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Development of 17 Novel Copy Number Variation (CNV) Reference Materials based on the Genome in a Bottle

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ABSTRACT

Introduction: Advancements in tumor characterization by solid and liquid biopsy are instrumental in the evolution of personalized medicine. Recent improvements in assay sensitivity for profiling DNA variants such as single nucleotide polymorphisms (SNP) and indels have facilitated expansion of both solid and liquid biopsy applications. However, measurement of copy number variations (CNV) is a relatively new application, and few reference materials exist to aid in assay development and optimization. In this study, we demonstrate for the first time the development of 17 whole gene CNV reference standards in a background of the highly characterized NIST Genome in a Bottle GM24385 genomic DNA.

Methods: DNA molecules containing full genes of MET, ERBB2, ERBB3, PIK3CA, EGFR, BRAF, FGFR1, FGFR2, FGFR4, KIT, KRAS, MYC-N, PDGFRA, MDM2, CD274, MYC-L and MYC were each spiked separately into background GM24385 genomic DNA to generate 2.8 copies of each gene. Copy number for each of the 17 CNV samples was determined using Bio-Rad® Droplet Digital™ PCR (ddPCR™). To assess linearity over a range of copy numbers, MET and ERBB2 CNV ladder reference materials with copy gains at 6 different levels 3, 6, 9, 12 and 15 copies were also generated. To mimic circulating tumor DNA, each CNV sample was fragmented and size selected to recover a population containing ~170 bp size fragments. Copy number was also verified post size selection using ddPCR™ and the Ion S5™ XL system. Multiplexed triple CNV controls with MET, EFGR and ERBB2 were also developed at 3, 6, 9, 12 and 15 copies and analyzed by ddPCR™.

Results: Copy number for all 17 genes measured at 2.80 ± 0.28 copies by ddPCR. When tested in a ctDNA copy number ladder format, MET and ERBB2 showed good linearity of R^2=0.99 for observed versus expected # of copies. Good correlation between ddPCR and NGS was also observed, with a slope of 0.979 for MET and 1.127 for ERBB2 across increasing copy numbers and standard deviation (SD) of ± 10%. Notably, SD increased at copy numbers higher than 12. The average size of fragmented ctDNA CNV reference standards were observed to be ~170 bp on the Agilent 2100 Bioanalyzer system. Finally, the multiplexed triple CNV control also performed as expected on ddPCR with SD of ± 10%.

Conclusions: A novel method for producing CNV and ctDNA CNV controls has been developed that enables preparation of any copy gain level with a known gDNA background. Simpler QC materials mimicking patient samples will enable simpler CNV test method development and analytical validation, which will be critical for laboratories to introduce CNV solid and liquid biopsy testing into the field.

INTRODUCTION

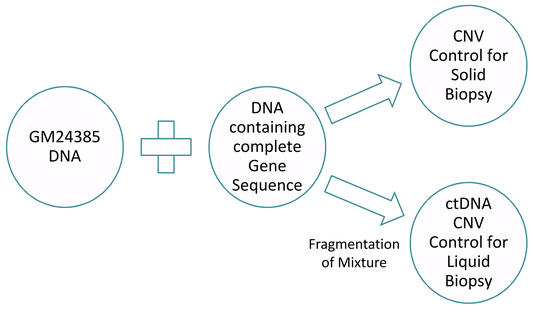

Figure 1. Workflow for manufacturing and qualifying CNV reference materials: DNA molecules containing full genes (introns and exons) are spiked into the background of GM24385 DNA for a specific target copy gain. For ctDNA CNV controls, each CNV sample is fragmented and size selected to contain a fragment size of ~170bp.

RESULTS

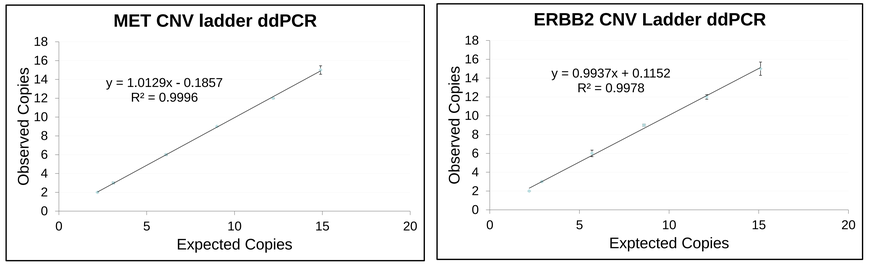

Figure 2. BioRad® Droplet Digital PCR (ddPCR™) data for MET and ERBB2 ctDNA copy number ladder reference materials. The ctDNA copy number reference ladder was developed with an intention to help with verification and validation of NGS panels targeting CNVs. The ctDNA ladder consists of 2, 3, 6, 9,12 and 15 copies. Both MET and ERBB2 showed good linearity with an R^2=0.99.

