# 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' |