3.A3.C Worksheet C – Nested Amplification of Nucleic Acid by PCR for the Confirmation of *Renibacterium salmoninarum*

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 49 µL		39.1	
10XBuffer		1X	10X	5.0	
$MgCL_2$		1.5 mM	50 mM	1.5	
dNTP's		0.4 mM	10 mM	1	
(+)Primer		20 pMole	20 pMole/µL	1	
(-)Primer		20 pMole	20 pMole/µL	1	
TAQ		2 units/Rx	5U/µL	0.4	
DNA[±] (round 1)		-	-	1 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 *R. salmoninarum* 2nd (Nested) Round Amplification

P4 (round 2 forward)	5'-AT TCT TCC ACT TCA ACA GTA CAA GG-3'
M38 (round 2 reverse)	5'-C ATT ATC GTT ACA CCC GAA ACC-3'

Gel Concentration		**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**