

The OhmX™ Platform: A novel system for whole-genome structural variant analysis



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Significance of Structural Variation

Structural Variation Is a Major Contributor to Genomic Function and Disease

- Structural variants (SVs) are defined as variants > 50 bp
- SVs affect 3–5x more DNA per genome than single-nucleotide variants (SNVs), making them the largest source of genetic variation¹
- Larger SVs (> 20 kb) are 50-fold more likely to affect gene expression than SNVs²
- ClinVar contains ~32,200 pathogenic or likely-pathogenic *clinical* SV entries

1. Chaisson, M.J., Sanders, A.D., Zhao, X. et al. Multi-platform discovery of haplotype-resolved structural variation in human genomes. *Nat Commun* 10, 1784 (2019).

2. Ebert P, Audano PA, Zhu Q, et al. Haplotype-resolved diverse human genomes and integrated analysis of structural variation. *Science*. 2021 Apr 2;372(6537):eabf7117. doi:10.1126/science.abf7117.

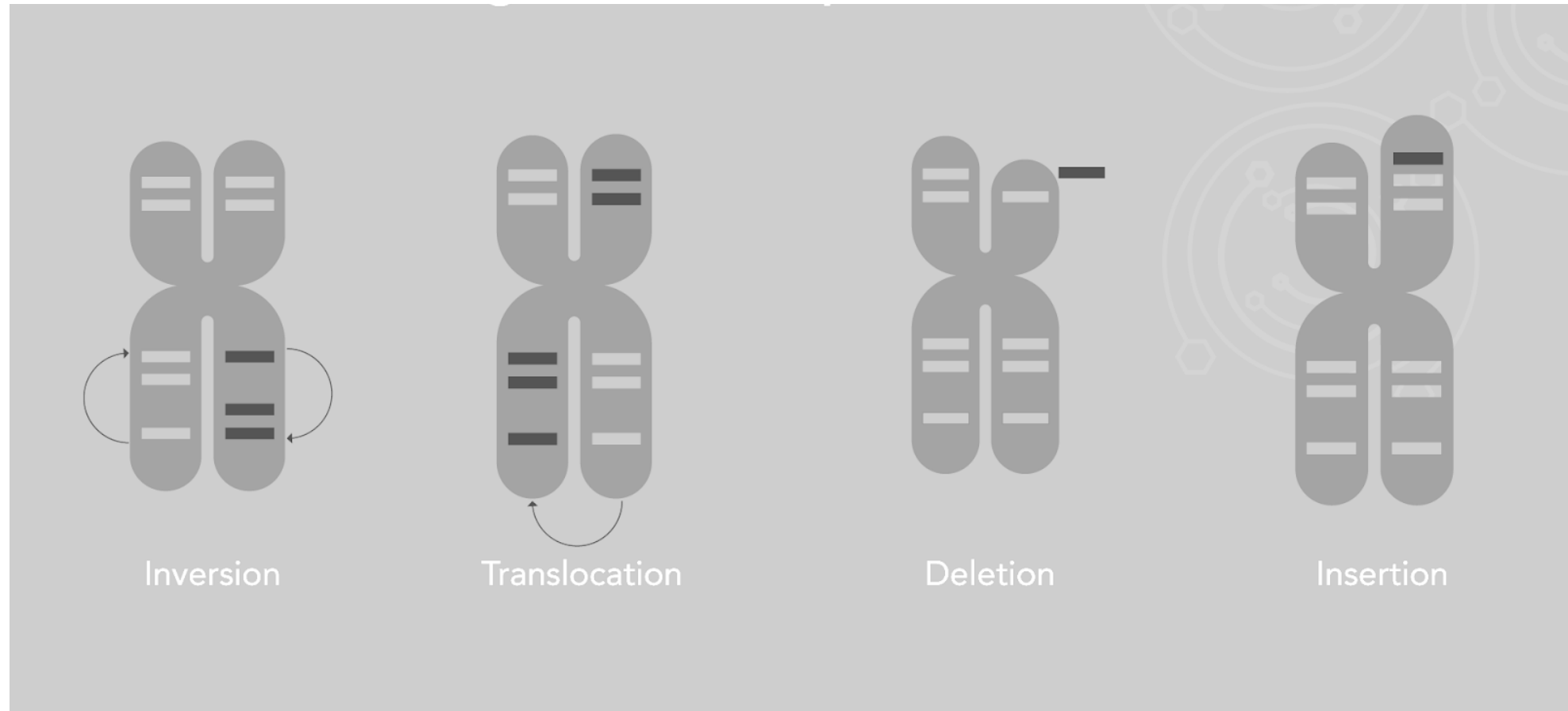


Figure 1: Types of structural variants (SVs). SVs come in several different forms, including inversions, translocations, deletions, insertions, and other, more complex chromosomal rearrangements.

