Evaluating Whole-Genome Sequencing as a Viable Replacement for Chromosomal Microarray Using the DRAGEN v4.4 Cytogenetics Module

Yueyao Gao[1], Francesco Brundu[2], Yeonghun Lee[2], Jialin Ma[2], Sarah South[3], Peter Bui[3], Mark Fleharty[1], Katie Larkin[1], Victoria Popic[1], Sean Truong[2], Sean Hofherr[1], Niall Lennon[1]

[1]Broad Clinical Laboratories, LLC, Burlington, MA [2]Illumina, Inc., San Diego, CA [3]Quest Diagnostics, Secaucus, NJ

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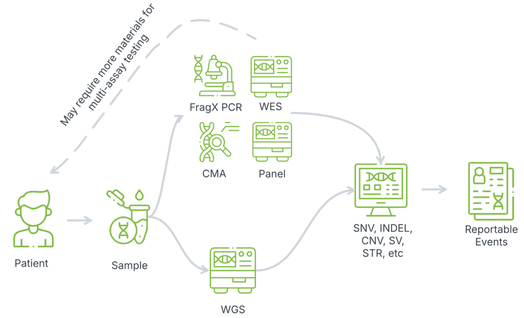
Introduction

Chromosomal microarray (CMA) has been the first-tier test for developmental disorders and congenital anomalies since the 2010 ACMG consensus.[1] It is now a clinical standard across settings from commercial reference labs to hospital in-house testing. However, CMA only captures copy number changes. Whole-genome sequencing (WGS), with falling cost and expanding capability, offers a single laboratory workflow that can identify all major variants type, including single nucleotide variants (SNVs), CNVs, loss of heterozygosity (LOHs), and short tandem repeats (STRs). The goal of our study is to evaluate whether WGS can effectively replace CMA, which remains one of the most commonly ordered genetic tests for patients with suspected hereditary disorders.



The DRAGEN™ v4.4 release includes a cytogenetics (cyto) module, a germline allele-specific copy number (ASCN) caller designed to produce CMA-equivalent results. This module leverages b-allele frequency for CNV detection, identifies LOH, smooths adjacent segments, detects mosaic events, and reports homozygosity index. In this study, we assessed whether WGS can capture the same events captured by CMA (large CNVs and homozygosity index), while providing additional insights into other variant types.

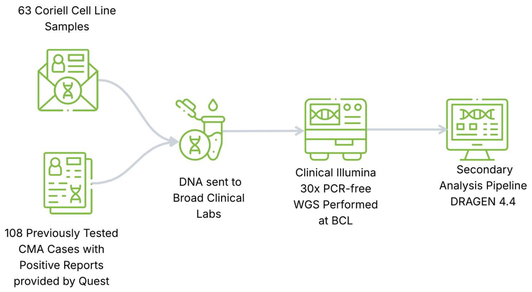
Figure 1: IGV view of a region with 1.6M DUP using DRAGEN v4.4.

Materials & Methods

Sample source:

- Coriell cell line: 63 samples
- Quest Diagnostics identified 108 samples with abnormal CMA reports. The study was approved by an IRB.

DNA was sent to Broad Clinical Labs (BCL) for clinical WGS (cWGS), which was performed using 30x PCR-free WGS.



We then compiled the CMA events from each sample into a VCF file and compared them against the WGS-based calls from the DRAGEN™ v4.4 cyto module using the SV benchmarking tool truvari.[2] In this analysis, we considered a WGS call concordant with a CMA-reported event if it matched its event type and showed at least 70% reciprocal overlap and 70% size similarity, calculated as follows:

- reciprocal overlap = (overlapping bases)/max(base_size, comp_size)
- size similarity = min(base_size, comp_size)/max(base_size, comp_size)

We also compared the homozygosity index between CMA and WGS, as it is an important metric in clinical reporting.

Figure 2: IGV view of a 540kb DUP event detected by CMA, showing 91.88% reciprocal overlap and 91.88% size similarity with DRAGEN v4.4 results.

Results

As shown in Table 1, the performance was consistent across 63 Coriell samples (99 events) and 108 Quest-identified samples (193 events). Concordance was **95.96% (95% CI: 90.06-98.42)** for Coriell samples and **96.89% (95% CI 93.38-98.57)** for Quest samples**.** We further reviewed all 10 discordant events listed in Table 2.

