Enhanced detection of SARS-CoV2 RNA species using signal amplification technologies in the context of DNA duplexes and DNA-RNA hybrids.

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Accurate detection of nucleic acids from certain biological pathogens is essential for the diagnosis of human disease. However, amplified detection of RNA molecules from a complex sample by direct detection of RNA:DNA hybrids remains a challenging quest. Here we explored the potential of a particular type IIS restriction endonuclease (RE) to digest DNA duplexes and DNA:RNA hybrids when assisted by a custom fluorescent sensing hairpin oligonucleotide. As a proof of concept, we designed a battery of sensing oligonucleotides against specific regions the of SARS-CoV-2 genome and interrogated the role of this RE as a potential nicking enzyme for fluorescent signal amplification. Interestingly, RE-assisted digestion of SARS-CoV2 probes increased the detection signal of ssDNA and RNA molecules and decreased the limit of detection by more than 3.5 fold (10 fmol) as compared to conventional molecular beacon approaches. The cleavage reaction is highly specific and can be multiplexed to facilitate the simultaneous detection of multiple human B-coronaviruses. We are currently coupling this signal amplification system with an array of isothermal amplification techniques to improve their sensitivity and specificity and overcome the inherent background detection problems associated with the use of these technologies.

FIGURES

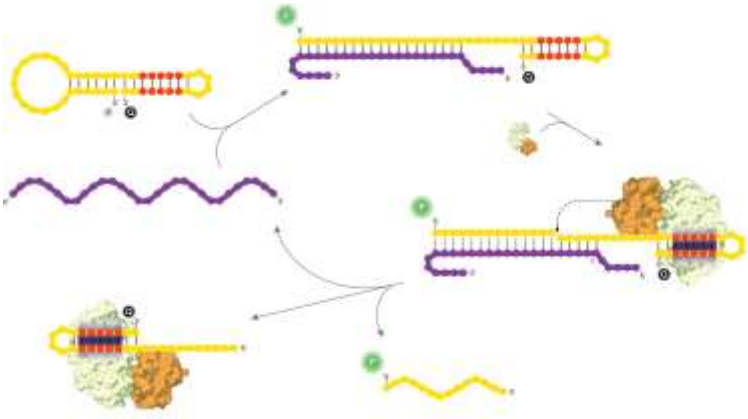

Figure 1: Working principle of Type IIS RE-assisted digestion of nucleic acids. Schema depicts the different stages of the signal amplification reaction. In the presence of a target molecule (purple), the custom hairpin oligonucleotide (yellow) triggers the hybridization step. In the presence of the RE, a feedback loop is activated, resulting in an enhancement of the fluorescent signal of the assay.