4.A1.B.6 Worksheet B.6 – Spring Viremia of Carp Virus (SVCV)

Case Number_____ **Date**_____

Reverse Transcription Master Mix (RTMM)

PCR Reagent	Lot #	Final Concentration	Stock Concentration	Volume per Reaction (µL) (to total 20 µL)	Volume for ____samples
d-H_2O[1]		-	-	10 µL	
RT buffer[2]		1X	5X	4 µL	
dNTP's		1 mM	10 mM	2µL	
M-MLV reverse transcriptase		20 Units/Rx	10 Units/µL	2 µL	
Primer SVCV R2 (below)		100 pmoles/Rx	100 pmoles/µL	1 µL	

1. Add nuclease free water to Master Mix first, M-MLV reverse transcriptase last.
2. 5X RT Buffer is 250 mM tris pH 8.3, 375 mM KCl, 50 mM DTT, 15 mM $MgCL_2$

PCR Master Mix (PCRMM)

PCR Reagent	Lot #	Final Concentration	Stock Concentration	Volume per Reaction (µL) (to total 50µL)	Volume for ____samples
d-H_2O[1]		-	-	36.75 µL	
PCR buffer (no $MgCl_2$)[2]		1X	10X	5 µL	
$MgCl_2$		2.5 mM	25 mM	5 µL	
dNTP's		0.2 mM	10 mM	1 µL	
TAQ		1.25 Units/Rx	5 Units/µL	0.25 µL	

1. Add nuclease free water to Master Mix first, TAQ last.
2. 10X PCR Buffer is 100 mM Tris HCl pH 9.0, 500 mM KCl, 1 % Triton X-100

Primer Sets for SVCV

	Forward	**Reverse**
RT Reaction	None	5'- AGA-TGG-TAT-GGA-CCC-CAA-TAC-ATH-CAN-CAY -3' (SVCV R2)
1st round	5'-TCT-TGG-AGC-CAA-ATA-GCT-CAR-RTC-3' (SVCV F1)	5'- AGA-TGG-TAT-GGA-CCC-CAA-TAC-ATH-ACN-CAY -3' (SVCV R2)

2nd round	5'-TCT-TGG-AGC-CAA-ATA-GCT-CAR-RTC-3' (SVCV F1)	5'- CTG-GGG-TTT-CCN-CCT-CAA-AGY-TGY -3' (SVCV R4)

Control Information

	POSITIVE CONTROLS		NEGATIVE CONTROLS	
	Extraction	PCR	Extraction	PCR
1st round PCR				
2nd round PCR				

Amplification (Thermocycle Process)

Round Number (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**