SVs Often Go Undetected Despite Decades of Innovation

- Traditional tools like karyotyping, fluorescence *in situ* hybridization (FISH), and chromosomal microarray (CMA) are low-resolution
- Next-generation sequencing (NGS) struggles with SVs and repetitive regions
- Long-read sequencing (LRS) is expensive and can miss larger SVs

SV Type	OhmX Platform	Karyotyping	FISH	Microarrays
Aneuploidy	✓	✓	✓ targeted	✓
Deletion	✓	✓ > 5–10 Mbp	✓ targeted	✓
Duplication	✓	✓ > 5–10 Mbp	✓ targeted	✓
Translocation	✓	✓ > 5–10 Mbp	✓ targeted	✗
Inversion	✓	✓ > 5–10 Mbp	✓ targeted	✗

Table 1: Comparing the ability of traditional cytogenetic methods and the OhmX Platform to detect SVs based on type. Traditional cytogenetic approaches, such as karyotyping, FISH, and chromosomal microarray, are limited in their ability to detect SVs genome-wide. In contrast, the OhmX Platform can detect several different types of SVs across size ranges in a targeted or holistic approach.

Introducing EGM and the OhmX Platform

Nabsys, the pioneer of electronic genome mapping (EGM), uses solid-state nanodetectors to survey long DNA molecules. The reads are used to construct high-density maps with long-range information that can be used to detect SVs. Optical DNA mapping technologies that rely on the use of expensive optical imaging are limited in the resolution of neighboring tags by the diffraction limit of light. EGM uses electronic detection to identify tags and does not have the same limitations as optical methods, providing superior resolution of small intervals.

