TT10. COLD-PCR in Emulsion Magnifies Low-Level Mutations Prior to Sequencing

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Introduction: Reliable sequencing of low-level mutations in certain classes of clinical samples (infiltrating, diffuse-type tumor specimens; tumor-margins; stromal cells; DNA from circulating-nucleic acids, circulating-cells, sputum or other bodily samples) is of paramount significance for personalized medicine based upon mutational profiling of individual patients. Nevertheless, Next-Generation-Sequencing (NGS) technologies are not sufficiently reliable for the discovery or validation of low-level mutations in clinical samples, and thus NGS integration within Clinical Molecular Diagnostics cannot be exploited effectively. Employing COLD-PCR, a technology that magnifies mutations at any sequence position of a given amplicon, enables enrichment of low-level mutations such that NGS technologies can easily identify them (see Abstract by Milbury et al, AMP 2011). Despite this advantage, COLD-PCR requires individualized optimization for each DNA target amplicon, and thus COLD-PCR is laborious and technically challenging for large-scale analyses. We examined the feasibility of simultaneously performing COLD-PCR in several sequences, by adapting COLD-PCR to operate in nano-PCR emulsion-based reactions in the presence of several copies of a distinct DNA target sequence. Accumulation of the target sequence within individual emulsions was enabled by creating magnetic beads coated with specially designed Reference Sequences (COLD-PCR-beads). **Methods:** COLD-PCR-beads were prepared using streptavidin-coated beads possessing several copies of a single-stranded Reference Sequence. The COLD-PCR-beads capture numerous copies (both mutant and wild type) of target sequences from a diverse DNA population. Genomic DNA from human cell-lines which harbor *TP53* mutations (c.847C>T, p.R283C and c.818G>A, p. R273H respectively), were serially-diluted into wild type DNA for subsequent experiments. Single-stranded target DNA, generated with an asymmetric PCR approach, was used for magnetic hybridization capture (MHC) on COLD-PCR beads. Following the capture of target sequences, COLD-PCR-beads were emulsified such that each emulsion contains at most one bead. Fast-COLD-PCR was performed at a denaturation temperature determined experimentally. **Results:** COLD-PCR performed within emulsion (eCOLD-PCR) resulted in the enrichment of mutations in all three sequences examined. Mutations located in different regions of *TP53* exon 8 showed an average 7-fold mutation enrichment during eCOLD-PCR. A second round of MHC and eCOLD-PCR produced additional 5-fold mutation enrichment. eCOLD-PCR produces similar mutation enrichment to that produced in-solution and following eCOLD-PCR DNA target amplicons with mutations at the 1% level or below could be directly sequenced via Sanger-sequencing. **Conclusions:** eCOLD-PCR enables miniaturization of COLD-PCR

and opens up the possibility for highly-parallel COLD-PCR reactions in several target sequences simultaneously, followed by reliable NGS-based detection of low-level mutations. eCOLD-PCR thus may facilitate broad inter-phasing of NGS with Clinical Diagnostics.