**Advancing SNV Diagnostics: Evaluating the efficiency of LNA-Tailored DNA Nanomachines**

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Nucleic acid detection represents a crucial area of molecular biology, with numerous methodologies developed to identify specific DNA sequences. Double-stranded DNA (dsDNA) detection offers distinct advantages, such as enhanced stability and feasibility. Conventional approaches for detecting dsDNA, however, face limitations. These include reliance on temperature changes or denaturation processes, which may compromise the practical utility of molecular probes—essential tools in genetic studies. Recent innovations in molecular techniques have further refined dsDNA detection capabilities, enabling its application in diverse environments, even within live cellular systems.

DNAzymes are synthetic catalytic single-stranded DNA-molecules capable of performing enzymatic reactions, such as substrate cleavage. The 10-23 DNAzyme consists of a conserved 15-nucleotide catalytic core that can be splitted and flanked by two substrate-binding arms and target-binding arms, forming a binary DNA-nanosensor. These arms hybridize to complementary regions of the target substrate, positioning the catalytic core for site-specific cleavage of a Fluorescent substrate molecule (F-sub). This releases the fluorophore from its quencher, producing a fluorescent signal [1].

 An improvement of the binary DNA-nanosensor with two additional outer arms is called DNA-nanomachine (DNM). Such a design possesses higher sensitivity and ability to work with complex secondary structures. In this study, the DNM’s two central binding arms form the catalytic core, while the two outer arms are modified with Locked Nucleic Acids (LNA). Incorporating LNAs into the DNAzyme’s binding arms enhances its thermal stability and hybridization specificity [2]. The rigid ribose conformation of LNAs increases the probe’s melting temperature (Tm), ensuring binding to dsDNA even at physiological temperatures (e.g., 37°C). This stability enables future cell-culture testing and FISH applications. Furthermore, LNA modifications reduce off-target interactions, improving the sensor’s accuracy in single-nucleotide variation (SNV) detection.

During this study, LNA-modified DNA sensors with optimized component lengths were developed for SNV detection, and a novel approach was designed to evaluate DNM efficiency in targeting dsDNA amplicons containing single-nucleotide mismatches.

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**Literature**

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