Optical Genome Mapping for Constitutional Postnatal SV, CNV, and Repeat Array Sizing: A Multi-site Clinical Study

N. Sahajpal1, V. Rodriguez2, L. Kanyo2, A. A. Stence3, S. Skinner4, M. Anwar Iqbal5, K. Awayda5, B. Levy6, U. Broeckel7, G. H. Scharer7, J. R. Hackman3, A. Mondal1, A. Bossler3, P. L. Nagy2, R. Kolhe1

1Augusta University, Augusta, GA, USA, 2Praxis Genomics, Atlanta, GA, USA, 3University of Iowa Hospitals and Clinics, Iowa, IA, USA, 4Greenwood Genetic Center, Greenwood, SC, USA, 5University of Rochester Medical Center, Rochester, NY, USA, 6Columbia University Irving Medical Center, New York, NY, USA, 7Medical College of Wisconsin, Milwaukee, WI, USA

Introduction

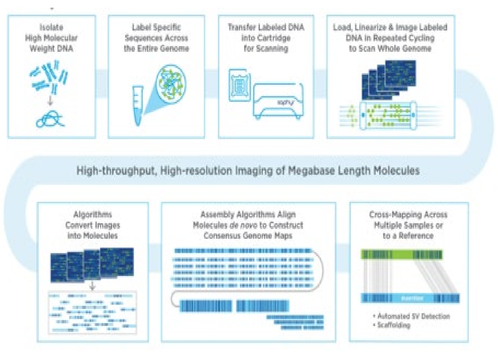
Optical genome mapping (OGM) is an emerging technology that is revolutionizing the clinical practice of cytogenetic characterization of samples that is currently obtained by a combination of technologies that include karyotyping, FISH, and chromosomal microarray (CMA). Recent reports have demonstrated the clinical utility of OGM in hematological malignancies and constitutional cases but lack a thorough investigation in solid tumors.

Samples and Data Analysis

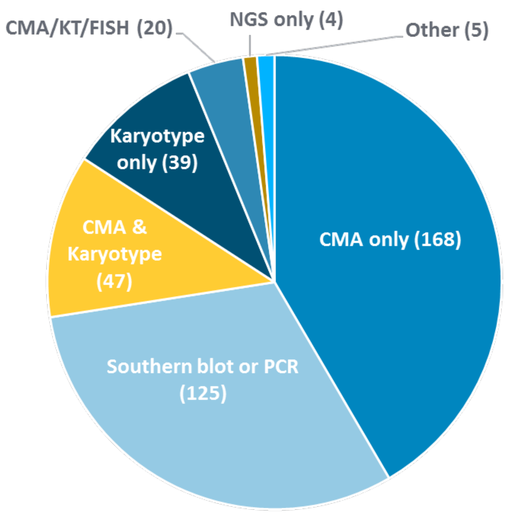
- This ongoing multi-institutional study includes a total of 520 sample runs that included 408 unique samples
- The results are provided for the first 331 samples that included 219 unique samples
- The operators and analysts were blinded to the known genetic abnormalities and only received the clinical phenotype information and classified variants as per ACMG classification.
- A standard operating procedure (SOP) was devised to enable analysts to systematically and efficiently select for rare (<1% population frequency) variants, with additional criteria's that included:
  - Filtering insertions and deletion >1500 bp.
  - Filtering for variants overlapping known disease-associated genes or overlapping known disease loci

Methods and Site-map of laboratory testing sites

Briefly, 1-1.5M cells were used to isolate high molecular weight DNA, labeled at specific 6bp motifs, followed by optical mapping. The de-novo genome assemblies were aligned against reference hg38 to call out structural variants.

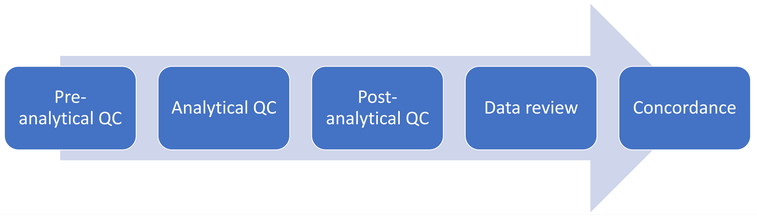


Standard-of-care test (n=408)



Standard of care test for concordance comparison (n=408)

Technical Concordance



DNA isolation and Data Generation Pass Rate				
	Samples run	Samples passed	First pass success	Final pass success (with repeats)
Total	331	328	93.8%	99.1%

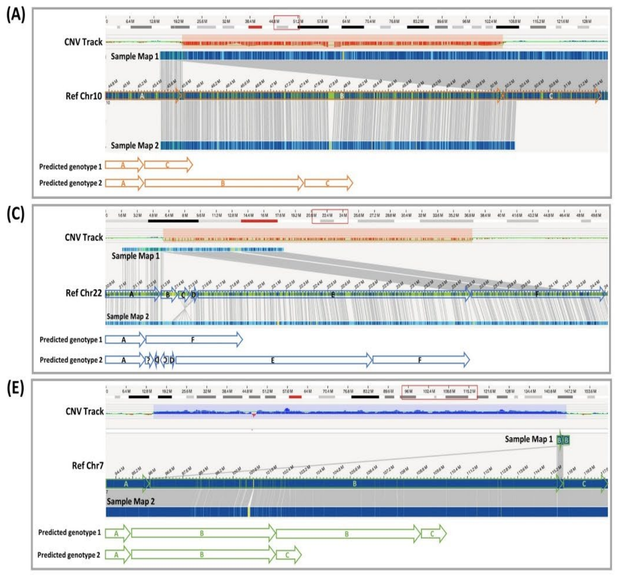
Analytical Performance Metrics								
N50				DNA output	Map Rate		Effective	
>230 kbp	>220 kbp	>210 kbp	>200 kbp	Average	Average	>70%	Average	>160X
92.1%	97.7%	98.2%	99.7%	805 Gbp	88.4	98.8%	223X	97.9%
*metrics are based on ≥150 kbp filtering								