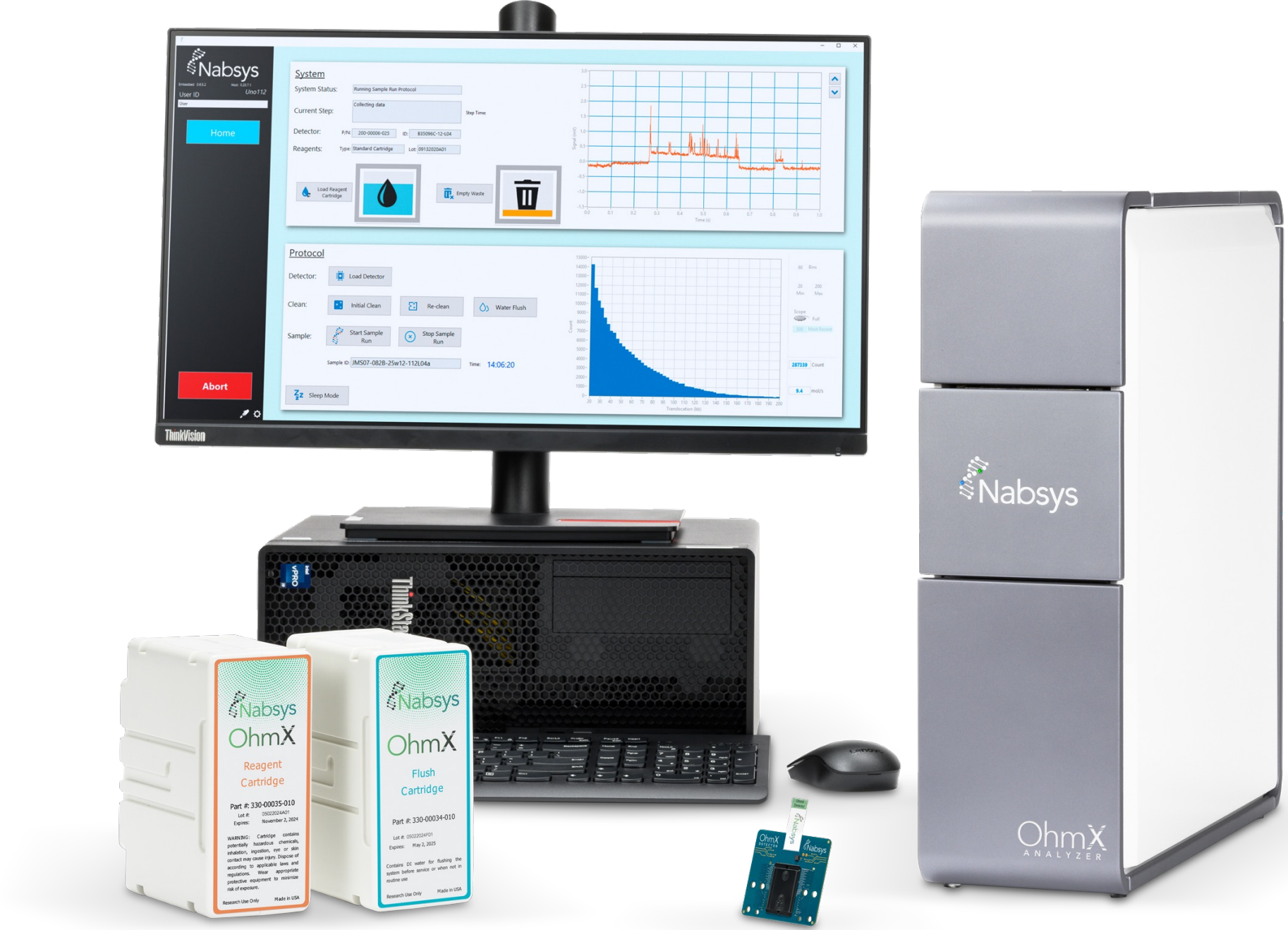


Figure 2: Components of the OhmX Platform. The OhmX Platform consists of the OhmX Analyzer, OhmX Controller (computer, monitor, mouse, and keyboard) loaded with OhmX software, and consumables (OhmX Detectors, OhmX Sample Preparation Kit, and OhmX Flush and Reagent Cartridges).

Features

- Genome-wide analysis
- Orthogonal validation of other technologies (NGS, LRS, CMA, FISH, karyotyping)
- 130 genomes per year
- 16-hour run time
- 300 bp resolution
- Small footprint—desktop size (5" x 17" x 17")
- Analytical software included
- 2–5x less expensive than competing technologies

EGM Workflow



Figure 3: Overview of the EGM workflow. The EGM workflow consists of the following steps: (1) high molecular weight (HMW) DNA isolation, (2) sample preparation using the OhmX Simultaneous Nicking and Labeling Sample Preparation Kit, (3) sample loading, (4) electronic detection of tagged molecules using the OhmX Analyzer, and (5) data analysis.

High Molecular Weight DNA Isolation

- Isolation of high molecular weight (HMW) DNA is performed using the New England Biolabs® Monarch® HMW DNA Extraction Kit for Cells & Blood
- Isolation results in HMW DNA from 65–500 kb

Sample Type	Storage	Input
Cells	Fresh/Frozen	1.0 million cells
Blood	Fresh/Frozen	1.0 million cells ~200 µL
Bone Marrow	Frozen	1.0 million cells variable

Table 2: Sample types compatible with the EGM workflow. The EGM workflow supports fresh or frozen cells, fresh or frozen blood, and frozen bone marrow for SV analysis.

Sample Preparation

- Simultaneous nicking and labeling
- No amplification or ligation steps for reduced bias
- 6-hour workflow with 1.5 hours of hands-on time

