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Identifying and discriminating of the non-coding regions of influenza A virus vRNA, cRNA and mRNA

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Abstract

Influenza A virus noncoding regions (NCRs) have been shown to play critical roles in replication and transcription. Although the NCR sequences are conserved, variations have been noted between strains. Previous methods have reported successful cloning and sequencing of influenza A virus gene segment NCRs using T4 RNA ligase-based approaches. In this study, a method employing poly(A) addition and SMART (switching mechanism at 5' end of RNA transcript) technology is described for directly cloning and sequencing both NCR ends of viral RNA (vRNA), complementary RNA (cRNA), or NCR and cap sequences from viral mRNA. This method may also be used to characterize the NCRs of influenza A virus samples in which the RNA has been degraded. During this study, polymorphisms of the first nucleotide of the IAV genome were identified at the 3′ vRNA NCRs of these representative viruses.