INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Development And Validation Of Icecap (Immobilized CRISPR Enriches Captured Target Pathogens) For On-demand Pathogen Detection

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The field of CRISPR-based molecular detection is experiencing rapid expansion, yet it remains largely reliant on a limited array of established chemistries. Like traditional PCR-based detection assays, these CRISPR-based systems can take weeks to months to develop and transition new assays onto closed platform devices. However, as Ebolavirus, SARS-CoV-2 and Mpox virus outbreaks have shown, novel, emerging, and re-emerging pathogens present an ever-evolving challenge. In this study, we present a novel chemistry, termed Immobilized CRISPR Enriches Captured Target Pathogens (ICECAP), that can be rapidly reconfigured on open platforms to accommodate new pathogens and/or new sequence variations within one week of discovery. ICECAP employs a "dead" Cas enzyme coupled with target-specific gRNA to selectively capture genomic targets of interest. Subsequently, it utilizes an innovative CRISPR-based fluorescent detection method to identify the captured targets. Herein, we detail the optimization process of ICECAP chemistry and provide validation data for a subset of high consequence targets, encompassing viruses, bacteria, antimicrobial resistance factors, pathogen variants, biothreats, and both endogenous and exogenous controls. Our findings underscore ICECAP's novelty and viability as a molecular detection chemistry.

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