A real-time polymerase chain reaction assay for the quantification of the Aleutian mink disease virus

A.H. Farid and P.P. Rupasinghe
Nova Scotia Agricultural College, Plant and Animal Sciences, Truro, Nova Scotia, B2N 5E3, Canada; hfarid@nsac.ca

Estimation of the Aleutian mink disease virus (AMDV) copy number in infected animals and environmental samples has a wide range of applications. For instance, while AMDV replication persists in some infected mink for a long period of time, the virus becomes latent in others. Estimate of viral copy number at specific time points in the mink is important for determining the dynamics of AMDV proliferation and designing selection strategies for establishing resistant or tolerant lines of mink. To develop and validate a SYBR Green quantitative real-time PCR assay (RT-qPCR) for estimating the AMDV copy number, 1,973 bp PCR products containing the entire VP2 gene (1,944 bp) of a local strain of the virus were ligated into the pGEM-T Easy plasmid. This 4,989 bp construct was propagated in *E. coli* and linearized with the PstI restriction enzyme. Five pairs of primers were designed for a conserved region of the VP2 gene and were tested in a Bio-Rad iQ5 cycler using nine 10-fold serially diluted samples of the purified plasmis-VP2 construct. One of the primer pairs resulted in a linear standard curve in the range of 11 to 11×106 dsDNA per µl of the sample, corresponding to 22 to 22×106 AMDV copies. The standard curve had high efficiency (98%-102%) and accuracy (R_2≥0.98) in multiple runs. Melt curve analysis confirmed specificity of the primer pair. Inter- and intra-assay variability were low when plasma and tissue samples from infected mink were tested in triplicate in multiple runs. This RT-qPCR method is highly sensitive and reproducible for the detection and quantification of AMDV over a 6-log range. Reproducibility of this standard curve was considerably higher than that when 532 bp PCR products of the VP2 gene or the circular plasmid-VP2 construct were used.