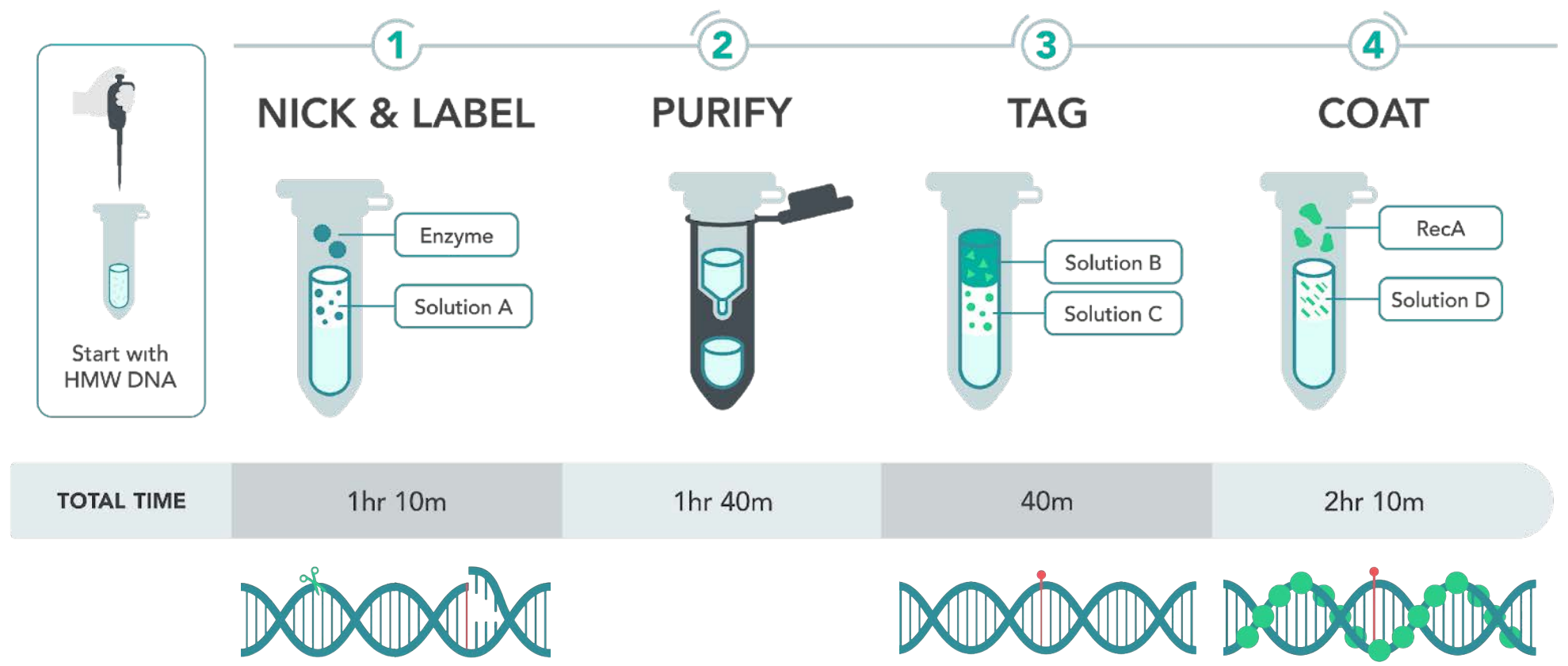


Figure 4: Overview of the OhmX sample preparation workflow. The EGM workflow consists of the following steps: (1) nick and label the HMW DNA with two nicking enzymes, (2) purify the sample to remove unincorporated label, (3) attach tags, and (4) coat the sample with RecA protein.

Sample Loading and Electronic Detection of Tagged Molecules

- < 1-hour analyzer initialization with 5 minutes of hands-on time
- < 15-minute sample loading followed by hands-off data collection

