DNA NANOSWITCH STRATEGY, A DEVICE-FREE PLATFORM FOR VIRAL RNA DETECTION

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Credible leads to Incredible

Abstract

The emergence of a highly transmissible novel coronavirus in 2019 led to an unprecedented need for widespread diagnostic testing. Challenges that followed, including reagent shortages, high costs, deployment delays, and prolonged turnaround times, underscored the need for an alternative set of affordable tests. Here, we investigated DNA nanoswitch, a nonenzymatic system for detecting viral RNA. The DNA nanoswitch is constructed using DNA self-assembly techniques, formed by hybridizing short oligonucleotides that are complementary to a single stranded M13mp18 DNA scaffold. Two distant "detector" strands were designed to contain overhangs complementary to the target viral sequences. Recognition and binding of the target RNA region reconfigure the switch from the linear "Off" state to the looped "On" state. The two states can be quantified using standard agarose gel electrophoresis. In this study, we constructed a DNA nanoswitch using SARS-CoV-2 specific detectors. Optimal conditions for annealing were determined, with the highest band intensity observed at room temperature and using Tris-Mg annealing buffer. The nanoswitch detected target RNA within 5 minutes, stabilizing after 60 minutes. Additionally, nanoswitches for detecting SARS-CoV-2 nucleocapsid and HIV-1 LTR RNAs were assembled. Specificity was confirmed through cross-testing, demonstrating the ability to detect the respective RNAs using the opposite nanoswitch. The systems showed high sensitivity, detecting target RNA down to 1.5pg with the SARS-CoV-2 nanoswitch. The DNA nanoswitch technology allows for the detection of viral RNA without the necessity of PCR amplification, labeling, or enzyme utilization. The assay involves minimal steps, rendering it suitable for diverse clinical or laboratory settings. This assay provides a third option between amplification-based RNA and viral antigen detections.

Method

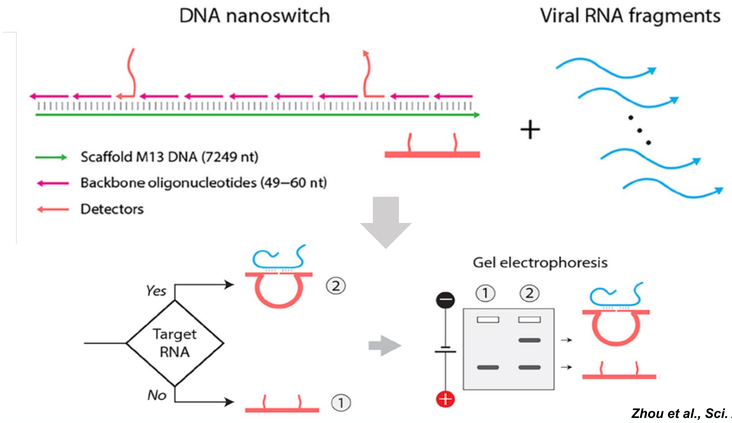
(1) DNA Nanoswitch construction:

- Linearization of genomic ssDNA (NEB, M13mp18) through targeted cleavage using a restriction enzyme, BtsCI.
- Mix the linearized ssDNA with a molar excess of a 121 short oligonucleotide mixture complementary to a single-stranded DNA scaffold.
- Mixed with two distant "detector" strands designed to contain overhangs complementary to different segments (typically halves) of the target viral RNA sequence and annealed from 90° to 20°C at 1°C/min.

(2) Following construction, RNAs of 30 nucleotides long targeted spike (T9) (provided by Dr. Ken), in addition, the RNAs targeted SARS-CoV-2 nucleocapsid (Nuc) and HIV-1 LTR were synthesized by Genscript. DNA nanoswitch and target RNA were mixed and incubated at different time points and conditions.

(2) DNA Agarose gel detection at 75 V for 45 min.

