4.A1.B.3 Worksheet B.3 – Infectious Salmon Anemia Virus (ISAV)

Case Number_____ **Date**_____

Master Mix

PCR Reagent	Lot #	Final Concentration	Stock Concentration	Volume per Reaction (µL) (to total 50 µL)	Volume for ____samples
d-H_2O*		-	-	26.75 µL	
PCR buffer (no $MgCl_2$)		1X	10X	5 µL	
$MgCl_2$		2.5 mM	25 mM	5 µL	
dNTP's		0.2 mM	2 mM	5 µL	
AMV reverse transcriptase		4.5 Units/Rx	9 Units/µL	0.5 µL	
(+)Primer		50 pmoles/Rx	50 pmoles/µL	1 µL	
(-)Primer		50 pmoles/Rx	50 pmoles/µL	1 µL	
TAQ		2.5 Units/Rx	5 Units/µL	0.5 µL	
RNasin		9.75 Units/Rx	39 Units/µL	0.25 µL	
RNA template		-	-	5 µL	-

*Add nuclease free water to Master Mix first, TAQ last.

Primer Sets for ISAV

Forward	**Reverse**
5'-GGC TAT CTA CCA TGA ACG AAT C-3'	5'-TAG GGG CAT ACA TCT GCA TC-3'

Control Information

POSITIVE CONTROLS		**NEGATIVE CONTROLS**	
Extraction	**PCR**	**Extraction**	**PCR**

Amplification (Thermocycle Process)

Date & Time	Program #	NOTES

Gel Preparation

Gel Concentration	Apparatus and Gel Size (Mini = 8-22 wells; Midi = 22-40)	Weight of Agarose (grams)	Volume of Buffer (mL)

Gel Template (Sample Placement Map)

Ladder Brand / Lot # _____ Loading Buffer Brand / Lot # _____

Enter sample ID below corresponding well number:

PCR Products (Loaded LEFT to RIGHT)														
1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**
16	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**	**28**	**29**	**30**