Virus isolation and sequencing

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Abstract

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Protocol

Step 1.
Viruses were isolated from cultured rhabdomyosarcoma (RD) cells and human epidermoid cancer cells (Hep-2) cells.

Step 2.
After the cells were inoculated with virally contaminated samples, they were cultured at 37°C and observed for three passages.

Step 3.
3.When the cytopathic effect (CPE) affected 75–100% of the cell monolayer, the virus was harvested. It needed to pass three generation when no CPE was observed in the initial cell culture.

Step 4.
Collected viral particles were analyzed using RT-PCR and the complete VP1 gene sequences from the EV71 and CA16 isolates were amplified.

Step 5.
The amplified products were sent to Sangon Biotech Co., Ltd (Shanghai, China) for DNA Sanger sequencing.