# Genomic Unity® Testing

## Detecting structural variants using WGS

Genomic Unity® testing comprehensively detects genome-wide structural variants, reporting those that are interpreted as pathogenic or likely pathogenic within the context of the patient's phenotypes.

### What role do structural variants play in genetic disease?

Structural variants have been shown to cause mendelian disorders such as Charcot-Marie-Tooth disease (duplication of the PMP22 gene) and Williams syndrome (deletion of the q11.23 region of chromosome 7). They've also been shown to contribute to complex disorders such as autism and schizophrenia. Although pathogenic structural variants are less commonly identified than pathogenic small sequence changes, recent studies of neurodevelopmental delay patients estimate that causal inherited or de novo structural variants account for ~15% of cases. With standard exome sequencing approaches, these genetic changes can be missed.

### How does Variantyx define structural variants?

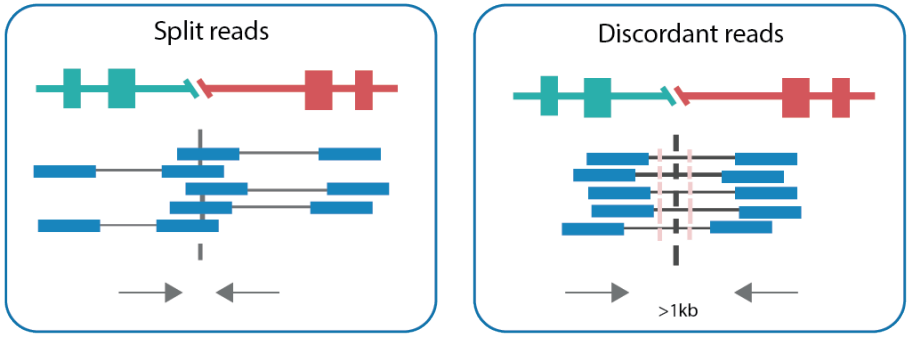
Structural variants are gross deletions (losses) or duplications (gains) of DNA that are greater than 50bp in size. Structural variants include small copy number variants (CNVs) that are often referred to as del/dup events and which are commonly accepted to range in size from a single exon to a full gene. Structural variants also include larger CNVs, including inversions, translocations and complex rearrangements, that have typically been detected by traditional cytogenetic methods.

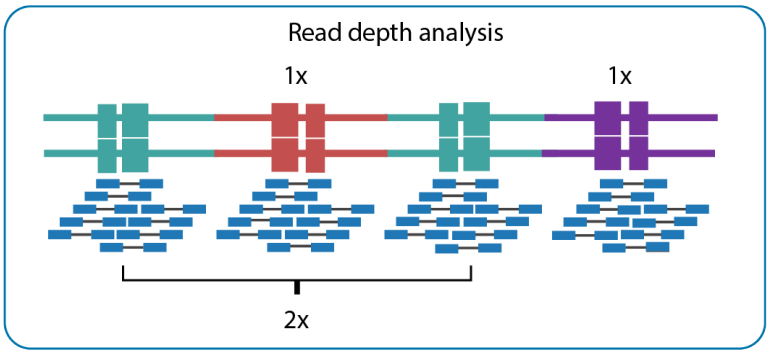
Insertions and/or deletions (indels) less than 50bp in size are considered to be small sequence changes. The methods that Variantyx uses to detect these small sequence changes are discussed in a separate document.

### How does Variantyx detect structural variants?

Variantyx uses whole genome sequencing (WGS) technology to provide consistent, comprehensive coverage of the entire genome. To analyze structural variants, two distinct analysis strategies are used: breakpoint analysis and read depth analysis.

Breakpoint analysis takes advantage of two types of reads: split reads and discordant reads. Under normal circumstances, a given paired sequence read will align to a single region of the genome. But for split and discordant reads, the paired read aligns to two distinct regions of the genome with little or no overlap. In the case of split reads, the breakpoint occurs within one of the reads and can be identified to the resolution of a single base pair. In the case of discordant reads, the breakpoint occurs in the insert between the reads, resulting in an unexpected span size or inconsistent






orientation. Both are indicative of structural variation.

Read depth analysis takes advantage of the expectation of consistent coverage across the genome. Regions with unexpected levels of coverage – both significantly higher (>=2X) and significantly lower (<=.5X) – are indicative of structural variation.

Taken together, the three signals generated by these two analysis methods, when considered alongside additional lines of evidence, makes it possible to robustly detect structural variants with high sensitivity and specificity. These variants are then interpretted for pathogenicity.

## What pathogenic structural variants have been indentified by Variantyx?

The following table highlights some representative examples of reported structural variants.

| Variant | Intersecting gene(s) | Disease association | Patient phenotype(s) |
|---|---|---|---|
| 5.9MB deletion chr4 | 21 genes including PCDH18 | Intellectual disability | Autism, intellectual disability, speech delay |
| 340KB duplication chrX | DMD exons 3-9 | Muscular dystrophy | Muscle weakness, progressive truncal ataxia |
| 9.7KB deletion chrX | MECP2 exons 3-4 | Rett syndrome | Developmental delay, developmental regression, hypotonia, seizures, breathing difficulties |

## What are the benefits of Genomic Unity® structural variant analysis?

With Genomic Unity® testing there is no need for a separate sample and separate assay.

Because WGS provides comprehensive coverage of the entire genome, all sequence data necessary for structural variant detection is present. Variantyx's custom-built, validated computer algorithms analyze the data, identifying structural variants across the full spectrum from small and large indels to small CNVs to large CNVs. Those structural variants interpreted as pathogenic or likely pathogenic are included in the clinical report alongside any other relevant variants.