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Functional DNA quantification guides accurate next-generation sequencing mutation detection in formalin-fixed, paraffin-embedded tumor biopsies

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Abstract

The formalin-fixed, paraffin-embedded (FFPE) biopsy is a challenging sample for molecular assays such as targeted next-generation sequencing (NGS). We compared three methods for FFPE DNA quantification, including a novel PCR assay ('QFI-PCR') that measures the absolute copy number of amplifiable DNA, across 165 residual clinical specimens. The results reveal the limitations of commonly used approaches, and demonstrate the value of an integrated workflow using QFI-PCR to improve the accuracy of NGS mutation detection and guide changes in input that can rescue low quality FFPE DNA. These findings address a growing need for improved quality measures in NGS-based patient testing.

Background

Approximately 120 years ago, formaldehyde was identified as a superior fixation agent to preserve tissue samples [1]. Since that time, preservation of tissues with formalin-fixed paraffin-embedding (FFPE) procedures has emerged as the method of choice for histological study and archival storage of clinical specimens. More than 400 million FFPE samples are thought to exist and many have clinical annotations such as primary diagnosis, therapeutic regimen, drug response, and recurrence status. These archives represent an invaluable repository of retrospective patient clinical data. Powerful new genomic technologies, such as next-generation sequencing (NGS), promise to unlock the molecular features of such samples and inform the linkage of genotype and phenotype. Achievement of this goal requires that the unintended consequences of the fixation and embedding process and the duration and conditions of storage on nucleic acid quality be accommodated by the profiling methodology to ensure reliable, accurate, and sensitive biomarker detection.

Unfortunately, the FFPE process causes fragmentation and chemical modifications in DNA, such as cross-linking, deamination and adducts [2-4]. These modifications reduce the number of DNA templates available for amplification, and pose significant challenges to efficient PCR. Factors such as type of fixative, fixation time, age and storage conditions of the FFPE block can contribute to problems in diagnostic testing [5]. In light of these challenges, pre-analytical methods such as end-point PCR using different reference gene amplicon lengths have been previously described to help qualify samples for molecular methods downstream [6-8].

Two common methods for DNA quantification are spectrophotometry and fluorometry using DNA-binding dyes. Spectrophotometry offers a simple and nimble way to accurately measure the bulk concentration of high quality DNA and instruments such as the NanoDrop Spectophotometer are readily available. Yet spectrophotometry cannot gauge the molecular damage and fragmentation caused by fixation, embedding, and/or long-term storage, nor can it anticipate the effects on PCR. For example, a recent study comparing the accuracy of different methods to quantify DNA following controlled degradation demonstrated up to a three-fold difference between

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