

Ultrasensitive detection of DNA based on three-way junction structure-induced isothermal amplification reaction

이서영, 장효원, 박현규[†]
한국과학기술원
(hgpark@kaist.ac.kr[†])

Herein, we describe a three-way junction (3WJ) structure-induced isothermal amplification (ThIsAmp) reaction for DNA detection. In this strategy, specially designed ThIsAmp template induces the formation of 3WJ-structure by associating the employed ThIsAmp primer but only in the presence of target DNA. Upon the formation of 3WJ-structure, the ThIsAmp primer is extended followed by nicking reaction by the activities of DNA polymerase and nicking endonuclease, consequently producing a large number of trigger strands. The produced trigger strand then promotes the amplification of the double-stranded DNA (dsDNA) products through two interconnected pathways. Finally, the produced dsDNA products can be monitored in real-time by detecting the fluorescence intensity resulting from SYBR Green I staining. Based on this strategy, we successfully detected target DNA with high specificity and sensitivity with a low detection limit of 78.1 aM. In addition, the diagnostic capability of this strategy was successfully demonstrated by reliably detecting normally long target DNA.