COPY NUMBER AND AOH DETECTION FROM NGS

Detecting high-quality CNV from NGS data has been a challenge for many years. BioDiscovery has perfected algorithms for the detection of CNV and AOH from almost all NGS assays with high sensitivity and low false-positive rates.

Algorithms for CNV and AOH Detection

BioDiscovery, a Bionano Genomics® company, has developed two algorithms for detection of CNV and AOH events from NGS using its decades long expertise in the area. One algorithm, the "Self-reference" algorithm, can be used for all WGS data regardless of sequencing depth. The second is the "Multi-Scale Reference" (MSR) algorithm that is applicable to all NGS data (WGS, WES, and Gene panels). The MSR algorithm is able to create "virtual" bins with sizes proportional to the expected number of reads offering high resolution detection of events in areas of interest (e.g. exons) while also providing a nice genome-wide backbone.

- **WES**
- **WGS**
- **SHALLOW SEQUENCING**
- **GENE PANELS**

Whole Exome Sequencing

Getting CNV calls from Whole Exome Sequencing has been one of the most challenging efforts in the community. There have been numerous algorithms proposed but they suffer either from poor sensitivity or too many false positive calls. The MSR algorithm has been able to offer the best balance of these competing measures, detecting small true-positives without generating many false-positives. The image in Figure 1 shows a small 12Kb deletion overlapping part of MECP2 gene resulting in only 2 virtual probes indicating a small copy number loss. At the same time, with such sensitivity, only four other CNVs were detected that passed the basic filtering stage demonstrating a very low false-positive rate.

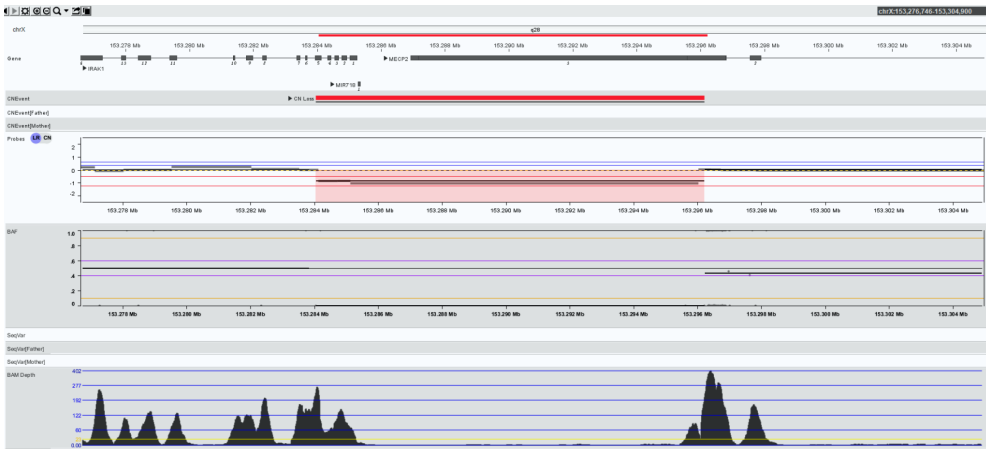

Figure 1. N$_x$Clinical was able to detect a 12 kb deletion that caused a validated pathogenic partial one exon deletion of MECP2 in WES data.

Whole Genome Sequencing

As described in Chaubey et al., Journal of Molecular Diagnostics, vol. 22, No. 6 June 2020, they used 10x WGS and validated that the NxClinical algorithm detected all CNVs and AOH that were found by high resolution SNP arrays. Figure 2. shows a small exonic deletion detected using 10x WGS with the MSR algorithm.

