CRISPR-Assisted Solid-State Nanopore Sensor for Rapid and Sensitive Point-of-Care amendable of Monkeypox Virus Detection Via RPA Amplification

*Md. Ahasan Ahamed, and Weihua Guan,\**

The Monkeypox virus (Mpox) represents a significant global health concern, requiring immediate, straightforward, and precise identification to control its transmission effectively. The Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) method has recently gained traction as a cutting-edge tool for molecular diagnostics for its high specificity. Here, We have developed a method that combines Solid-state CRISPR-Cas12a Assisted Nanopore with isothermal recombinase polymerase amplification (RPA-SCAN) to achieve high sensitivity and specificity in detecting Mpox. The RPA-SCAN method can detect the Mpox virus more sensitively than unamplified SCAN, and it overcomes the challenges of using PCR-SCAN for point-of-care testing. We show that we can use size-counting of single molecules to analyze how the cleaved reporter is distributed over time during the reaction. Our RPA assay for Mpox achieves a detection limit of 19 copies in a 50 μl reaction system. The SCAN sensor determines that incorporating 2 μl of RPA amplified samples into a 20 μl CRISPR reaction yields a detection capacity of 16 copies/μl (26.56 aM) for MPOX with a 95% confidence interval. We also established that RPA-SCAN accurately differentiates MPOX from the cowpox virus without any errors, indicating 100% specificity. These results indicate that the isothermal RPA-SCAN device offers high sensitivity and specificity for MPOX detection. With its electronic capabilities and potential for miniaturization, the RPA-SCAN system provides a platform for diagnosing various infectious pathogens at the point of care.