The OhmX™ platform: electronic genome mapping

A novel system for human whole-genome structural variation analysis

Overview

- Obtain the full-view of structural variants including molecules greater than 100kb
- Capture balanced and unbalanced SVs at high resolution, as small as 300bp, in a single run
- Lower cost per sample than long-read sequencing, OGM, or traditional cytogenetic techniques

INTRODUCTION

Structural variants (SV) are queried far less frequently than single nucleotide variants (SNV) in both clinical and research settings. SVs are widely recognized as drivers of human constitutional disease and cancers as well as being responsible for >25% of protein truncating events that can cause disease.[1] Current limitations in the genomics tools landscape yield cost-prohibitive, long-read data required to detect variants of scale.

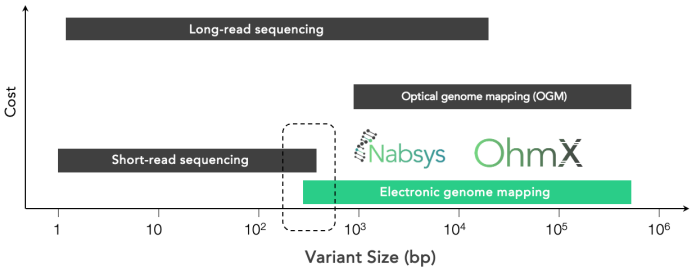

Figure 2: OhmX closes the gap between short-read ensemble sequencing and OGM while expanding the length-scale of long read sequencing at a lower cost per sample.

Short-read sequencing platforms offer low cost but read lengths are limited to ~500bp making the technology adept at detecting SNVs and small indels, but not well suited for SVs. Hi-C and long-read single molecule sequencing enable the detection of some SVs but at a higher cost. Optical genome mapping (OGM) improves price performance and length-scale but cannot consistently resolve shorter SVs.

Figure 1: The OhmX Analyzer's design and unique chemistry deliver cost-effective throughput allowing human whole-genome analysis on an instrument the size of a desktop computer

These tradeoffs result in continued reliance on legacy cytogenetic technologies, such as karyotyping, fluorescence in-situ hybridization (FISH), and chromosomal microarrays (aCGH) which are used reflexively or in parallel to capture the complexity of balanced and unbalanced SVs.

Nabsys's OhmX platform is a novel system for high-definition human whole-genome structural variation analysis (Figure 1). By utilizing electronic detection as opposed to optical detection, OhmX can capture balanced and unbalanced SVs at high resolution, as small as 300bp. The higher resolution captures repeat expansions and contractions at small enough intervals to complement short-read sequencing with reads long enough (over >100kb) to capture large chromosomal rearrangements at a lower cost per sample. (Figure 2).

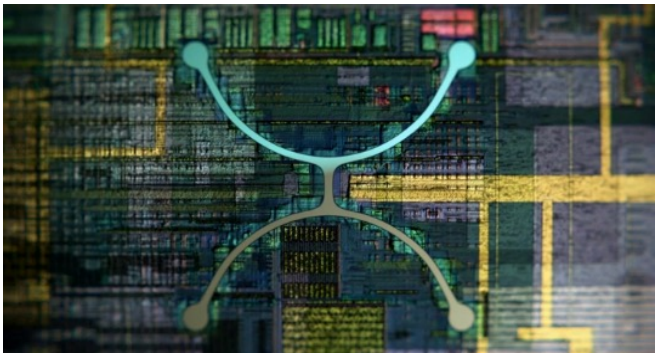

Figure 3: 256 electronic nanochannels are tightly configured to allow high-resolution mapping and high-throughput, at-scale.

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OhmX OVERVIEW

The Nabsys OhmX platform detects tagged high molecular weight DNA through changes in electrical resistance as single molecules pass through solid-state nanochannels (Figure 3). Two hundred and fifty-six nanochannels are tightly configured into a single reusable detector to enable the processing of millions of DNA molecules in a single run.

High molecular weight (HMW) DNA is isolated, and the molecules are labelled at known recognition sites. Once a detector is loaded on the instrument and the sample is injected, single DNA molecules are electrophoretically moved through a solid-state nanochannel where the tags are electronically detected by changes in resistance. The results are single molecule maps with the location of each tag. The maps are assembled into contigs to produce a genome-wide map of all tagged locations for the sample which are aligned against a reference genome for SV analysis (Figure 4).

While the OhmX platform does not sequence single nucleotides, the electronic detection method captures a broader length-scale (300bp - >100kb) of variation short read sequencing and more comprehensive than cytogenetics and OGM at a lower cost per sample.

