Insights Derived From The First 500+ Clinical Cases Run Utilizing Low Pass Genome Sequencing (LP-GS) As An Alternative To Traditional Microarray

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BACKGROUND

Chromosomal microarrays were defined as a first-tier clinical diagnostic test for individuals with developmental delay and/or congenital anomalies in a consensus statement published in 2010. Since that time array technologies have continued to improve, but their capabilities remain limited with regards to breakpoint resolution and flexibility in design due to the inherent restrictions of probed-based assays. With increased breakpoint resolution and decreasing costs, low pass genome sequencing (LP-GS) is a promising alternative to traditional microarrays in detecting traditional CNV and AOH events. Here we present our experience from our first 500+ clinical cases referred for clinical diagnostic testing for LP-GS analysis.

METHODS

DNA samples were extracted from 519 clinical specimens (including blood, saliva, CVS, AF, and POC), prepared using KAPA HyperPlus PCR-free library preparation kit, then sequenced at ~5X average coverage per sample using the NovaSeq 6000 platform. Raw sequence reads were converted to FASTQ format, then mapped to the GRCh Build 37 (hg19) reference sequence. Copy number and AOH analysis was performed using NxClinical v5.0 software.

RESULTS

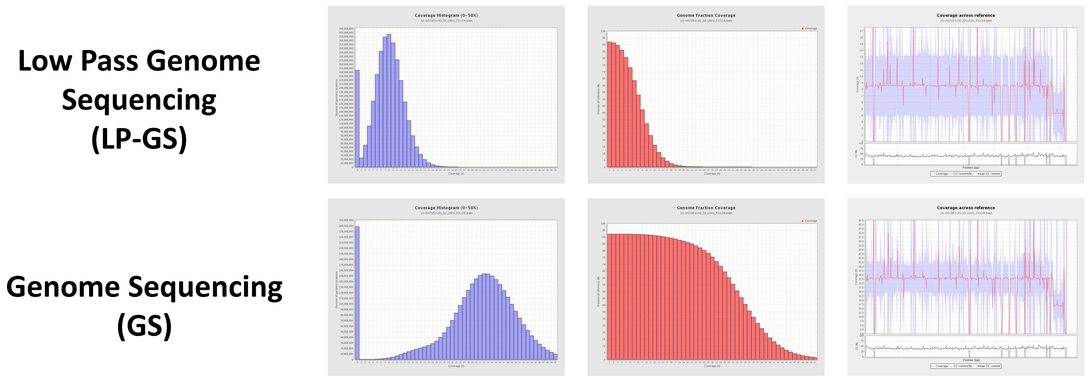
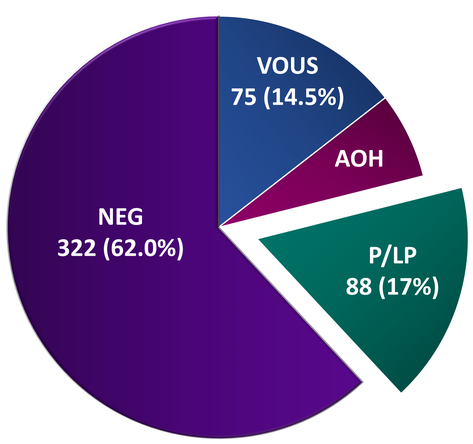


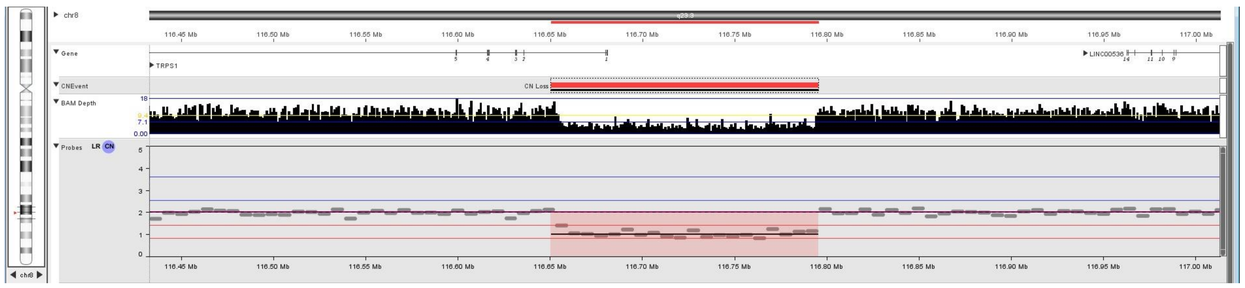
Fig 1. LP-GS provides coverage of ~5X across the genome (top panels) compared with ~30X for genome sequencing (GS; bottom panels). LP-GS and GS coverage is compared via (A) Coverage histograms, (B) genome fraction coverage, and (C) coverage across the reference. LP-GS technology is designed for CNV and AOH detection, while GS technology can capture CNV, AOH, and sequencing variants.



