TT18. Ice-COLD-PCR: An Improved Version of COLD-PCR That Enriches All Types of Low-prevalence Unknown Mutations Using a Wild-Type-Blocking Reference Sequence

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Introduction: Molecular profiling of somatic mutations in cancer often requires the identification of low-prevalence DNA mutations in an excess of wild-type (WT) DNA; however, the method selectivity and sensitivity are often limiting factors. COLD-PCR (CO-amplification at Lower Denaturation Temperature) resolves several limitations in low-level mutation detection by using critical denaturation temperatures to enrich mutant-containing amplicons during PCR. However, certain mutation types are not enriched by some COLD-PCR formats. We report a novel enhancement in COLD-PCR that enables Improved and Complete Enrichment (ice-COLD-PCR) for all mutation types in an efficient and robust manner. **Methods:** A Reference Sequence (RS) that preferably forms double-stranded structures with WT sequences, but not with mutant sequences, was added to COLD-PCR. A RS was designed to bind to one WT-sequence strand, avoid primer binding and prevent polymerase extension. To validate the use of a RS in COLD-PCR, we evaluated segments of TP53 exon 8. Serial dilutions of mutant cell-line DNA, or human lung tumor DNA, in WT DNA were analyzed. Mutations that increase, decrease, or retain the amplicon melting temperature were tested. Following conventional-PCR, COLD-PCR (full or fast COLD-PCR formats), and ice-COLD-PCR methods amplicons were sequenced and the degree of mutation enrichment was compared. Several clinical lung adenocarcinoma samples with known low-level mutations were also analyzed with ice-COLD-PCR. **Results:** ice-COLD-PCR yielded ~13-fold enrichment for Tm-increasing and Tm-equivalent mutations, and ~15-fold enrichment for Tm-reducing mutations. In contrast, Full-COLD-PCR demonstrated ~5-fold enrichment for all mutations. Further, fast-COLD-PCR, which can only enrich Tm-reducing mutations, exhibited ~17-fold enrichment for these types of mutations, while the Tm-increasing and Tm-equivalent mutations remained undetectable. Regardless of mutant type and position, after ice-COLD-PCR amplification, all mutation types are strongly enriched and can be reliably sequenced down to a level of 1-3%. Ice-COLD-PCR duration is ~1hour, compared to several hours for full-COLD-PCR. **Conclusions:** The inclusion of an appropriately designed RS within COLD-PCR selectively inhibits WT amplification throughout PCR, while preferentially enriching mutants and reducing time-intensive hybridization times. Ice-COLD-PCR combines high sensitivity, speed, and low-cost, and facilitates direct sequencing for all types of unknown low-prevalence mutations in clinical cancer samples.