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## Urinary exosomal mRNA detection using novel isothermal gene amplification method based on three-way junction

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### Abstract

Exosomal messenger RNA (mRNA) has emerged as a valuable biomarker for liquid biopsy-based disease diagnosis and prognosis due to its stability in body fluids and its biological regulatory function. Here, we report a rapid one-step isothermal gene amplification reaction based on three-way junction (3WJ) formation and the successful detection of urinary exosomal mRNA from tumor-bearing mice. The 3WJ structure can be formed by the association of 3WJ probes (3WJ-template and 3WJ-primer) in the presence of target RNA. After 3WJ structure formation, the 3WJ primer is repeatedly extended and cleaved by a combination of DNA polymerase and nicking endonuclease, producing multiple signal primers. Subsequently, the signal primers promote a specially designed network reaction pathway to produce G-quadruplex probes under isothermal conditions. Finally, G-quadruplex structure produces highly enhanced fluorescence signal upon binding to thioflavin T. This method provides a detection limit of 1.23 pM (24.6 amol) with high selectivity for the target RNA. More importantly, this method can be useful for the sensing of various kinds of mRNA, including breast cancer cellular mRNA, breast cancer exosomal mRNA, and even urinary exosomal mRNA from breast cancer mice. We anticipate that the developed RNA detection assay can be used for various biomedical applications, such as disease diagnosis, prognosis, and treatment monitoring.

Keywords : Exosome; RNA; Isothermal gene amplification; Three-way junction; G-quadruplex; Cancer diagnosis

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