Expanding the length-scale

OGM relies on the optical detection of fluorescent labels. Light diffraction makes it difficult to detect labels in close proximity, limiting resolution. Missing tags and the associated reduction in resolution increase error in SV-sizing and create uncertainty in the breakpoint locus. The OhmX platform does not use optics or fluorescence, allowing detection of tags in close proximity to be resolved. The increased tag density in the OhmX approach leads to greater resolution compared to OGM.

OhmX allows detection of smaller SVs in the 300-1000bp range as well as ultra long-range genomic data with read lengths over >100kb, long enough to span large SVs including chromosomal rearrangements.

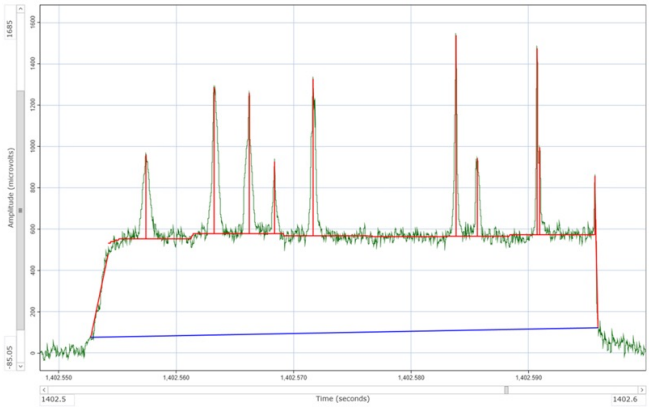

Figure 6: The OhmX platform achieves a high signal-to-noise ratio (in the above case SNR=30) without slowing or stretching the molecule which is traveling up to 1 megabase per second.

Cost-effective throughput

The OhmX platform electronic nanochannel approach reduces design complexity, consumable requirements, and associated costs. Nanochannel, solid state electronic or biological, are typically sized such that the majority of the channel is occupied by a single DNA molecule in order to create a detectable signal. The large resistance change that occurs when DNA passes through a nanochannel results in crosstalk in adjacent channels as often seen in array-based approaches. Nabsys has developed an approach to minimize resistance while isolating each signal through the use of nanochannels that create physical barriers that limit cross talk.

OhmX's detectors (Figure 5) uses a solid-state nanochannel that only requires a <1% change in resistance for a label to be detected. The low change in electrical resistance, as well as the isolating qualities of the nanochannels, eliminate crosstalk concerns. This, combined with OhmX's unique protein coating of the DNA molecule, produces a high signal-to-noise ratio (SNR) (Figure 6). Because of the high SNR and low crosstalk, the nanochannels can be tightly configured. Further, the high SNR is achieved without having to slow or stretch the DNA molecule as it passes through the detection channel.

Figure 5: Detector

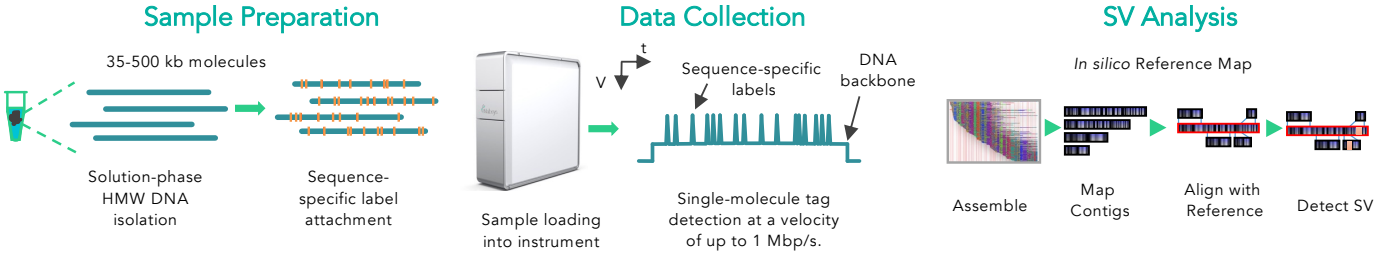

Figure 4: Human SV analysis workflow – The HD-Mapping platform enables whole-genome SV analysis using a straightforward workflow with minimal hands-on time.

