CLONING AND EXPRESSION OF FUSION (F) PROTEIN GENE OF NEWCASTLE DISEASE VIRUS IN EUKARYOTIC EXPRESSION SYSTEM

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CERTIFICATE

This is to certify that the thesis entitled **"CLONING AND EXPRESSION OF FUSION (F) PROTEIN GENE OF NEWCASTLE DISEASE VIRUS IN EUKARYOTIC EXPRESSSION SYSTEM"** submitted in partial fulfillment for the requirement of the degree of **MASTER OF VETERINARY SCIENCE** in **ANIMAL BIOTECHNOLOGY** to the Tamil Nadu Veterinary and Animal Sciences University, Chennai, is a record of bonafide research work carried out by **MANJUNATH.S.** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma, fellowship or the other similar titles or prizes and that the work has not been published in part or full in any scientific or popular journal or magazine.

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ABSTRACT

Title	:	**Cloning and Expression of Fusion (F) Protein Gene of Newcastle Disease Virus in Eukaryotic Expression system.**
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The present study was carried out to clone and express the fusion protein gene of Newcastle disease virus (2K3) in eukaryotic expression system and to characterize it by SDS-PAGE and western blotting.

The Newcastle disease virus (2K3) isolate from pigeon was propagated in embryonated chicken eggs and the infected allantoic fluid was collected for RNA isolation. The c-DNA was synthesized using RNA as a template by reverse transcription polymerase chain reaction (RT-PCR). The fusion gene was amplified with c-DNA as template using specific primers with restriction sites *Nco*I in forward primer and *Xho*I in reverse primer. The amplicon of 1.68 Kb was gel purified and cloned into TOPO pCR 2.1vector and the clone was confirmed by colony PCR, restriction digestion and sequencing. The pTriEx Neo1.1 expression vector was linearised with restriction enzymes *Nco*I and *Xho*I. The fusion protein gene released from the recombinant TOPO pCR 2.1 after digestion with restriction enzymes was ligated with linearised pTriEx vector using T_4 DNA ligase. The ligation reaction was transformed into *E.coli* DH5α cells using ampicillin as selectable marker and the recombinant colonies were identified by colony PCR. Further the clone was confirmed by digestion of the recombinant pTriEx plasmid with *Nco*I and *Xho*I enzymes. The recombinant

pTriEx colony was induced with 1 mM Isopropyl-1-thio-β-D-galactosidase (IPTG) to analyse the expression of the fusion protein in prokaryotic host (BL21DE3). The expressed protein was analysed by using 12% SDS-PAGE. The expressed protein of 55 kDa was obtained with the overnight induced cultures which further reacted with NDV anti serum raised in rabbits by immunoblot analysis showing protein with the molecular weight of 55 kDa.

The recombinant pTriEx plasmid was transfected in Vero cells to analyse the expression of the fusion protein. The cell lysate was collected at 48 hrs and 72 hrs post transfection. The expressed protein was analysed using 12% SDS-PAGE. On SDS-PAGE protein with the molecular weight of 55 kDa was obtained at 48 hrs and 72 hrs post transfection. The protein was further confirmed to be NDV specific, by its immunoreactivity with NDV anti serum raised in rabbits showing a protein with the molecular weight of 55 kDa. The expression of the fusion protein on the transfected Vero cells was further confirmed by immunofluorescence assay (IFA) which exhibited a bright cytoplasmic fluorescence in transfected Vero cells when compared to non transfected cells which did not exhibit any fluorescence which served as compared control.

Key words: *Newcastle disease virus - Fusion protein gene – TOPO cloning – Sub-cloning in pTriEx - Expression in Prokaryotic host – Expression in Eukaryotic host – SDS PAGE – Western Blot – Immunofluorescence Assay.*