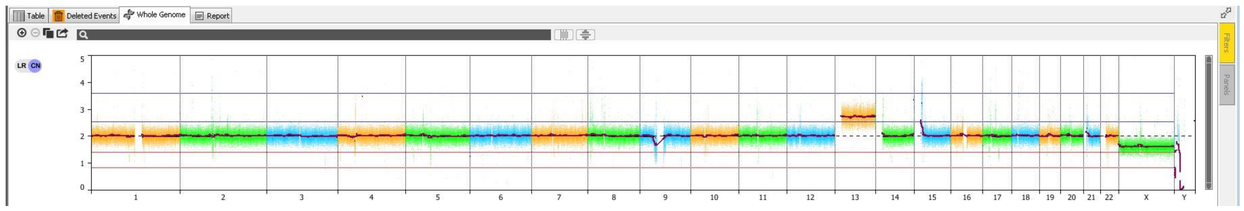
Pathogenic & Likely Pathogenic Findings		
	Diagnostic	**Carrier**
Aneuploidy	9 (10.2%)	0
Microdel/dup	48 (9.2%)	3 (0.6%)
Intragenic CNV	13 (2.5%)	12 (2.3%)
UPD	1 (0.2%)	0
Suspected unbalanced translocation	2 (0.4%)	0
Total	**73 (14.1%)**	**15 (2.9%)**

Figure 2. Summary from 519 clinical cases analyzed for CNV and AOH detection by LP-GS. CNVs for each case were analyzed and classified according to standard ACMG recommendations. Cases were then classified according to main variant type detected: Pathogenic or Likely Pathogenic (P/LP), Variant of uncertain significance (VOUS), Absence of Heterozygosity (AOH), or negative (NEG) for reportable CNVs or AOH regions. Pathogenic CNVs were further stratified by aberration type (see table).

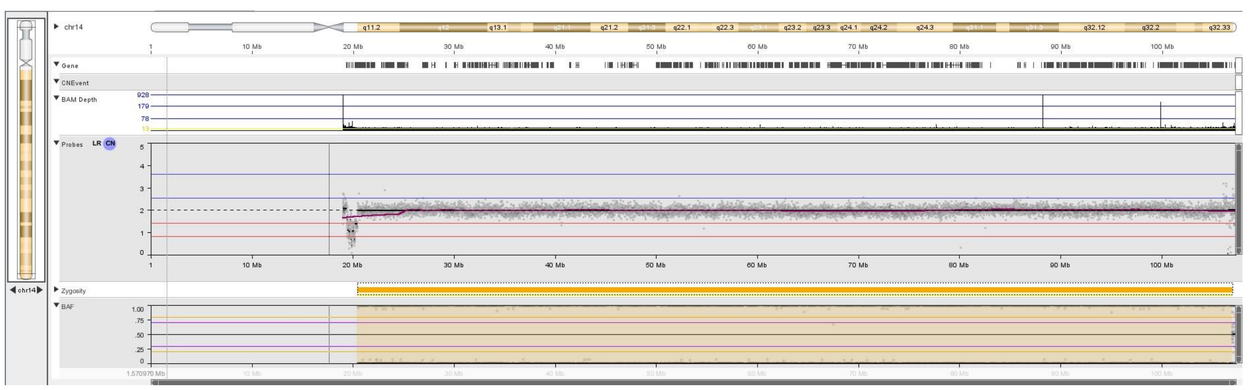
REPRESENTATIVE CLINICAL CASES



Case 1: Intragenic copy number loss of *TRPS1* gene on chr 8q23.3 in a patient with clinical features of trichorhinophalangeal syndrome (TRPS). The BAM depth track depicts the sequencing reads in this sample. The Probes track portrays virtual probes calculated using the multi-scale reference (MSR) algorithm. The copy number (CN) information shows a deletion (CN=1) encompassing the 5'UTR and exon 1 of the *TRPS1* gene. Nucleotide-level breakpoint resolution is achievable.



Case 2: Dual diagnosis of a double aneuploidy in a POC specimen. Whole genome view from NxClinical software in a product of conception (POC) specimen shows both trisomy 13 and mosaic monosomy X, both of which are common aneuploidies in pregnancy loss. The Copy Number (CN) track shows virtual probes across the genome with different alternating colors per chromosome. A red line depicts the moving-average of the copy number of the probes. Chr 1-22, X and Y are shown from left to right. Chr 13 shows a CN state of 3 while Chr X shows a CN state of ~1.5 (mosaic monosomy X).



Case 3: Detection of chromosome 14 AOH indicating uniparental isodisomy 14 (UPD14). The yellow bar highlights regions of AOH. BAM depth and probes depict a copy number (CN) state of 2 while the B-allele frequency (BAF) track shows loss of heterozygous probes (BB=1.00; AA=0).

DISCUSSION

- LP-GS can detect a wide range of genomic changes, including aneuploidies, microdeletion/ microduplication syndromes, intragenic CNVs and absence of heterozygosity (AOH)
- Compared to the traditional microarray, LP-GS shows:
  - Higher resolution for more accurate breakpoint detection
    - Nucleotide-level resolution is achievable
  - More uniform coverage over the entire genome
  - Equivalent or higher diagnostic yield
  - Freedom from limitations of probe placement

LP-GS is a competitive alternative to traditional microarrays as the standard-of-care for CNV and AOH detection.