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STRUCTURAL VARIATION ANALYSIS USING OhmX

Using OhmX's onboard field-programmable gate array (FPGA), single molecule reads are processed in real-time into signal-processed files which are transferred to the cloud for SV analysis. The single-molecule reads derived from the sample are assembled into map contigs. Before SV analysis can continue, a comparable reference is created. To accomplish this, a sequence-based reference genome is labelled *in silico* using the same sequence specific recognition sites as the sample to generate a reference genome map. Once a reference is created, the map contigs are aligned to the reference genome map. This alignment is loaded into the SV analysis pipeline. SVs are then identified by comparing the observed label pattern of the sample map contigs with the expected pattern of the reference genome. Changes to the loci of these labels represent structural changes in the genome (Figure 7). For example, two label sites measurably farther apart on a sample map contig compared to the corresponding locations on the reference map could indicate an insertion (Figure 8).

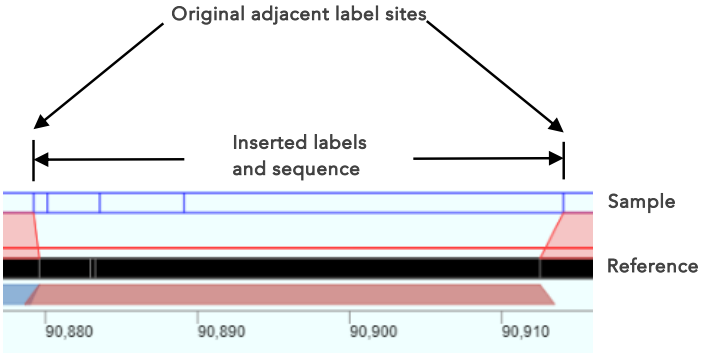


Figure 7: OhmX using Human Chromosome Exporer detects novel insertion in sample genome.

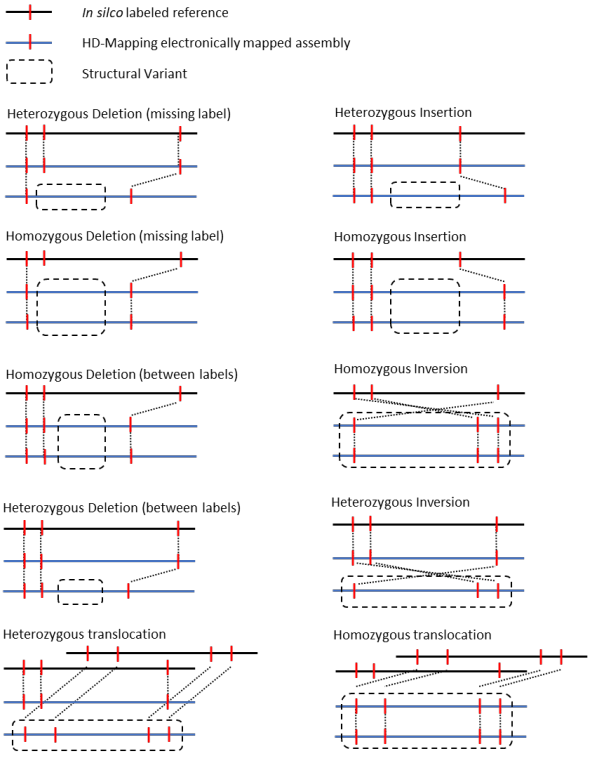


Figure 8: Comparing label patterns between sample map contigs and a reference genome map reveals structural variation in the sample.

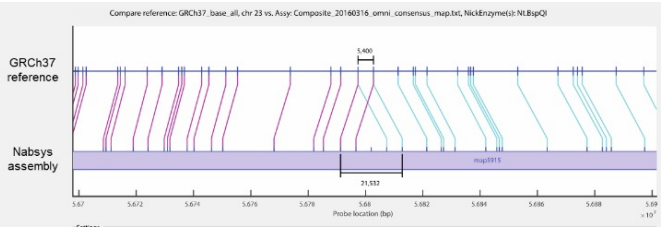


Figure 9: OhmX assembly of NA24385 compared to GRCh37 reference showing a collapsed repeat region on the reference.

NABSYS AND GENOME IN A BOTTLE

Nabsys has demonstrated the utility of the OhmX platform for SV analysis in part through its work with the Genome in a Bottle (GIAB) Consortium. A study comparing NA24385 relative to GRCh37 examined a collapsed repeat region on the X chromosome (Figure 9). The OhmX platform was able to detect a 16,132bp insertion where no call had been made previously. The team was also able to pick up a 34kb balanced inversion on chromosome 10, a 1,531bp insertion in chromosome 11, and a 301bp deletion in chromosome 18 demonstrating the range of structural variation detectable by OhmX. This range is further demonstrated when comparing the concordance in deletion sizing to a GIAB high confidence call-set (Figure 10).