Table 1: Comparison of results between CMA and WGS on known events

Event Type	Size	Coriell Cell Line			Quest Identified Samples		
		CMA Detected	WGS Recovered	Concordance	CMA Detected	WGS Recoved	Concordance
DEL	25kb-100kb	1	1	100.00%	5	5	100.00%
	100kb-500kb	10	9	90.00%	17	16	94.12%
	500kb-1Mb	2	2	100.00%	9	9	100.00%
	1Mb-10Mb	24	23	95.83%	24	23	95.83%
	10Mb-25Mb	14	14	100.00%	4	4	100.00%
	25Mb+	6	6	100.00%	4	4	100.00%
	Overall	57	55	96.49%	63	61	96.83%
DUP	100kb-500kb	8	8	100.00%	4	4	100.00%
	500kb-1Mb	2	2	100.00%	28	27	96.43%
	1Mb-10Mb	8	7	87.50%	18	17	94.44%
	10Mb-25Mb	7	7	100.00%	1	1	100.00%
	25Mb+	16	15	93.75%	1	1	100.00%
	Overall	41	39	95.12%	52	50	96.15%
LOH	5Mb-10Mb	0	0	NA	30	29	96.67%
	10Mb-25Mb	0	0	NA	36	35	97.22%
	25Mb+	1	1	100.00%	12	12	100.00%
	Overall	1	1	100.00%	78	76	97.44%
All		99	95	95.96%	193	187	96.89%

Table 2: Summary of discrepancies between orthogonal truth and WGS

Event Type	Source	Event Size	Total Recovered Length / Truth Length (%)	Reasons for Suboptimal Matches
DEL	Coriell	410kb	0	KMER-NON-UNIQUE region
		4.34Mb	8	KMER-NON-UNIQUE region
	Quest	1.9Mb	47.12	KMER-NON-UNIQUE region
		312kb	66.11	WGS has better resolution than CMA
DUP	Coriell	3.16Mb	6.6	KMER-NON-UNIQUE region
		29.8Mb	>99.9	Fragmented due to GAINLOH
	Quest	1.1Mb	68.26	WGS has better resolution than CMA
		2.5Mb	62.5	KMER-NON-UNIQUE region
LOH	Quest	15.8Mb	65.51	WGS has better resolution than CMA
		7Mb	56.19	WGS has better resolution than CMA

Figure 3: integrated visualization of a 312kb DEL event identified by CMA with improved resolution and calling results by DRAGEN v4.4

Typically, samples with a homozygosity index above 2% are reported. We have compared the homozygosity index of seven samples previously reported with high homozygosity index. The homozygosity index is consistent across both platforms, indicating DRAGEN™ v4.4 can reliably report this metric from WGS data.

Table 3: Homozygosity index computed by CMA and WGS

Samples	CMA: ChAS		WGS: DRAGEN v4.4	
	Total_autosome_LOH_kbp	Homozygositylndex	Total_autosome_LOH_kbp	Homozygositylndex (1M)
Sample 1	218677	7.86	222327	7.72
Sample 2	212547	7.64	223549	7.76
Sample 3	20684	0.74	21373	0.74
Sample 4	550342	19.8	563288	19.55
Sample 5	143740	5.17	154084	5.35
Sample 6	202434	7.28	207274	7.19
Sample 7	26302	0.95	25328	0.88

Conclusion

In this work, we demonstrated that the DRAGEN™ v4.4 cytogenetics module achieves performance comparable to the current CMA standard. Performance was consistent across Coriell cell line samples and real clinical samples from blood and buccal swabs. This module reliably detects CNVs, including large DUP and DELs, and accurately reports the homozygosity index, an important metric in clinical reporting. Unlike CMA, WGS can detect a broad range of variant types without additional laboratory assays including STR expansion. Although not shown in this poster, we have tested integrating DRAGEN STR results to rule out negative FragX cases (results included in the preprint below). These results highlight that WGS with DRAGEN™ v4.4 secondary analysis can reduce the need for multiple assays in routine clinical setting.

References

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