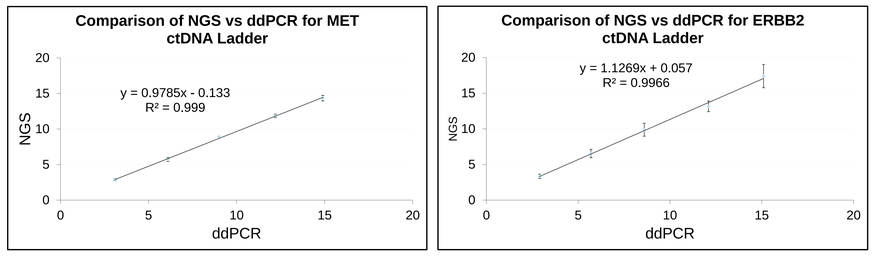

Figure 3. NGS data for MET and ERBB2 ctDNA copy number ladder reference materials. The CNV ladder reference material created with copy gains of 3, 6, 9, 12 and 15 copies was also sequenced using the Ion S5™ System. Copies observed from sequencing 3 libraries for both Met and ERBB2 showed good correlation with an R^2=0.99. Variability in the NGS data was noticed for >12 copies.

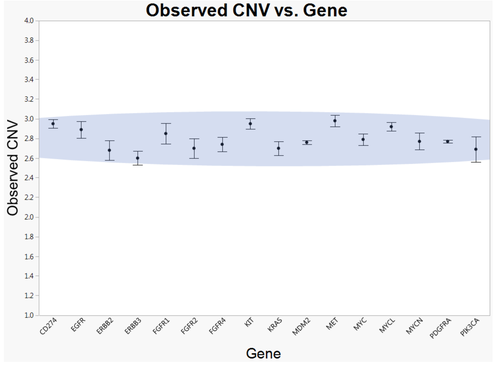

Figure 4. ddPCR™ 2,8 copy gain controls for 17 genes. The copy numbers for each of the 17 genes was measured using BioRad ® Droplet Digital PCR (ddPCR™). Data was analyzed using JMP14 and standard deviation was determined by using data from 3 replicates. All genes were measured to be within 2.8± 0.28 copies by ddPCR. The ellipse encompasses the area of 10% variation and all targets are within this range.

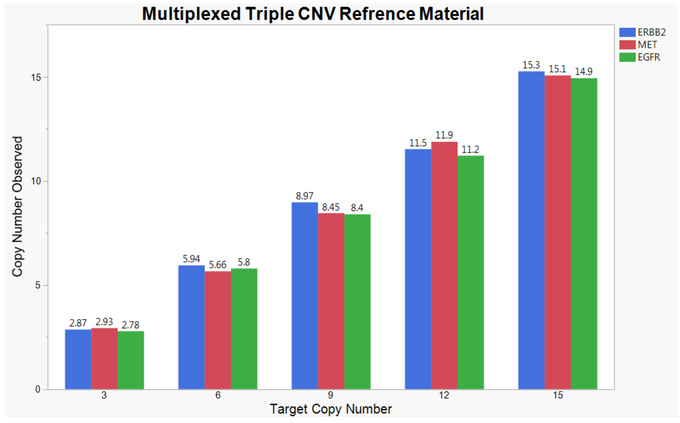

Figure 5. Multiplexed Triple CNV Reference Material results are as expected. These control consists of ERBB2, EGFR and MET genes with copy gains of 3, 6, 9, 12 and 15 in background GM24385 DNA. The copy numbers are calculated based on the data generated using the BioRad® Droplet Digital PCR (ddPCR™). All three genes showed consistent copy gains as expected with SD of ± 10%.

CONCLUSIONS

•QC materials that mimic patient samples for both solid and liquid biopsy would enable laboratories to develop and validate CNV test methods.

•A multiplexed triple CNV ladder format control with different copy gains provides the capability of a comprehensive study of multiple genes from a single tube.

•The consistency of CNV controls across 17 genes at 2.8 copies facilitates LOD studies for various CNV test methods.

ACKNOWLEDGEMENTS

The authors thank Dan Mazur and his team for efforts put in generating NGS data.

TRADEMARKS

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