

Virus identification and discovery using metagenomics

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Technological innovations in DNA sequencing have increased the rate of viral discovery. The same technologies can be applied to detecting adventitious viruses in biological products.

Viral metagenomics provides several advantages over more traditional methods of viral discovery. Classical methods have typically relied on cell culture replication followed by nucleotide hybridization, PCR or antigenic cross reactivity. Cell culture amplification requires that a susceptible cell line be available. While a large panel of cell lines may be used, this slow and expensive approach consumes large volumes of clinical samples, without guarantee of viral replication. Furthermore replicating viruses do not always induce CPE and even for known viruses in vitro replication is still not possible. Consensus PCR targeting related viruses based on highly conserved genomic regions has also been successful but the genome of distantly related viruses may not show cross-hybridization. Similarly, antibodies able to cross-react to closely related virus may not bind to antigens from different species, much less to different genus. The very large and growing number of human viral species and genera also makes PCR or antibody cross reactivity based methods impractical. Microarrays while highly effective for identifying known viruses require a high level of nucleotide similarity over the oligonucleotide probes used and will also fail to detect highly divergent viruses. Viral metagenomics by analyzing randomly amplified and massively sequenced nucleic acids followed by similarity searches of in silico translated proteins against all known viral proteomes allows the detection of more highly divergent novel viruses than nucleotide hybridization based methods.

We have developed methodologies for the synthesis of viral DNA and RNA libraries for 454 pyrosequencing and developed a bioinformatics pipeline dedicated solely to the identification of known and novel viruses. Several examples of viral discovery and detection of contamination by PCV2 in a live attenuated vaccine will be shown. The general applicability and limitation of viral metagenomic approaches will be discussed.