Designing a Novel Primer Blocker Using Bioconjugation of Single Stranded Binding Protein and Oligonucleotides for Improved KRAS Mutation Detection

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The Kirsten rat sarcoma viral (KRAS) gene is the most frequently mutated oncogene, responsible for many types of highly fatal cancers — notably, pancreatic, colorectal, and non-small cell lung cancers (NSCLC). An improved primer-blocker mechanism was designed to detect the KRAS G12D mutation subtype in qPCR method liquid biopsies. Early detection is paramount to increasing the chance of patient survival. In order for liquid biopsies to be a reliable method of cancer diagnosis, especially during earlier stages, there needs to be improvements in accuracy and sensitivity to detect the low amounts of tumor genetic material (ctDNA) in the blood when tumors are still localized. This project provided a proof of concept for a novel design: replacing oligonucleotides with single stranded binding protein to act as the anchor in primer-blocker technologies, eliminating the need for a longer primer length for competitive accuracy. A shorter, more competitive primer blocker with greater differentiation ability was designed with an SSB protein acting as a thermostable anchor, raising the primer blocker melting temperature. The primer-blocker was constructed using copper-catalyzed azide-alkyne click chemistry, in order to connect the SSB protein anchor with KRAS G12D mutation specific primers. Multiple primers of different lengths were tested to determine the best parameters and results of qPCR trials show that the novel SSB protein primer blocker was able to selectively amplify mutant DNA when connected to the medium length (18 bp) primer, specifically in 1.0uM and 2.0uM concentrations. The RT-qPCR charts also showed that the controls (water, just oligo, or just protein) did not show any selective amplification.

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