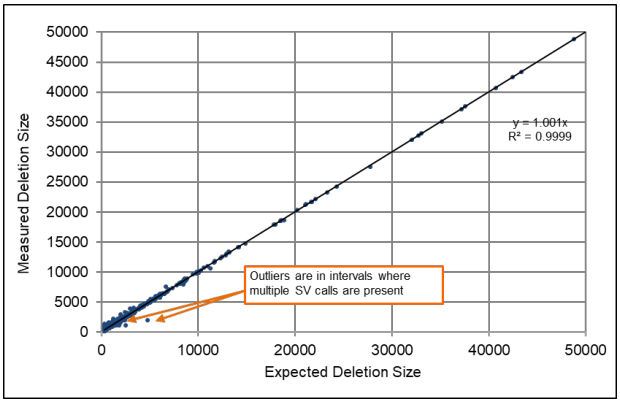


Figure 10: Concordance of OhmX deletion size to GIAB high confidence call set. (Note: expected deletion size is an aggregate when multiple SVs are found between two label sites.)

NABSYS AND HITACHI HIGH-TECH

In furthering its analytics, Nabsys has partnered with Hitachi High-Tech on a collaboration to develop Human Chromosome Explorer™ (HCE) (Figure 11). HCE™ exploits multiple threads, clusters, and compute nodes in Google Cloud's High Performance Computing cloud infrastructure to distribute the generation of long-range uniform map contigs into a haplotype aware assembly of a human whole-genome map for SV analysis through the web. For a detailed discussion of HCE & SV analysis, see *"Human Chromosome Explorer for OhmX: Structural variation analysis using electronic whole-genome mapping data."*

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 nearly eliminates 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 HCE™, the system captures and visualizes repeat expansions and contractions at small enough intervals to be complimentary to short-read sequencing with reads long enough to capture large structural rearrangements.

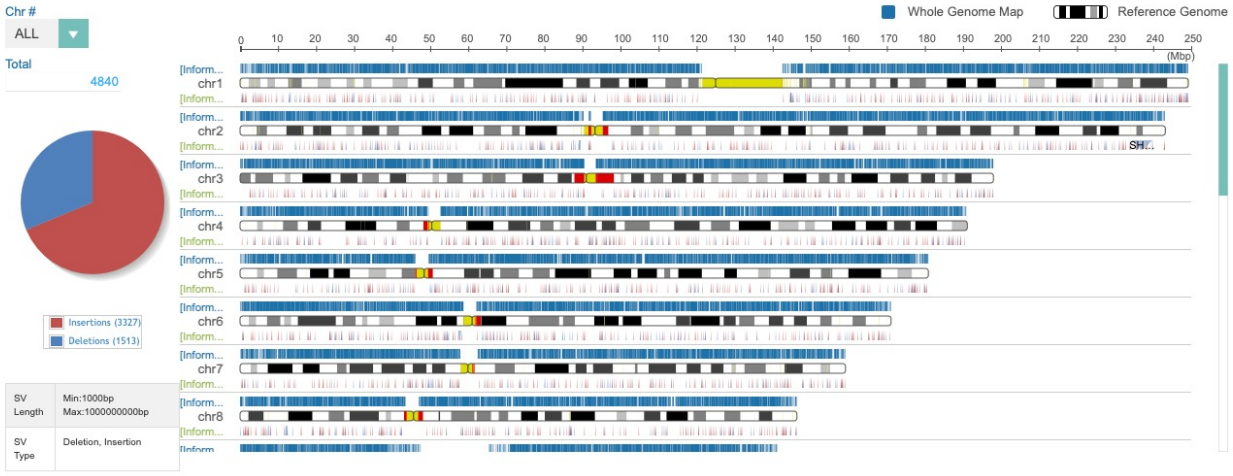
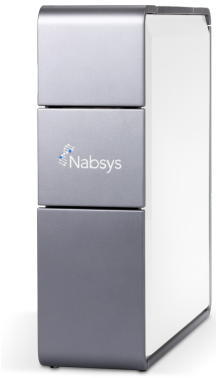


Figure 11: Result Summary View of structural variants visualized in HCE™



LEARN MORE

To learn more about Nabsys the OhmX platform visit https://nabsys.com

1. Collins et al Nature (2020) 581: 444-451. A structural variation reference for medical and population genetics