6.1 Polymerase Chain Reaction (PCR) Introduction

The polymerase chain reaction technique employs oligonucleotide primers to amplify segments of genes specific for the target pathogen. Reverse transcriptase-PCR (RT-PCR) employs an initial reverse transcription step so that complimentary DNA can be amplified from viral RNA. DNA or RNA is extracted from various fish tissues and laboratory assay products, such as cell culture supernatant containing viral agents, and amplified using forward and reverse primer sets. In some instances for either method, the initial amplified product may be re-amplified using an additional "nested PCR" technique. The DNA products are then visualized by gel electrophoresis. Quantitative PCR (QPCR) and reverse-transcriptase quantitative PCR (RT-QPCR) can be used to quantify copy number of a target pathogen in a sample. QPCR typically employs two oligonucleotide primers and an internal probe dual-labeled with a reporter and quencher dye. Alternatively, QPCR can be performed by using only primers and incorporating a fluorescent dye into the PCR product. Fluorescence produced during the QPCR reaction is detected by a QPCR instrument.

Specific details for sample preparation, DNA/RNA extraction, primers and cycle conditions appear in each chapter under the specific pathogens. However, certain PCR protocols and precautions are pertinent to all assays and these are described in this chapter. The PCR quality assurance and control procedures outlined below are extremely important when performing all PCR assays (Ennis et al. 1990). Further information on QPCR can be found in Bustin et al. (2004).

DISCLAIMER: Mention of specific brands or manufacturers does not warrant endorsement by the U. S. Fish and Wildlife Service, the United States government, and/or the American Fisheries Society. Any comparable instrument, laboratory supply, or reagent may be substituted in this protocol if operation and performance are deemed comparable to the items specified.