OCT 06, 2019

OPEN ACCESS

DOI:
[dx.doi.org/10.17504/protocols.io.7zehp3e](https://doi.org/10.17504/protocols.io.7zehp3e)

External link:
<https://doi.org/10.1371/journal.pone.0225863>

Protocol Citation: Norfitriah Mohamed Sohaimi, Mohd Hair Bejo, Abdul Rahman Omar, Aini Ideris, Nurulfiza Mat Isa 2019. Sequencing and sequence analysis. **protocols.io** <https://dx.doi.org/10.17504/protocols.io.7zehp3e>

MANUSCRIPT CITATION: Sohaimi NM, Bejo MH, Omar AR, Ideris A, Isa NM (2019) Molecular characterization of fowl adenovirus isolate of Malaysia attenuated in chicken embryo liver cells and its pathogenicity and immunogenicity in chickens. PLoS ONE 14(12): e0225863. doi: [10.1371/journal.pone.0225863](https://doi.org/10.1371/journal.pone.0225863)

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Sequencing and sequence analysis

PLOS One

Norfitriah Mohamed Sohaimi¹, Mohd Hair Bejo¹, Abdul Rahman Omar¹, Aini Ideris¹, Nurulfiza Mat Isa²

¹Faculty of Veterinary Medicine, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.;

²Faculty of Biotechnology and Biomolecular Sciences, Universiti Putra Malaysia, Serdang, Selangor, Malaysia.

Norfitriah Mohamed Sohaimi

MATERIALS

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BigDye™ Terminator v3.1 Cycle Sequencing Kit **Applied Biosystems**

Plasmid from original isolate and series of propagated isolates were subjected to DNA sequencing on an automatic sequencer, ABI PRISM 3730x/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].

Protocol status: Working
We use this protocol and it's
working

Created: Oct 06, 2019

Last Modified: Oct 06, 2019

PROTOCOL integer ID:
28422