INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Pathogen Identification Using Isothermal Amplification And Next-generation Sequencing Technologies

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The ability to characterize and respond to novel emerging threats requires a multifaceted approach including both primary detection and prolonged routine testing capabilities. Primary detection ideally involves agnostic or massively multiplexed technologies that, upon identification, would be replaced with cheaper, easy-to-use, target specific capabilities. Currently, this is because primary agnostic approaches are more complicated, expensive, and less sensitive than target specific approaches and, during an outbreak scenario, are no longer required since the pathogen of interest has been identified. Ideally, a single platform that performs both tasks, as well as others, would be fielded to limit reagent, equipment, and protocol burdens on the operator. Defense Threat Reduction Agency (DTRA) funded projects, such as Far-Forward Advanced Sequencing Technology (FFAST), have already demonstrated the potential for field forward agnostic and targeted sequencing approaches on the same platform detecting numerous pathogens of consequence. However, these approaches are not conducive to routine high throughput sample testing requiring secondary devices, protocols, and reagents to fill that critical gap.

Utilization of isothermal amplification approaches to detect pathogens has become a backbone of rapid molecular detection technologies such as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) and lateral flow technologies to name a few. Isothermal amplification methods can include simple colorimetric or fluorescent readouts; however, spurious background amplification results in increased false positives and decreased sensitivity. Here we demonstrate the combination of isothermal amplification approaches and next generation nanopore sequencing developing a rapid protocol with sample to answer times less than that of real-time PCR. Utilizing sequencing as a readout for isothermal amplification serves several purposes including 1) significant reduction in background noise 2) increased multiplex capabilities and 3) specific amplification/detection of genetic variants of interest. Importantly, the combination of these approaches affords the operator a universal platform capable of multiple outputs including primary agnostic detection and routine detection of known agents. Taken together the transition and development of protocols that can build on established sustained technologies and is adaptable to multiple use cases is essential especially in forward operations where resources are limited.