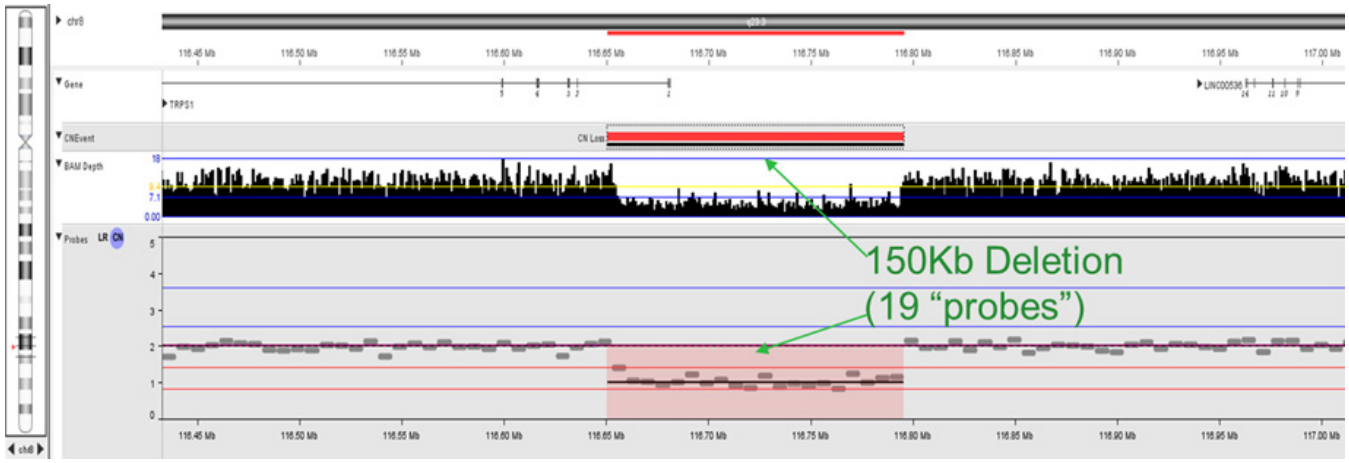

Figure 2. A deletion on chromosome 8 which includes the first exon of TRP51 is shown along with the BAP depth.

With higher depth NGS, smaller CNVs can be detected and integrated with sequence variants to provide a wholistic view of the sample. In Figure 3, the ideogram shows regions of copy number gain (blue bars), loss (red bars), AOH (yellow shading), Allelic Imbalance (purple shading), as well as various types of Sequence Variants (e.g., SNV, In/Del, etc.) as colored "lollipops".

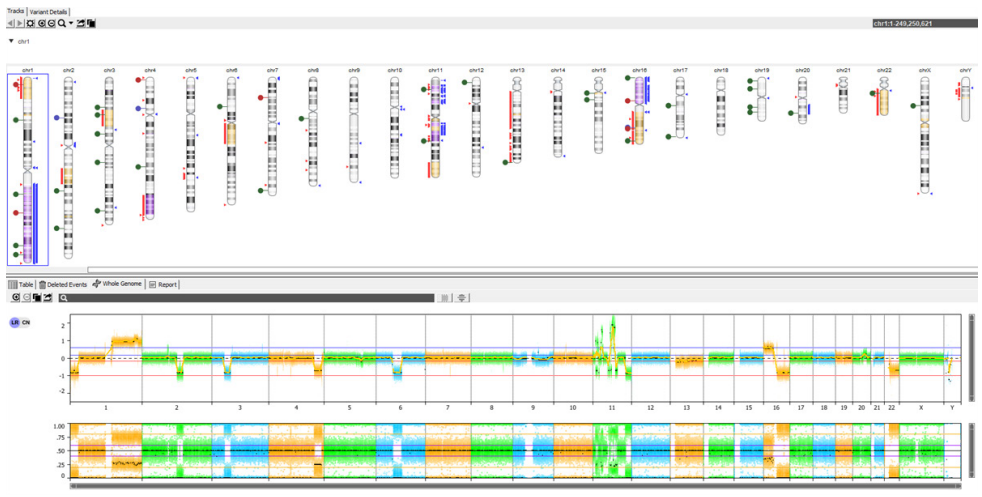

Figure 3. Whole genome view in NxClinical. The top shows the cytogenic view in an ideogram of all of the chromosomes overlayed with CN loss (red), CN gain (blue), AOH (yellow highlight), and Sequence Variants (lollipops) and the bottom shows the molecular view of the whole genome in log ratio and B allele frequency plots.

