Pyrimidine-dependent UV-mediated crosslinking magnifies minor genetic or epigenetic changes in clinical samples

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Detection of minor DNA allele alterations is becoming increasingly important for cancer early detection, monitoring, and treatment selection. As applications increase, so does the need for methodologies that provide improved sensitivity combined with robust performance and specificity. We describe a new method that uses ultraviolet light to eliminate wild-type DNA alleles and enables improved detection of minority genetic or epigenetic changes in tissues and liquid biopsies [1]. Pyrimidine-Dependent UV-based Minor-allele Enrichment (PD-UVME) employs oligonucleotide-probes that hybridize to wild-type (WT) sequences and incorporate a UVA-sensitive molecule (CNVK) placed directly opposite interrogated pyrimidines, such as thymidine (T) in targeted DNA. Upon UVA illumination CNVK crosslinks with T, preventing subsequent amplification of WT DNA strands. Mutations that remove the T escape crosslinking and are readily amplified and detected. Similarly, when CNVK is placed opposite cytosines in CpG dinucleotides it discriminates between methylated and unmethylated cytosine, enabling direct enrichment of unmethylated DNA targets. PD-UVME was applied for detecting *BRAF* V600E mutations in model systems, thyroid patient cancer samples and plasma-cfDNA from melanoma patients. When PD-UVME was applied in serial dilutions of mutated *BRAF* V600E DNA into WT DNA, the mutation abundance changed from 0.01%, 0.1% and 1% to 1.74%, 3.41% and 25.6% respectively. Out of 9 thyroid cancer tissue samples, PD-UVME applied prior to ddPCR revealed a low-level mutation in 1 of the samples classified as negative by conventional ddPCR. Similarly, when 7 plasma circulating-free DNA (cfDNA) samples from melanoma patients were examined for *BRAF* V600E mutations, PD-UVME-ddPCR identified 6 positive samples versus 1 positive sample detected via conventional ddPCR. All 10 plasma-cfDNA samples obtained from normal volunteers were negative via both approaches. PD-UVME mutation/methylation enrichment performed prior to ddPCR magnifies signals from low-level mutations or epigenetic changes and can increase confidence in the results.

References:

1.Yu, Farzana Ahmed, Smilkou et. al *Clinical Chemistry*, 2024