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GI Pathology

**LigAmp: Sensitive Detection of Single Nucleotide Differences**

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Sensitive and accurate detection of small number of tumor cells in the presence of a vast excess of normal cells is a problem common to many cancer research and clinical applications, but it is difficult since the mutant DNA often differs from the wildtype DNA by only a single base. The aim of present study is to develop a novel strategy that converts a single base substitution to a distinct molecule so that it can be detected in a sensitive and linear fashion. This strategy (designated LigAmp) employs two unique oligonucleotides that contain regions specific to the target gene and M13 tails. In addition, the upstream oligonucleotide also contains a region of completely foreign DNA. These two oligonucleotides should be ligated at the mutation site only when a perfectly matched target is present. Ligated products are then amplified by realtime quantitative PCR using M13 primers and the foreign DNA region as a probe. To test this strategy, K-ras mutant SW480 genomic DNA was 10 fold serially diluted into wildtype K-ras Hela DNA. We demonstrated the ability to detect one mutant DNA molecule in the presence of a background of 10,000-100,000 wildtype molecules. We envision that with this level of sensitivity and accurate quantification, this approach may find many cancer applications, including early detection, minimal residual disease testing and molecular relapse monitoring.