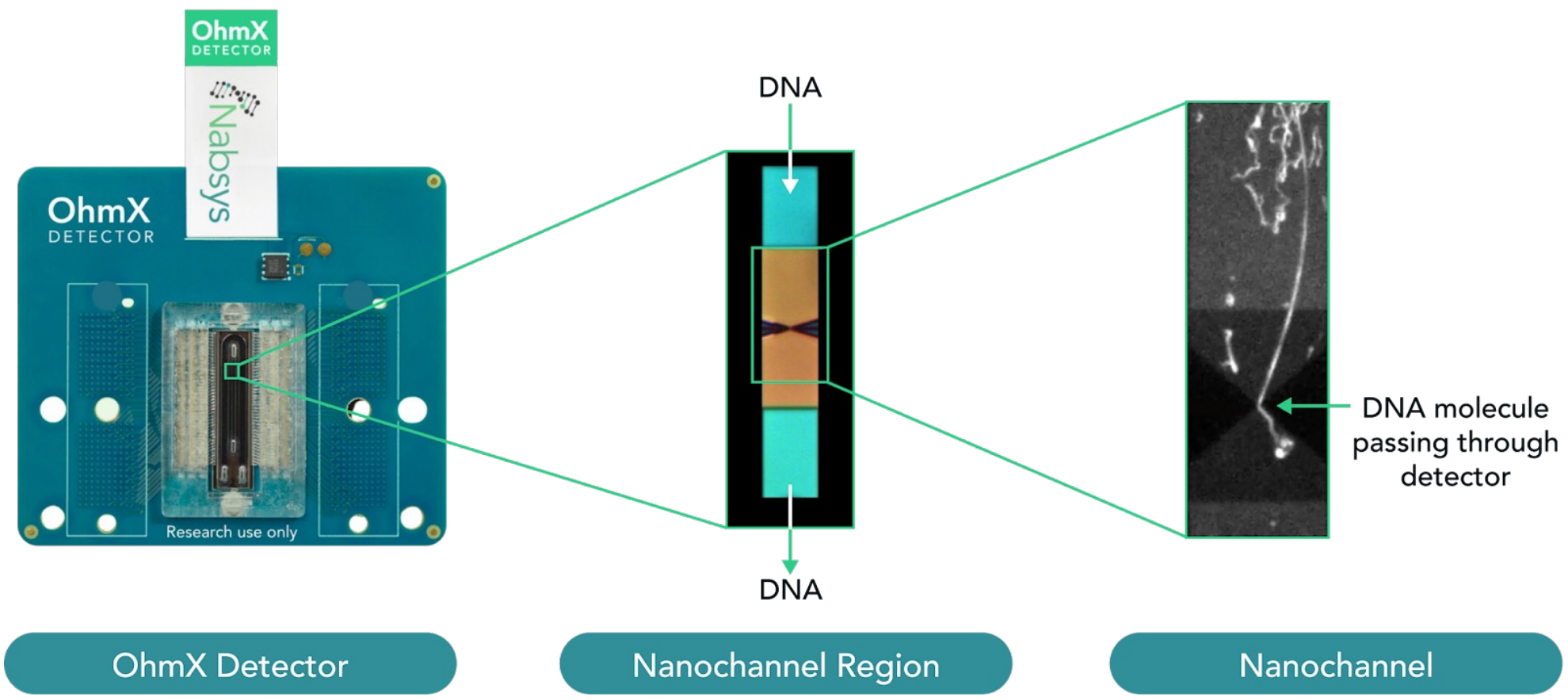


Figure 5: OhmX Detector. The OhmX Detector houses 256 parallel nanochannels, each with its own electronic sensor. Each sensor detects changes in voltage as a function of time as molecules pass through the detector. The righthand image depicts fluorescently labeled DNA molecules passing through the detector. Fluorescent detection is not part of the EGM workflow and is only shown for demonstrative purposes.

SV Detection using EGM

Primary Analysis Using OhmX Software

- Time between sequence-specific tags is converted to distance in bp
- Molecules or “maps” are created

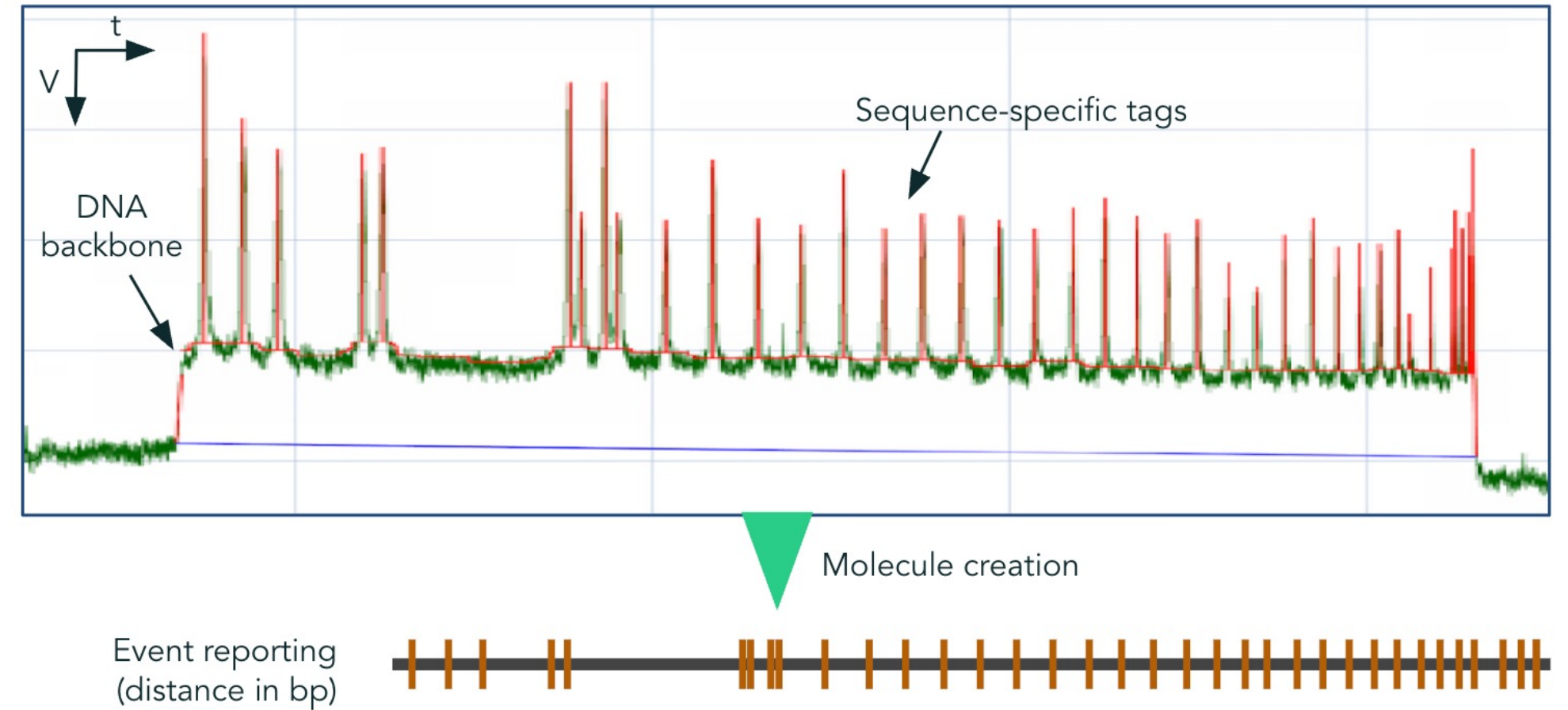


Figure 6: Primary analysis using OhmX Software. During data acquisition, the time at which tags pass through the OhmX Detector is recorded. This time domain is converted to a distance in bp, and molecule reads that specify the distance between tags are created.

