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NOVEL STRATEGY FOR THE SEQUENCING AND DISCOVERY OF CIRCULAR DNA VIRUSES

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The emergence of COVID-19 and recent zoonotic outbreaks highlight the need of early pathogen detection, thus the development of quick and reliable diagnostic strategies. In this study, we developed an agnostic and cost-effective unbiased sequence-independent enrichment (USIE) protocol for the complete genome sequencing of circular DNA viruses.

DNA extracts from different hosts and biological samples are enriched by multiply primed rolling circle amplification (RCA). RCA products are debranched using T7 endonuclease and used as input for libraries and sequencing by Oxford Nanopore Technology™. The generated reads are analyzed using different metaviromic tools and customized pipelines.

Thus far, this diagnostic strategy has been successfully used for the complete genome sequencing and discovery of the following viruses; hepadnaviruses in domestic dogs (Diakoudi et al, 2022), Iberian lynxes (Diakoudi et al., 2025), and passerine birds (unpublished data), emerging papillomaviruses in horses (unpublished data), several CRESS DNA viruses in cats (Vasinioti et al., 2023), squamates (Capozza et al, 2022), Iberian lynxes (Castro-Scholten et al., 2024), and wolves (unpublished data), and avian polyomaviruses (unpublished data). Overall, the USIE protocol is a host- and tissue-independent strategy that can be used to sequence circular DNA viruses. Updating and implementing the diagnostic algorithms is crucial for the effective prevention and control of emerging and re-emerging viruses.

Keywords

Circular DNA viruses, sequencing, diagnostics, USIE, virus discovery

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