

1.2.12.2 Worksheet B – 2nd Amplification of Nucleic Acid by PCR for the Confirmation of *Francisella* spp.

Case Number _____

Date _____

PCR Reagent	Lot#	Final Concentration	Stock Concentration**	Volume per Reaction (µL) (to total 50 µL)	Volume for _____ samples
d-H ₂ O*		Add to total 45 µL		32.5	
10XBuffer		1X	10X	5.0	
MgCL ₂		1.5 mM	25 mM	3	
dNTP's		0.2 mM	20 mM	5	
FLB16S180f		1 µM	50 µM	1	
FLB16S465r		1 µM	50 µM	1	
Taq		2.5 units/Rx	5U/µL	0.5	
DNA [‡]		-	-	2 µL of initial Rx	-

*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 *Francisella* spp. 2nd Round Amplification

FLB16S180f	5'-GCG-GAT-TAA-AGG-TGG-CCT-TTG-C-3'
FLB16S465r	5'-CCT-GCA-AGC-TAT-TAA-CTC-ACA-GG-3'