3.A3.D Worksheet D – Initial Amplification of Nucleic Acid by PCR for the Confirmation of *Piscirickettsia salmonis*

Case Number_____ **Date**_____

PCR Reagent	Lot#	Final Concentration	Stock Concentration**	Volume per Reaction (µL) (to total 50 µL)	Volume for ____samples
d-H_2O*		Add to total 45 µL		35.0	
10XBuffer		1X	10X	5.0	
$MgCL_2$		1.5 mM	50 mM	1.5	
dNTP's		0.2 mM	10 mM	1	
(+)Primer		1 mM	1 mM	1	
(-)Primer		1 mM	1 mM	1	
TAQ		2.5 units/Rx	5U/µL	0.5	
DNA[±]		-	-	5 µL	-

*Add water to Master Mix first, TAQ last.

**Change "Stock Concentration" parameters as necessary. Different reagent sources supply varying stock concentrations.

[±]Do not add DNA template until Master Mix reaction tubes have been removed from the reagent mixing (MM) area.

Primer Sets for *P. salmonis* 1st Round Amplification

EubA (round 1 forward)	5'- AAG-GAG-GTG-ATC-CAN-CCR-CA -3'
EubB (round 1 reverse)	5'- AGA-GTT-TGA-TCM-TGG-CTC-AG -3'