INNOVATING CROSS-DOMAIN SOLUTIONS TO DETECT EMERGING BIOLOGICAL THREATS

Streamlining Nanopore Sequencing Protocols For Untargeted Rna Virus Identification In The Field

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RNA sequencing in the field is a difficult and time-consuming process despite recent advancements in portable sequencing technology. RNA extraction requires the use of hazardous reagents, a large number of consumables, and lengthy protocols for cDNA conversion involving the use of thermocyclers with larger power requirements. To improve accessibility of RNA virus sequencing, and potentially allow for field-ready capabilities, these roadblocks need to be alleviated. Previous work in our laboratory demonstrated an alternative protocol that decreased total preparation time to under 90 minutes and utilized safer reagents. This new approach utilizes a magnetic bead-based process for RNA extraction, with modifications to replace more hazardous reagents and reduce incubation times and steps. This adjusted protocol also eliminates the need for a microcentrifuge that is required for spin column-based RNA extraction kits. To make RNA sequencing more field-friendly, even to inexperienced users, we modified the protocol to utilize syringes and remove the need for pipettors. Fieldable PCR devices were introduced to allow for necessary temperature-dependent incubation steps without the need for a large power source. With the use of the previously streamlined protocol, the introduction of a syringe-based process, as well as small low-power devices, we have developed a fieldable untargeted RNA sequencing system. This system was successfully tested in the field at Dragon Spear Research, Development, and Acquisition Experiment (RDAX) 23 to obtain feedback from SOCOM operators on usability in realistic scenarios.

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