Секция «Молекулярная биология»

THE AMPLIFICATION FREE DETECTION OF THE NOVEL CORONA VIRUS IN THE CLINICAL SAMPLES

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Abstract:

In spite of the superb sensitivity of the binary Deoxiribozimes in comparison with molecular beacons and different hybridization sensors, it's still not enough for a successful viral diagnosis approach because of the insufficient Limit of Detection. In this study, we present different evolved Deoxiribozime-based apparatuses with LOD 1 and 0.1 pM. In an attempt to enable the competition with the real-time PCR.

Due to the fact that Ribonucleic acid-based viruses are highly mutable. Therefore, it infrequently directs to more dangerous symptoms. The earlier the virus is detected the better epidemiological control and safe recovery are achieved. Yet, it is challenging due to the cost and sophistication of the detection procedure. These days, RT-qPCR is typically used in viral detection and especially the Novel coronavirus. This approach is entrusted and admirable but still demands complex equipment and pricey reagents.

Thanks to DNA-Nanotechnology we could develop an advanced generation DNA-apratus that can be the light of hope to open the door for transforming diagnostics of viral diseases to be much simpler and with less cost. Deoxiribozimes are demonstrating promising evolution and improvement in the past years they evolved from a single detection probe to the Bi-Deoxiribozime [2]. The Binary DZ sensor consists of two separate strands that find and bind to a certain region in the viral RNA and initiate the catalytic core to cleave a fluorogenic reporter substrate (F-sub) after binding. The binary DNAzyme is not only giving a signal but also can amplify it by cleaving several substrates by binding to one viral RNA molecule. In addition, to the high selectivity of RNA identification compared to other probes. Nonetheless, Bi-DZ has drawbacks with the sensitiveness over the long RNA molecules due to the self-folding feature in the RNA analytes. We overcame this Drawback by transforming the Bi-DZ into different advanced DNA apparatus by joining several binding arms and (or) cores together to a shared double-stranded DNA platform. These Different DNA apparatuses can unwind the folded RNA secondary structures.

Results:

The different DNA- apparatuses proved successfully enhanced sensitivity, with a limit of detection below 1 pM, which is 20 times lower than that of Binary Deoxyribozyme and other apparatus with LOD 0.1 pM which is less than 200 times than the Bi-DZ[1]. Significant selectivity was confirmed, This directed us to the successful viral detection directly in the clinical samples with no any amplification steps.

References

1) Gerasimova Y.V., Cornett E., Kolpashchikov D.M. RNA-Cleaving Deoxyribozyme Sensor for Nucleic Acid Analysis: The Limit of Detection // Chembiochem Eur. J. Chem. Biol. – 2010. – Vol. 11. – № 6. – P. 811–729.

2) Kolpashchikov D. M. Evolution of Hybridization Probes to DNA Machines and Robots // Accounts of Chemical Research. 2019. № 7 (52). С. 1949–1956.

Illustrations

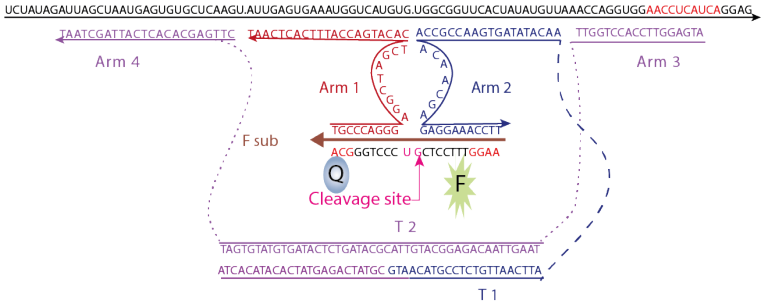

Рис. 1. The 1st generation of the advanced DNA apparatuses with 4 Arms DNA Machine consist of 3 main parts the separated Arm 1, T1, and T2. T1 contains arm 2 while T2 has 2 arms (3 and 4) both are connected with spacers to Tile 2. Both T1 and T2 are complementary to each other to form a double stranded platform that holds all the three arms together.

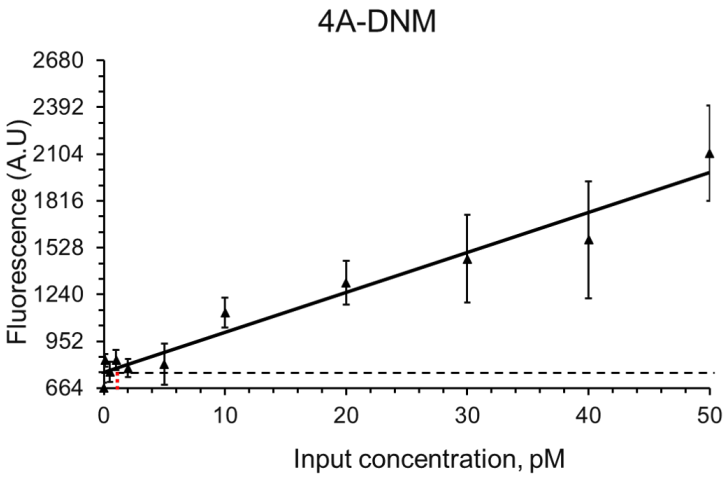

Рис. 2. Limit of detection of 1st generation of the Advanced DNA apparatuses, the reaction was incubated at 55oC for 3 h, followed by fluorescence spectrophotometry recording and analyzing. The data are average values of three independent experiment.