

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 ₂ O 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 MgCl₂

PCR Master Mix (PCRMM)

PCR Reagent	Lot #	Final Concentration	Stock Concentration	Volume per Reaction (µL) (to total 50µL)	Volume for _____ samples
d-H ₂ O 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)
1 st 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)

4.A1.B.6 Worksheet B.6 – Spring Viremia of Carp Virus (SVCV) - 2

2 nd 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
1 st round PCR				
2 nd 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)														
<u>1</u>	<u>2</u>	<u>3</u>	<u>4</u>	<u>5</u>	<u>6</u>	<u>7</u>	<u>8</u>	<u>9</u>	<u>10</u>	<u>11</u>	<u>12</u>	<u>13</u>	<u>14</u>	<u>15</u>
<u>16</u>	<u>17</u>	<u>18</u>	<u>19</u>	<u>20</u>	<u>21</u>	<u>22</u>	<u>23</u>	<u>24</u>	<u>25</u>	<u>26</u>	<u>27</u>	<u>28</u>	<u>29</u>	<u>30</u>