3.2.17.2 Quantitative PCR to identify *Nucleospora (Enterocytozoon) salmonis* DNA within Fish Tissue Appendix 2

A. Reference

Protocol targets the *N. salmonis* small subunit (SSU) of the rDNA complex. Protocol is modified from: Badil, S. B., D. G. Elliott, T. Kurobe, R. P. Hedrick, K. Clemens, M. Blair and M. K. Purcell. 2011. Comparative evaluation of molecular diagnostic tests for *Nucleospora salmonis* and prevalence in migrating juvenile salmonids from the Snake River, USA. J. Aquatic Animal Health 23: 19-29.

B. Primer sets

Primer name	**Sequence (5' – 3')**
Ns-332-F	AGGCGTGATTAAAAGAGCGAAGTG
Ns-458-R	ACTTTTTAACTGCAGCATCCACCA
Ns-360-T	6'Fam-CACCTTCGTGTAACGCAATT-NFQ/MGB

C. Quantitative PCR amplification

PCR Reagents	Lot No.	Stock Concentration *	Final Concentration	Volume/Reaction (Total Volume = 12 µl	Volume for ____
Commercial Master Mix**		2X	1X	6 µl	
Forward Primer		50 µM	0.9 µM	0.22 µl	
Reverse Primer		50 µM	0.9 µM	0.22 µl	
Taq Man Probe		10 µM	0.20 µM	0.24 µl	
Water				0.32 µl	-
DNA			10-100 ng	5 µl	
Total				12 µl	

*Change "Stock Concentration" parameters as necessary depending on reagent source.

**Protocol assumes commercial master mix contains uracil-N-glycosylase (UNG).

1. Add PCR reagents except the template DNA into the "Master Mix" tube.
2. Aliquot **7** µl of Master Mix to the wells.
3. Move to DNA extraction area and add **5** µl of extracted DNA to the wells. Add DNA standards and seal plate.

4. Centrifuge plate for 3 minutes at 3000 rpm in the centrifuge to eliminate air bubbles in wells.
5. Program quantitative PCR with the following parameters

   Activation of uracil N-glycosylase: 50°C for 2 min

   Deactivate uracil N-glycosylase: 95°C incubation for 10 minutes

   Amplification for 40 cycles:

   a. Denaturing at 95°C for 15 seconds.

   b. Anneal/Extend at 60°C for 60 seconds.

D. Analysis

• Refer to quantitative PCR instrumentation documentation on analyzing run results.

• Absolute quantitative standards in the form of a plasmid DNA encoding the target SSU region is available from the Western Fisheries Research Center, 6505 NE 65th St. Seattle, WA 98115. Attn. Maureen Purcell.