

S007 Cold-PCR: A new platform for highly improved mutation detection in cancer and genetic testing

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PCR is widely employed as the initial DNA amplification step for genetic testing and cancer biomarker detection. However, a key limitation of PCR-based methods, including real-time PCR, is the inability to selectively amplify low-levels of variant alleles in a wild-type background. As a result, downstream assays are limited in their ability to identify subtle genetic changes that can have profound impact in clinical decision-making and outcome and can serve as cancer biomarkers. We developed Co-amplification-at-Lower Denaturation-temperature (COLD-PCR, Nature Medicine May 08), a novel form of PCR that amplifies minority alleles selectively from mixtures of wild-type and mutation-containing sequences irrespective of the mutation type or position on the sequence. Consequently, COLD-PCR amplification from genomic DNA yields PCR products containing high prevalence variant alleles that can be detected. Since PCR comprises a ubiquitous initial step for almost all genetic analysis, COLD-PCR provides a general platform to improve the sensitivity of essentially all DNA-variation detection technologies. COLD-PCR combined with Real Time PCR provides a new approach to boost the capabilities of existing real-time mutation detection methods, such as the Taqman method. Using COLD-PCR we identified low-level somatic mutations in lung and colon cancer samples that appeared to be mutation-free using established technologies. COLD-PCR will have applications in the fields of biomarker identification and tracing, genomic instability, infectious diseases, methylation testing and pre-natal identification of fetal alleles.