Analytical Concordance

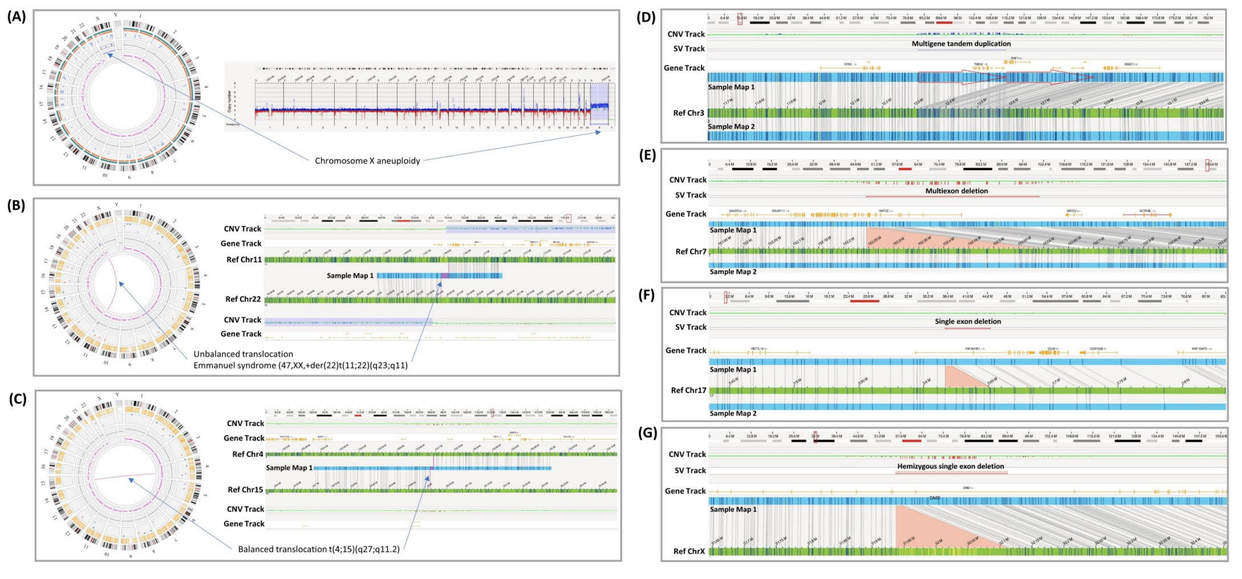
Categories	Number of Samples N (%)
Concordant	214 (97.7%)
Partially Concordant	5 (2.3%)

Variant	Variant Types		Variant Description	Bionano OGM
Aneuploidy	Monosomy		Chromosome loss	✓
	Trisomy		Chromosome gain	✓
	Triploidy		Whole genome Triploidy	Constitutional
	Tetraploidy		Whole genome Tetraploidy	Not currently
	Ring Chromosome		CNV and fusion	≥500 kbp + fusion break
Structural Variants	Copy number Variants	Deletions/Duplications	Interstitial	≥500 bp
			Terminal	≥500 kbp
		Insertions	Interstitial (unknown sequence)	≥500 bp
	Translocations		Balanced	✓
			Unbalanced	✓
	Inversions		Pericentric	✓
			Paracentric	≥30 kb
Regions of Homozygosity	ROH		ROH	Constitutional
Macrosatellite/Microsatellite	Repeats		Contractions/Expansions	≥500 bp
Sequencing Variants	Single nucleotide variants, INDELs		Transitions/transversions Insertions/deletions <50bp	-

List of variant classes and OGM performance across each class



Representative examples of concordant microduplication and microdeletions.



Representative examples of different SVs (aneuploidy, translocations, and interstitial duplication and deletions).

Conclusion

The current results showed that OGM detected all the expected SVs and CNVs with 97.7% accuracy, sensitivity, and 100% specificity. Inter-run, inter-chip, and inter-site reproducibility was assessed and all expected SVs and CNVs were reliably detected across different runs, reagents, and sample preparations. This clinical validation study is ongoing and is extended with additional samples. The current data demonstrate the potential of OGM as an alternative to the current standard of care methods in detecting the structural variants of clinical significance for many constitutional postnatal diseases.

Acknowledgements

We would like to thank Alka Chaubey, Alex Hastie, Hayk Barseghyan, Andy Pang, Rena Vanzo, Ben Clifford, James Yu, Mike Gallagher, Ben Kellman, Jillian Burke, Alberto Reyes for their data analysis support and valuable help in preparation of this poster.

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