identify compound mutations in amplicons up to 20 kb in length
RNA SEQUENCING	Get a complete view of isoform diversity with the Iso-Seq® method by sequencing full-length transcripts up to 10 kb with no assembly required Employ a hybrid technology approach to distinguish fusion from non-fusion isoforms

EXPLORE THE HIDDEN LANDSCAPE OF CANCER VARIANTS

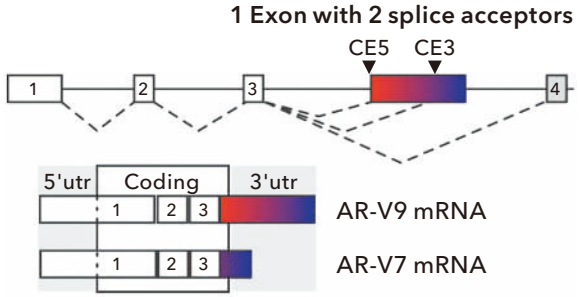
- Develop a complete picture of somatic variants of all sizes and determine the allelic distribution of low-frequency mutations[2]
- Precisely map the genomic context of CNVs including the exact breakpoints of inversions, insertions, deletions, and translocations, and access the full sequence of microsatellite instability biomarkers



SMRT Sequencing revealed four times as many total variants than short-read sequencing including variants >10 kb (A), insertions and deletions between 50 bp -1 kb (B), and variants >1 kb (C). As seen in panel B, short reads are particularly poor at detecting insertion events, even at high coverage[3].

FULLY RESOLVE ISOFORM DIVERSITY WITH THE ISO-SEQ METHOD

- Discover hidden biology by fully resolving cancer isoform diversity, including gene fusions, alternative splice sites, and retained introns[4,5]
- Eliminate ambiguity around isoform variants with full-length cDNA sequencing to characterize the whole transcriptome[6]



Targeted SMRT Sequencing of androgen receptor (AR) isoforms revealed that the structure of AR-V9 was previously mischaracterized and had omitted a cryptic exon that was thought to appear only in AR-V7. AR-V7 has been identified as a potential biomarker for drug resistance based on knock-down experiments targeting both isoforms. These data suggest AR-V9 may in fact be the more predictive isoform[7].

KEY REFERENCES

1. Ashby, M. (2017) Whitepaper: Structural variation in the human genome.
2. Ardui, S., et al. (2018) Single Molecule Real-Time (SMRT) sequencing comes of age: applications and utilities for medical diagnostics. *Nucleic Acids Research, 46(5).*
3. Nattestad, M., et al. (2018) Complex rearrangements and oncogene amplifications revealed by long-read DNA and RNA sequencing of a breast cancer cell line. *Genome research, 28(8), 1126–1135.*
4. Tseng, E. (2018) ASHG PacBio Workshop: The Iso-Seq method for discovering alternative splicing in human diseases. *68th Annual Meeting of the American Society of Human Genetics. San Diego, CA.*
5. Weirather, J. L., et al. (2015) Characterization of fusion genes and the significantly expressed fusion isoforms in breast cancer by hybrid sequencing. *Nucleic Acids Research, 43(18), e116.*
6. Ashby, M., et al. (2018) Scalability and reliability improvements to the Iso-Seq analysis pipeline enables higher throughput sequencing of full-length cancer transcripts. *American Association for Cancer Research Annual Meeting 2018. Chicago, IL.*
7. Kohli, M., al. (2017) Androgen receptor variant AR-V9 is co-expressed with AR-V7 in prostate cancer metastases and predicts abiraterone resistance. *Clinical Cancer Research, 23(16), 4704–15.*