Highly Sensitive Single Mutation Detection of EGFR by Bridged Nucleic Acid

Kim, Edward

Highly sensitive detection of mutations is crucial for early action against cancers and other genetic diseases. Real-time quantitative PCR (qPCR) and a newly developed bridged-nucleic acid (BNA) clamp technique together provide a means of highly sensitive single mutation detection that suggests a novel method for cancer diagnosis. One of the most recently developed DNA mimics, bridged-nucleic acid (BNA) displays exceptional hybridization with DNA, making it an effective choice for clamp design. The BNA clamp, an oligonucleotide composed of both BNA and DNA monomers, allows for detection of 1 mutant species out of 10,000 wild-type species. With optimization, a 13-mer clamp containing 9 BNA bases was deemed most effective in distinguishing the mutant genes from the wild-type genes for the single mutation of T790M of epidermal growth factor receptor (EGFR), which poses issues for cancer treatment. In order to aid with design and analysis of the mutation detection technique, molecular dynamics simulations were performed via an umbrella sampling method to approximate the free energy of binding (deltaG) between the BNA clamp and a wild-type or mutant template strand. The script, ONECLICK, was designed and written for the first time to automate the umbrella sampling process. The results showed that the deltaG values for the wild-type and mutant helices were significantly different, suggesting that molecular dynamics simulations could ease the BNA clamp designing process and increase accessibility. This BNA-clamping qPCR technology coupled with computationally predictive software may offer a promising new avenue for early detection of clinically important mutations in the future.

Awards Won:

Intel ISEF Best of Category Award of $5,000

First Award of $5,000