Secondary Analysis Using De Novo Assembly and Alignment-Based Pipelines

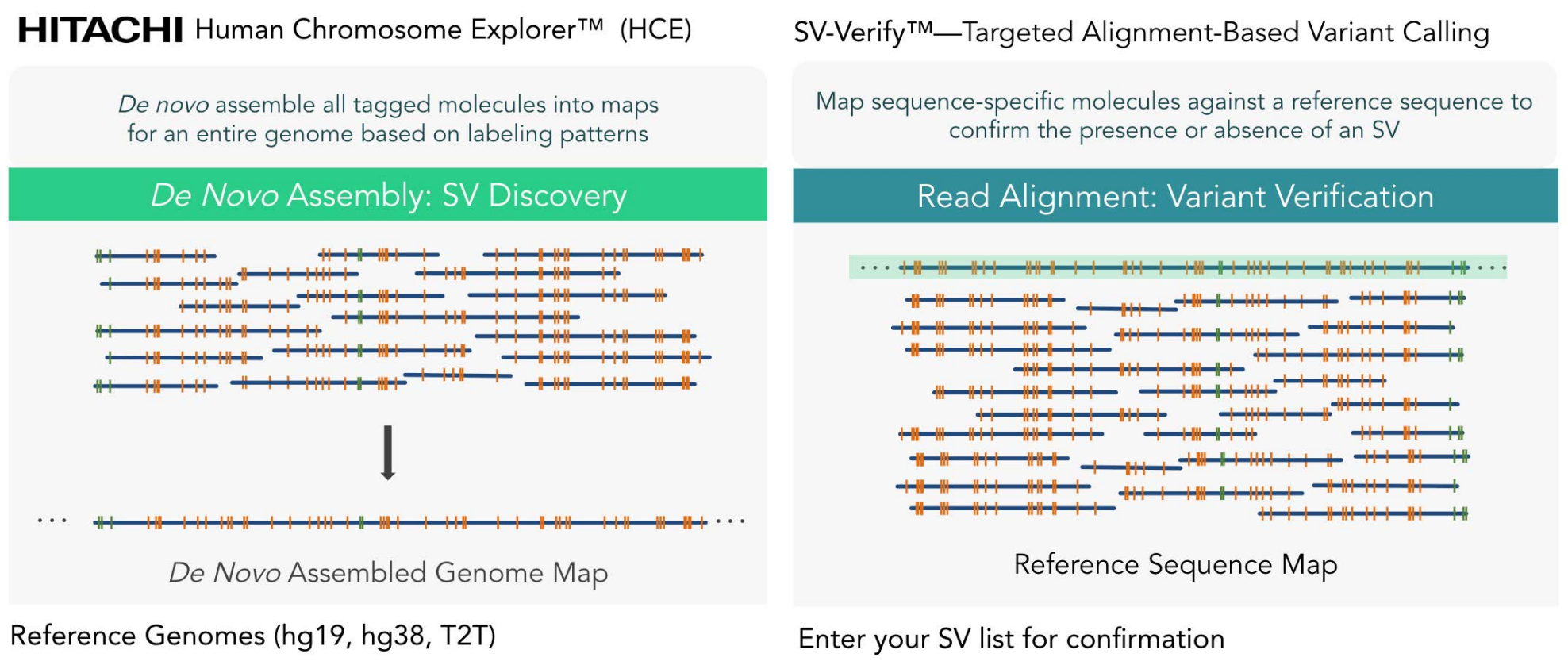


Figure 7: Secondary analysis using HCE™ and SV-Verify™. Nabsys has two pipelines for secondary analysis. Human Chromosome Explorer (HCE) was created in collaboration with Hitachi and uses an assembly-based approach to call SVs, while SV-Verify uses a targeted alignment-based approach and a candidate SV list to confirm or refute the presence of hypothesized SVs.

