Experience from the First 330 Cases of Low Pass Genome Sequencing (5X) Demonstrates Clinical Utility and Provides Potential Alternative to Traditional Microarray in the Clinical Settings

Alka Chaubey[1], Justin Leighton[1], Kate Hanauer[1], Leah Stansberry[1], Edward Szekeres[1], Zeqiang Ma[1], and Madhuri Hegde[1]

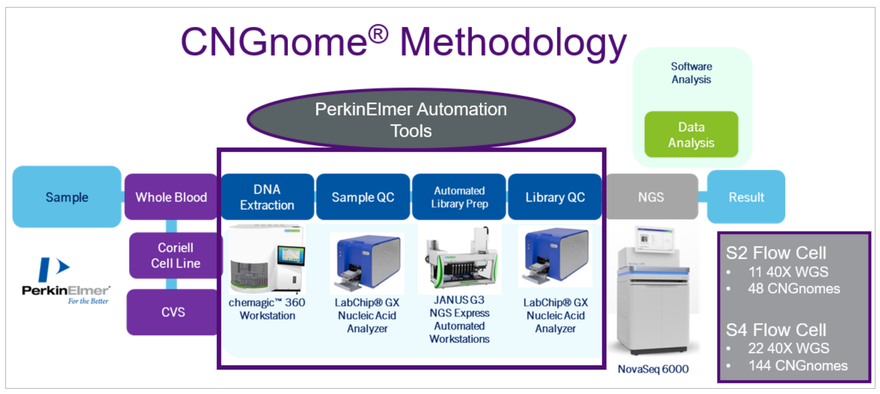
1. Revvity Omics, Pittsburgh, PA

Background

High resolution whole genome sequencing (WGS) is increasingly being utilized as an approach to investigate single nucleotide variants and copy number variants (CNVs) across the genome with improved diagnostic yields over traditional targeted NGS methods. However, numerous studies have also suggested that low resolution genome sequencing techniques can reliably be used to detect larger genomic CNV events traditionally detected with microarrays. Here we present data on the utilization of our low-resolution (5x) WGS assay, the CNGnome® test, for the detection of both large and intragenic CNVs along with absence of heterozygosity (AOH) in our first 331 clinical cases, which included 180 males and 151 females.

Methods

The CNGnome® test methodology involved automated DNA extraction using the Chemagic 360 workstation, followed by a library preparation using the KAPA HyperPlus PCR-free library construction kit before direct sequencing using 2X150 bp reads on Illumina's NovaSeq6000 system at a mean coverage of 5X.



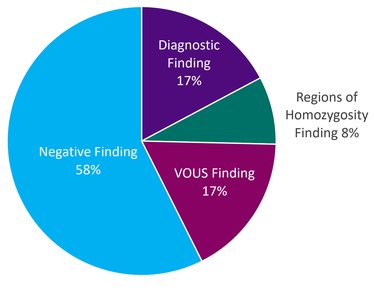
Results

- Pathogenic or Likely Pathogenic findings were identified in 17.2% of cases, ranging from small intragenic changes to whole chromosome changes (Trisomy and Triploidy) and UPD.
- These findings are defined as "Diagnostic Findings" in Figure 1.
- Variants of uncertain significance were identified in 17.2% of cases in our cohort.
- Regions of homozygosity were identified in an additional 8.2% of cases in our cohort.
- The majority of cases (57.4%) were reported as negative.

Table 1: Breakdown of result classification from the first 331 cases analyzed with the CNGnome® Test

Type of Result	Number of Cases (%)
Pathogenic/Likely Pathogenic	57 (17.2%)
Variant of Uncertain Significance (VOUS)	57 (17.2%)
Area of Homozygosity (AOH)	27 (8.2%)
Negative	190 (57.4%)

Figure 1: Breakdown of result classification from the first 331 cases analyzed with the CNGnome® Test



Types of Findings

- The CNGnome® test was able to identify multiple types of variants in our cohort of 331 cases submitted for clinical testing.
- Breakpoint accuracy was achieved for large-scale CNVs including a 700 kb deletion involving the *SQSTM1* gene, a 1.9 Mb chromosome 5q35.2q35.3 microdeletion and a 4.8 Mb chromosome 15q11q13 duplication.
- Several smaller intragenic CNV events were detected including pathogenic heterozygous intragenic deletions involving the *DMD*, *NOTCH2*, and *SHANK3* genes, and an intragenic homozygous deletion involving the *TMCO1* gene.

Table 2: Breakdown of Pathogenic/Likely Pathogenic findings identified in this cohort

Type of CNV	Diagnoses Related to Phenotype (%)	Carrier Finding (%)	Diagnoses Unrelated to Phenotype (%)
Characterized Del/Dup Syndrome	33 (67.4%)	1 (14.3%)	-
Intragenic Change	10 (20.4%)	6 (85.7%)	1 (100%)
Trisomy	4 (8.2%)	-	-
Triploidy	1 (2.0%)	-	-
Uniparental Disomy	1 (2.0%)	-	-
Total (57)	49 (86.0%)	7 (12.3%)	1 (1.7%)

Figure 2: Breakdown of Pathogenic/Likely Pathogenic findings identified in this cohort

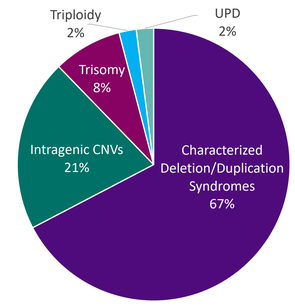
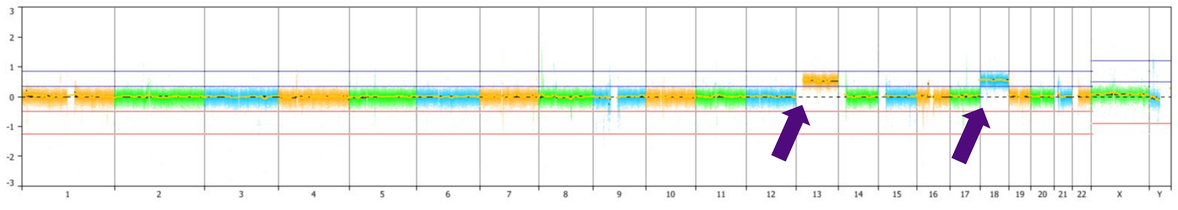


Figure 3: Representative CNGnome® case with a double trisomy: Trisomy 13 and Trisomy 18



Discussion

- The data presented here suggests that the CNGnome® test has a diagnostic yield that is equivalent to, or higher than, current diagnostic array-based assays available in the clinical diagnostic market.
- The CNGnome® test has a demonstrated ability to detect a wide range of changes, including unbalanced translocations, microdeletion/ microduplication syndromes, intragenic CNVs and absence of heterozygosity.
- This study provides continued evidence that low resolution genome sequencing can meet or exceed the performance of traditional microarrays by providing more uniform coverage of the entire genome without the limitations of probe placement all while maintaining a low cost and fast turn-around-time; thus, the CNGnome® test may be considered as a potential alternative to traditional microarrays as the standard-of-care for CNV detection.

Acknowledgement

We would like to thank Dr Suresh Shenoy, Trey Wilson and Ruby Liu for their assistance in compiling all of the clinical CNGnome® test data.