Ultrasensitive isothermal amplification method based on nicking and extension chain reaction system for RNA detection

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We herein developed an ultrasensitive version of nucleic acid sequence-based amplification (NASBA) called Nicking and Extension chain reaction System-Based Amplification (NESBA). The NESBA primer set employed in this technique was designed by incorporating nicking site at the 5'-end of NASBA primer set containing T7 promoter region. The use of NESBA primer set would integrate nicking and extension chain reaction into conventional NASBA system and enable the exponential amplification of T7 promoter-containing double-stranded DNA (T7DNA) through the continuously repeated nicking and extension chain reaction catalyzed by nicking endonuclease (NE) and reverse transcriptase (RT). The amplified T7DNAs are then transcribed to produce a large amount of RNA amplicons which could generate high fluorescence signal through binding to fluorescent molecular beacon probes. With this strategy, we successfully determined the respiratory syncytial virus A (RSV A) genomic RNA (gRNA) with much higher sensitivity than that of conventional NASBA method.