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Overcoming limitations in the sequencing of whole viral genomes

The identification and analysis of pathogenic viruses, especially the Ebola virus, has recently received significant attention. The sequencing of newly identified viral genomes has presented historical challenges as existing technology fails to capture the 3’ and 5’ terminal ends of the viral genome. The authors described a new technique based on next-generation sequencing to capture and sequence the full genome of positive and negative single-stranded RNA viruses. The new method involves RNA extraction and removal of background mRNA, rRNA, and DNA followed by random fragmentation of the relatively large viral RNA using divalent cations. The fragmented RNA termini are cleaned up using phosphatase and kinase treatment, and 3’ adapters followed by 5’ adapters are added to the RNA. After ligation, reverse transcription is performed, followed by polymerase chain reaction amplification and sequencing using an Illlumina MiSeq instrument. The authors tested the new methodology on a positive-stranded RNA virus (hepatitis C virus) and a negative-stranded RNA virus (Ebola virus). They included an assessment of sequencing of both the unstructured termini in the Ebola virus and the more highly structured termini in the hepatitis C virus. Testing of a number of conditions showed that effective sequencing of the terminal ends could be achieved while ensuring a good depth of coverage across the entire viral genome. The ability to more fully sequence viral genomes, including capture of the terminal ends, within approximately 12 hours makes the new technology relevant to virus discovery, viral evolution, and antiviral resistance.

Alfson KJ, Beadles MW, Griffiths A. Overcoming limitations in the sequencing of whole viral genomes. *J Virol Methods.* http://dx.doi.org/10.1016/j.jviromet.2014.07.023.

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