Concordance with Previously Reported Datasets

SV-Verify vs. HG002 Truth Set

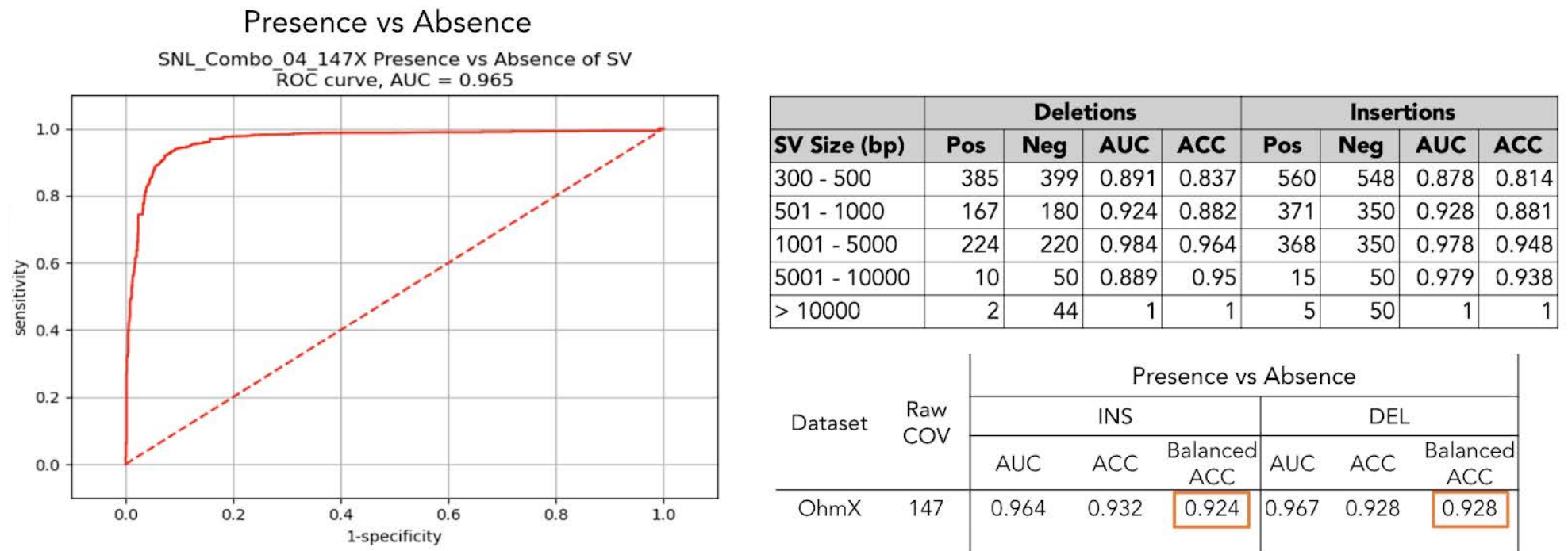


Figure 8: Receiver operating characteristic (ROC) curve, area under the curve (AUC), and accuracy (ACC) for EGM measured against the Genome in a Bottle HG002 truth set. Using EGM and the OhmX Platform, balanced ACC values of 0.924 and 0.928 were achieved for insertions and deletions, respectively.

