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Competitive DNA Calibrators as Quality Reference Standards (QRS™) for Germline and Somatic Copy Number Variations/Variant Allelic Frequencies Analyses

Authors : Eirini Konstanta, Cedric Gouedard, Aggeliki Delimitsou, Stefania Patera, Samuel Murray

Abstract : Introduction: Quality reference DNA standards (QRS) for molecular testing by next-generation sequencing (NGS) are essential for accurate quantitation of copy number variations (CNV) for germline and variant allelic frequencies (VAF) for somatic analyses. Objectives: Presently, several molecular analytics for oncology patients are reliant upon quantitative metrics. Test validation and standardisation are also reliant upon the availability of surrogate control materials allowing for understanding test LOD (limit of detection), sensitivity, specificity. We have developed a dual calibration platform allowing for QRS pairs to be included in analysed DNA samples, allowing for accurate quantitation of CNV and VAF metrics within and between patient samples. Methods: QRS™ blocks up to 500nt were designed for common NGS panel targets incorporating ≥ 2 identification tags (IDTDNA.com). These were analysed upon spiking into gDNA, somatic, and ctDNA using a proprietary CalSuite™ platform adaptable to common LIMS. Results: We demonstrate QRS™ calibration reproducibility spiked to 5–25% at ± 2.5% in gDNA and ctDNA. Furthermore, we demonstrate CNV and VAF within and between samples (gDNA and ctDNA) with the same reproducibility (± 2.5%) in a clinical sample of lung cancer and HBOC (EGFR and BRCA1, respectively). CNV analytics was performed with similar accuracy using a single pair of QRS calibrators when using multiple single targeted sequencing controls. Conclusion: Dual paired QRS™ calibrators allow for accurate and reproducible quantitative analyses of CNV, VAF, intrinsic sample allele measurement, inter and intra-sample measure not only simplifying NGS analytics but allowing for monitoring clinically relevant biomarker VAF across patient ctDNA samples with improved accuracy.

Keywords : calibrator, CNV, gene copy number, VAF
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