DNA nanoswitch strategy for viral RNA detection



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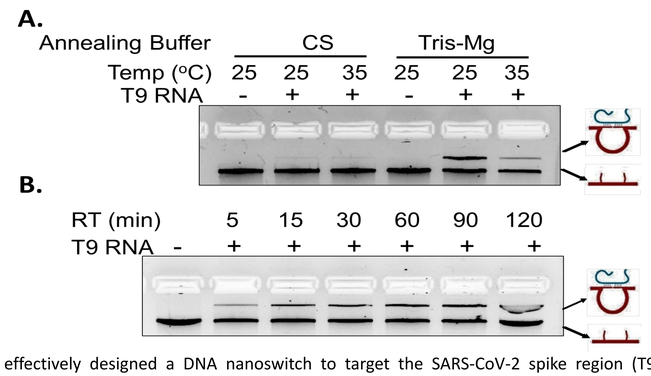
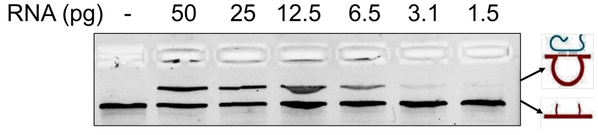
Results

Fig. 1. Condition optimization of DNA nanoswitch viral RNA detection



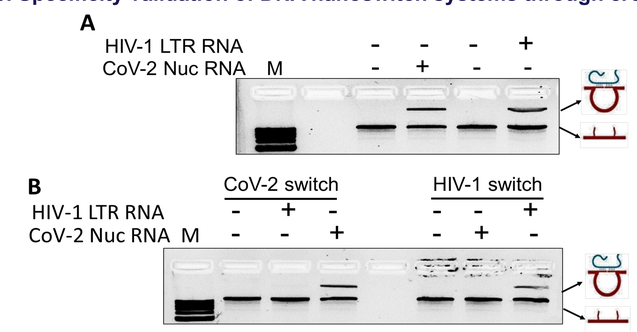
This study effectively designed a DNA nanoswitch to target the SARS-CoV-2 spike region (T9). Optimized annealing conditions showed notable loop formation when incubated with target RNA at RT in Tris-Mg buffer, observable within 5 minutes and stabilizing within 60 minutes.

Fig. 2. High sensitivity detection of target RNA by DNA nanoswitch



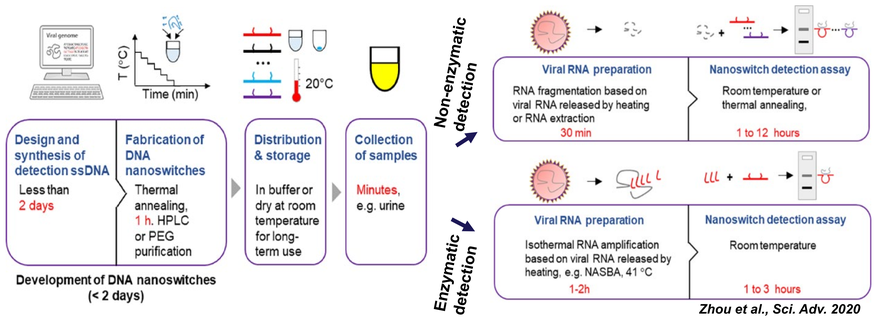
The DNA nanoswitch system demonstrates the capability to detect target RNA as low as 1.5 pg.

Fig. 3. Specificity validation of DNA nanoswitch systems through cross-testing



DNA nanoswitches were constructed for SARS-CoV-2 and HIV-1, targeting Nuc and LTR, respectively, with specificity confirmed through cross-testing.

Fig. 4. The Development cycle for DNA nanoswitch-based detection of viral RNAs



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DNA nanoswitches have demonstrated and validated the ability to swiftly detect viral RNA in human samples. The method is fundamentally nonenzymatic but can be supplemented with an isothermal amplification process, making it suitable for deployment in regions with limited resources.

Conclusions

The DNA nanoswitch technology offers a cost-effective, efficient means of detecting viral RNA with high precision and adaptability, potentially revolutionizing viral detection and control efforts, with applicability spanning from clinical diagnostics to surveillance of emerging viral threats.

Acknowledgements

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References

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