Orthogonal Confirmation of LRS Calls Using EGM

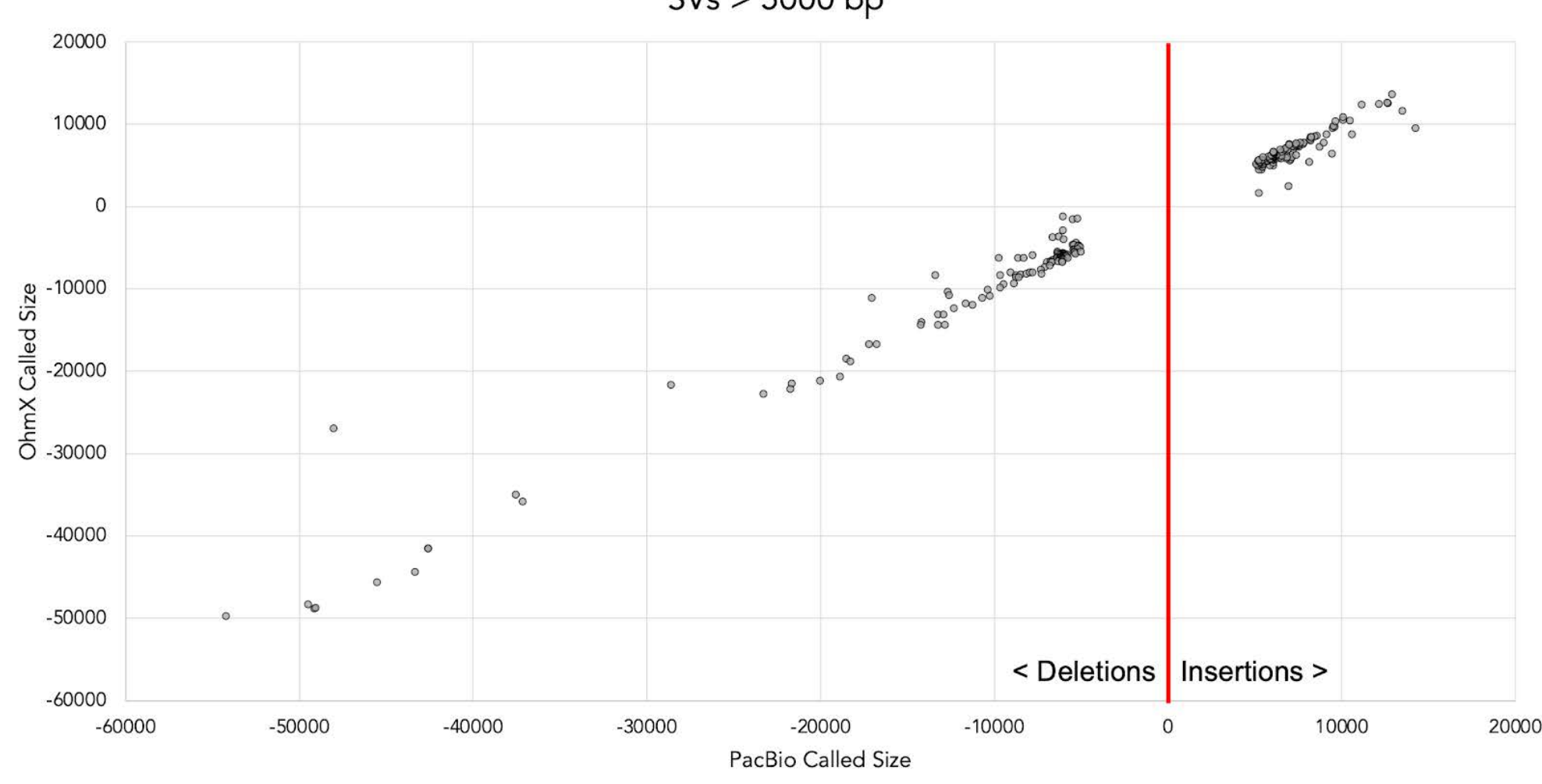


Figure 9: Genome-wide concordance between LRS and EGM SV calls. Nabsys analyzed 3 HG002 technical replicate SV call sets from PacBio HiFi and compared them to EGM calls using HCE. OhmX Called Size and PacBio Called Size are reported in bp. Genome-wide SV calls exceeding 5 kb are shown, demonstrating high concordance.

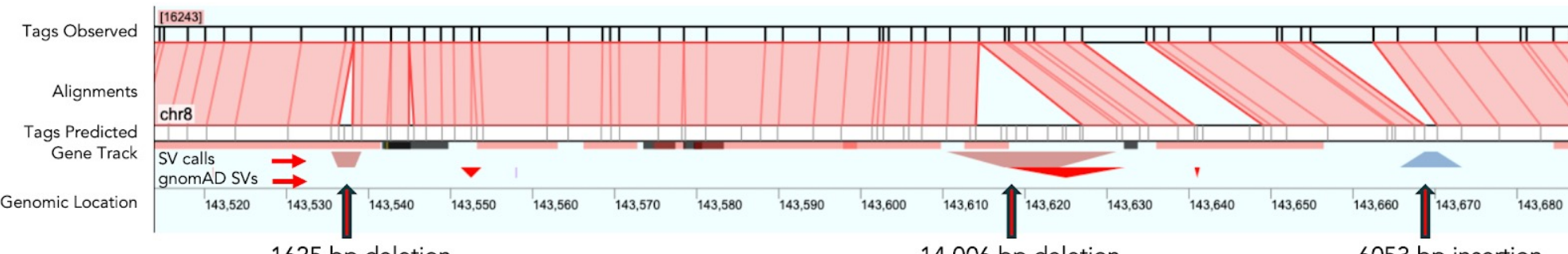


Figure 10: Individual SV call concordance between LRS and EGM. Nabsys analyzed 3 HG002 technical replicate SV call sets from PacBio HiFi and compared them to EGM calls using HCE. Both EGM and LRS called two deletions (red) and one insertion (blue).

Orthogonal Confirmation of ONT Calls Using EGM



Figure 11: Individual SV call concordance between Oxford Nanopore Technologies (ONT) and EGM. The HCE pipeline was used to analyze the EGM data and compare to ONT data. Both EGM and ONT called a ~1.8 kb deletion (red) on Chromosome X.

Conclusion

The genomic instrumentation landscape is characterized by a proportional increase in cost as the length scale of detecting genomic variation increases. The OhmX Platform's use of electronic detection and solid-state nanochannels combine to provide high signal-to-noise ratios and no detectable crosstalk, capturing a broader length-scale of variation at a lower cost per sample than any other whole-genome analysis platform from an instrument the size of a desktop computer. When combined with analytics, the platform captures and visualizes SVs at small enough intervals to be complementary to short-read sequencing with reads long enough to capture large structural rearrangements.

Learn more at [Nabsys.com](https://nabsys.com)