Gene Panels

The MSR algorithm can be applied to any gene panel from single gene (e.g. DMD test) to large panels having thousands of genes. Figure 4 below is from the Illumina TruSight™ Oncology 500 (TSO500) panel showing a somatic cancer profile. The cytogenetic complexity of the tumor sample is clearly evident with a large copy number gain of 8p and loss of a large section of 13q. Aberrations associated with genomic scarring, such as Loss of Heterozygosity (LOH), telomeric allelic imbalances (TAI), and large-scale state transitions (LST) can be visualized and manually called with confidence.

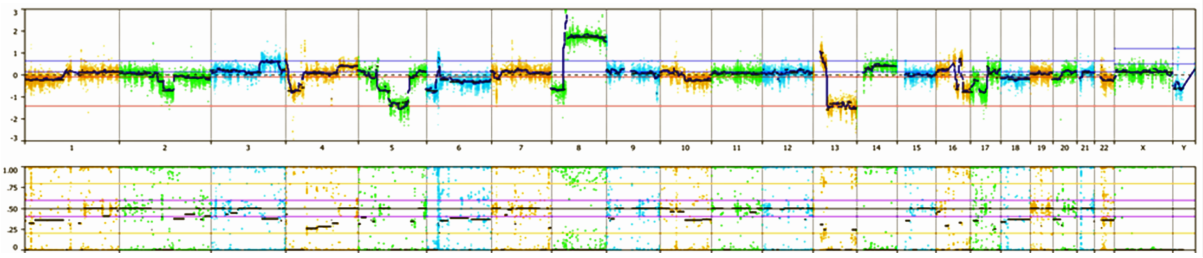

Figure 4. Shows the log R whole genome view of TSO-500 panel in N_xClinical. By using a virtual probe strategy, N_xClinical gives a much richer view of the whole genome than would be expected by a 500 gene panel.

Shallow Sequencing

The MSR algorithm can also be applied to detect CNVs from shallow sequencing, including very low-level mosaic events seen in NIPS or ctDNA samples. Figure 5 shows a sample with trisomy 21 detected using 1x WGS. CNVs are an important contributor to disease and are required for accurate reporting. For clinical sequencing to be fully accepted as a replacement for microarrays and other widely used techniques, it must provide high quality CNV information. N_xClinical can easily and accurately provide that information from various approaches using NGS data.

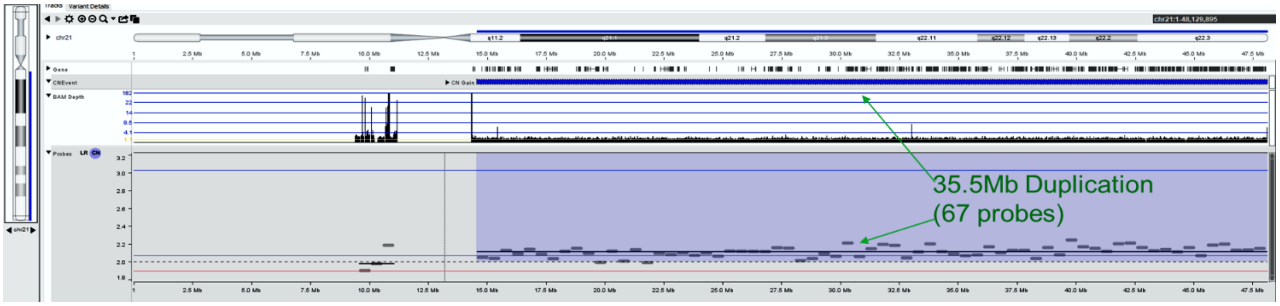

Figure 5. A large duplication event affecting the q arm of chromosome 21.

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