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Sequencing and sequence analysis

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MATERIALS

NAME	CATALOG #	VENDOR
BigDye™ Terminator v3.1 Cycle Sequencing Kit	View	Applied Biosystems

MATERIALS TEXT

Plasmid from original isolate and series of propagated isolates were subjected to DNA sequencing on an automatic sequencer, ABI PRISM 3730*x*/Genetic Analyzer (Applied Biosystems, USA) using the BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, USA). Sequencing was conducted at least three times to obtain consensus sequences using universal primers, T7 promoter and M13R_pUC26 for both direction [Norfitriah et al., 2018]. The sequence was verified as fowl adenovirus Group E based on NCBI BLAST GenBank. Nucleotide sequences were assembled, edited and analyzed using BioEdit Version 7.2.5. Sequence was translated into amino acid sequences using online ExPASy tool program. Multiple sequence alignment was carried out using ClustalW program in BioEdit version 7.2.5 package to compare nucleotide and deduced amino acids sequence between original isolate (E2) and propagated isolates in CEL cells. The E2 FAdV designated as UPM1137E2 with GenBank accession number KF866370 (hexon) and KY305950 (fiber). Sequence difference count matrix was carried out to calculate nucleotide and amino acid changes between each passage before and after attenuation. In addition, location for variable L1 loop hexon gene and knob region in fiber gene was identified by alignment with reference FAdV gene, HG strain [Grgic et al., 2011]. L1 loop of hexon gene corresponding to residue 101 to 298 of HG strain, while for fiber gene as follows: Tail: 1-75, Shaft: 76-356, Knob: 369-523 [Grgic et al., 2014].