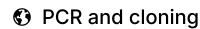


Oct 06, 2019



PLOS One

DOI

dx.doi.org/10.17504/protocols.io.7zfhp3n

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

Create & collaborate more with a free account

Edit and publish protocols, collaborate in communities, share insights through comments, and track progress with run records.

Create free account

OPEN ACCESS



DOI: https://dx.doi.org/10.17504/protocols.io.7zfhp3n

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. PCR and cloning. protocols.io https://dx.doi.org/10.17504/protocols.io.7zfhp3n



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

License: This is an open access protocol distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited

Protocol status: Working

We use this protocol and it's working

Created: October 06, 2019

Last Modified: October 06, 2019

Protocol Integer ID: 28423

Keywords: pcr

Materials

MATERIALS



TOPO™ TA Cloning™ Kit, with pCR™ 2.1-TOPO™, One Shot TOP10 Chemically Competent E. coli, and PureLink™ Quick Plasmid Miniprep Kit Thermo Fisher Catalog #K450002

Amplification of DNA was performed by two set of published hexon gene primers, H1/H2 and H3/H4 [Raue and Hess, 1998] and fiber gene primer, FibF/FibR, FibF: 5'-GGTCTACCCCTTTTGGCTCC-3' and FibR: 5'-GCGTCGTAGATGAAGGGAGG-3` [Norfitriah et al., 2018] according to manufacture protocol (Bioline, UK). The PCR products were analyzed by electrophoresis in a 1% agarose gel stained with RedSafe™ Nuclei Acid Staining solution (iNtRON, Korea) at 70 volts for 45 minutes and visualized under U.V. transillumination. Purification of PCR products were carried out by using MEGAquick-spin™ Total Fragment DNA Purification kit (iNtRON) based on the manufacture recommendation. Purified PCR products from H1/H2 and FibF/FibR were cloned into the pCR™ 2.1-TOPO® vector using TOPO TA Cloning kit (Invitrogen, USA). Positive cloned was analyzed by colony-PCR prior plasmid extraction using DNA-spin™ Plasmid DNA Purification kit (iNtRON) and stored at -20°C until used.

Troubleshooting

