VV968 - CLONED NUCLEOPROTEIN (NP) GENE OF AVIAN INFLUENZA VIRUS FOR USING AS A POSITIVE CONTROL IN SYBER GREEN I REAL TIME RT-PCR (RRT-PCR)

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RRT-PCR has been extensively adopted for the diagnosis of AIV infection, due to high sensitivity and rapidity, and the occurrence of low cross contamination, compared to conventional RT-PCR technique. However, the RRT-PCR has the potential for false-negative results, which may be caused by the several factors, such as the presence of RT-PCR inhibitors, low RNA recovery after purification process, degradation of RNA, poor optimization of this reaction. Thus, the presence of a positive control could benefit the RRT-PCR performance by providing positive signal and to determine a standard curve of SYBR Green I real-time PCR. The purpose of this study is to clone the NP gene of H4N2 strain of AIV in Escherichia coli, in order to use the plasmid with this insert as target in RRT-PCR. A region of 1180 bp of NP from subtype H4N2 of AIV gene were amplified by PCR from cDNA of AIV. The PCR product was cloned into pGEM T-easy vector (PROMEGA) for subsequent transformation of chemically competent DH10B E. coli. The positive clones carrying the NP insert were identified by nucleotide sequencing. After purification and quantitation, plasmid DNAs were seeded into SPF biological samples, such as allantoic fluid, and cloacal swabs, which were ten-fold serially diluted to test in RRT-PCR, using primers specific for NP gene. The standard curve displayed a linear relationship between the Ct values and the related numbers of copy of plasmid DNA containing the insert of NP gene of AIV, giving good correlation between Ct values and template concentrations. The detection limits for these AIV seeded samples were approximately of 103 copies of plasmid DNA and the assay appears to be highly reproducible based on standard deviations of the CT values. Thus the inclusion of the plasmid DNA containing the insert of NP gene of AIV can contribute to the standardization of RRT-PCR for the detection of AIV, and could be used to measure the viral load in clinical samples submitted to this technique.
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VV971 - FIRST MOLECULAR DETECTION OF BOVINE KOBUVIRUS IN DIARRHEIC CALVES FROM DAIRY AND BEEF HERDS IN BRAZIL

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