Appendix 1. Detailed methods of sample processing of effluent from fish processing plants

Multiple replicates of 1.7 mL aliquots of each sample were immediately preserved (within 5 hours of collection) by flash freezing 2 mL cryovials in liquid nitrogen, and then storing at -80 ^{o}C **(LN2 Cryovial) (Table 1)**. These samples were preserved in case there is a future need to establish viability of any detected virus through infectivity trials.

Within 72 hours of sample collection, an additional 40 mL aliquots of each sample were frozen and stored at -80 ^{o}C in 50 mL centrifuge tubes **(-80C Direct)**. As well, 40mL of sample was aliquoted into 50 mL centrifuge tubes, spun at 2500g for 10 minutes, and the supernatant separated from the pellet. Supernatant was filtered using a Millipore Express 0.22um bottle-top filter (volume depended on the sample). Filters were removed from the filter apparatus using a sterile scalpel and forceps. Each filter was divided into 4 pieces, and each piece put into a 5ml Eppendorf tube. Filters were used immediately or stored at -80C for storage or until further processing **(Filter at -80C)**. An aliquot of the filtrate was frozen in a 50mL centrifuge tube and stored at -80C in case further precipitation of virus was necessary (as only cellular-bound virus will be captured on the 0.22 ul filter). The pellet was re-suspended using a small amount of remaining supernatant, and transferred to a 1.5 mL Eppendorf tube. Pellets from 2- 40mL aliquots were combined, and this was then analyzed immediately or frozen at -80 for storage or until further processing **(Pellet -80 C)**. (Note J3231 had little to no visible pellet)

Table 1. Samples processed and stored from effluents collected at salmon processing plants.

Sample	LN2 Cryovial	-80C Direct	Filter at -80C	Filtrate	Pellet -80 C
J3205	22 x 1.7ml (5 aliquoted onsite, 17 in lab)	10x40ml	Filter A–250ml total/4=62.5ml/piece Filter B–200ml total/4=50ml/piece	40 mLs	2
J3231	28 x 1.7 mL	5x40ml	Filter A–200ml total/4=50ml/piece Filter B–160ml total/4=40ml/piece	40 mLs	2
J3232	28 x 1.7 mL	5x40ml	Filter A–400ml total/4 =100ml/piece	40 mLs	2

RNA Extraction Method

RNA was extracted from the filters and the pellet (in table below) using the MoBio PowerWater RNA Extraction Kit (Cat#14700-50-NF) according to the manufacturer's directions. Adjustments from the protocol were to include the suggested 10 min incubation at 55C during the initial lysis step. RNA was eluted in 75uL volume. RNA was quantified using the Invitrogen Qubit fluorometer, with either the RNA HS or BR assay kit. An extraction control was processed in-step with the samples.

RNA Sample Name	Description	RNA concentration (ng/uL)

J3205 BB Filter 1	¼ of filter A = 62.5mL sample volume	130
J3205 BB Filter 2	¼ of filter A = 50mL sample volume	88.3
J3205 BB Filter 3	¼ of filter A = 62.5mL sample volume	1181
J3205 BB Pellet 1	Pellet from 2x40mL=80mL sample column	3531
J3205 BB Pellet 2	Pellet from 2x40mL=80mL sample column	421
J3231 LG-V Filter 1	¼ of filter A = 50mL sample volume	45
J3231 LG-V Filter2	¼ of filter A = 40mL sample volume	31.5
J3231 LG-V Filter3	¼ of filter A = 50mL sample volume	58.5
J3231 LG-V Pellet 1	Pellet from 2x40mL=80mL sample volume	Below detection limit
J3232 LG-P Filter 1	¼ of filter A = 100mL sample volume	Below detection limit
J3232 LG-P Pellet 1	Pellet from 2x40mL=80mL sample column	116
J3232 LG-P Pellet 2	Pellet from 2x40mL=80mL sample column	27.5
Extraction Control (EC)	Extraction using blank filter and sddH2O	Below detection limit

cDNA synthesis

cDNA synthesis was performed using Invitrogen Superscript Vilo according to manufacturer's protocol, using a 20uL reaction containing 4uL of Vilo Enzyme Master Mix and 16 ul of RNA. RNA was diluted to 62.5 ng/uL, so that 16uL of input equals 1ug of RNA input. Where [RNA] was below 62.5ng/uL, 16uL of the undiluted RNA extraction was used. 16 uL of sddH2O was used as a cDNA synthesis negative control.

qRT-PCR

Real-time quantitative polymerase chain reaction (qRT-PCR) was used to test for PRV on the Applied Biosystems 7900ht platform using a validated TaqMan assay for the PRV L1 segment. Primers and probes were diluted fresh from stock solutions. 1ul of cDNA or control material was used in each reaction mixture. Sterile water was used for a No Template Control (NTC). A set of standards for PRV was run and used to generate a standard curve. Each sample was run six times for the PRV assay, and each control was run 4x. Each sample and control was also run with a No Assay Control (NAC) assay using sddH2O in place of primers and probes.

Forward Reverse Primer Mix (10pmol/uL each)	0.2
Probe (10uM)	0.3
AB Gene Expression Master Mix 2x	4.5

Sterile water	6.0
cDNA	1.0
Total Volume	12.0

Thermocycling conditions were:

Step 1	50C/2 min
Step 2	95C/10 min
Cycle x 45	95C/15 sec
	60C/60 sec

Results were analyzed using the ABI SDS software designed for the ABI7900ht platform.

Copy number calculation (abundance of virus) for each qPCR replicate was calculated by the ABI SDS software based on the standards curve generated by the known clone standards. Copy number per mL sample was calculated by:

(((Copies per Reaction x 20 uL total cDNA)/ uL RNA input into cDNA synthesis) x (75 uL RNA Extraction)) /Volume Sample used for extraction