

The following supplement accompanies the article

Quantifying chelonid herpesvirus 5 in symptomatic and asymptomatic rehabilitating green sea turtles

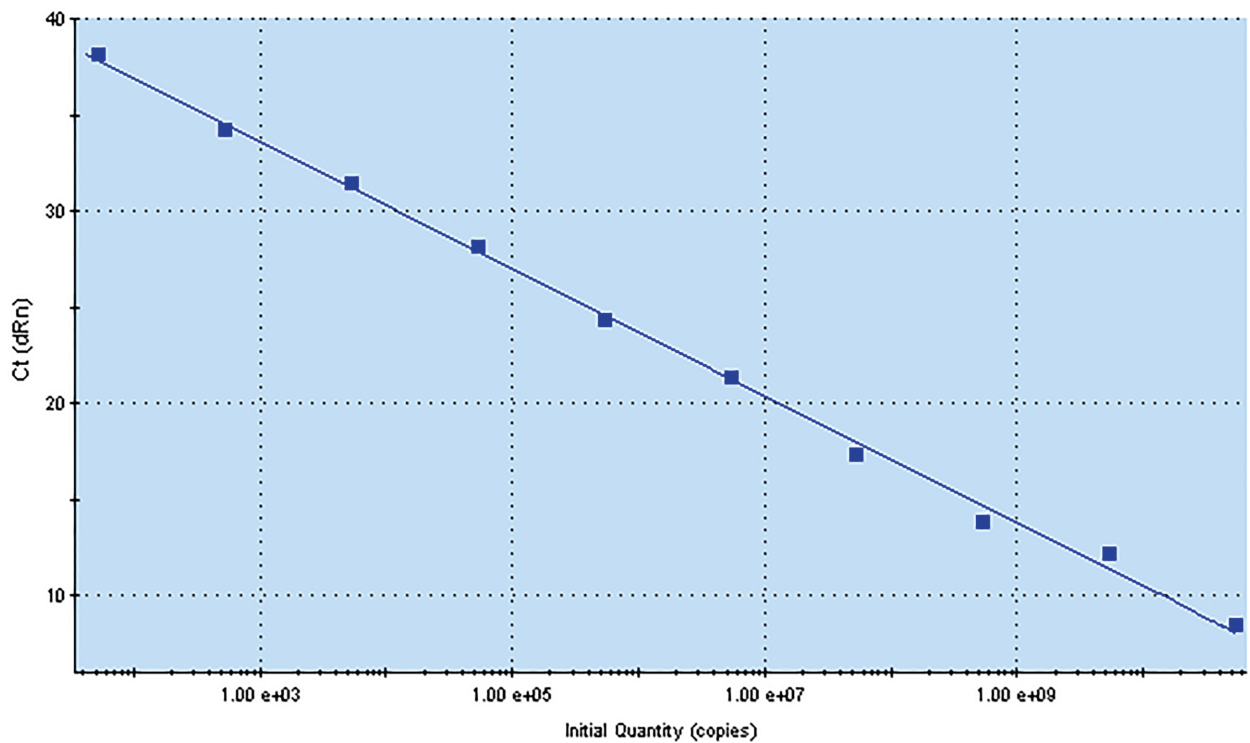
Annie Page-Karjian*, Terry M. Norton, Branson Ritchie, Corrie Brown, Carmen Mancía, Mark Jackwood, Nicole L. Gottdenker

*Corresponding author: anniep@uga.edu

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Supplement.

Figure S1. ChHV5 plasmid standard curve. Example of a ChHV5 plasmid standard curve used in qPCR assay validation.



Dataset S1. Reaction efficiency and R² data. Reaction efficiency and coefficient of determination (R²) values of ChHV5 plasmid standard curves used in qPCR assay validation.

Dilution Series #	qPCR Run #	Efficiency	R ²
1	1-1	98.6	0.996
1	1-2	106	0.998
1	1-3	103	0.999
1	1-4	111.4	0.977
1	1-5	102.7	0.962
1	1-6	105.3	0.986
1	1-7	94.8	0.992
1	1-8	90.3	0.992
1	1-9	91.4	0.998
1	1-10	91.7	0.991
2	2-1	97.2	0.991
2	2-2	94.2	0.999
2	2-3	90.8	0.998
2	2-4	108	0.928
2	2-5	96.3	0.992
2	2-6	93.1	0.997
2	2-7	92.8	0.996
2	2-8	94.1	0.994
2	2-9	91.1	0.996
2	2-10	92.6	0.975
3	3-1	100.7	0.997
3	3-2	98.8	0.994
3	3-3	104	0.924
3	3-4	101.8	0.996
3	3-5	93.7	0.993
3	3-6	92.2	0.989
3	3-7	97	0.989
3	3-8	97.6	0.986
3	3-9	97.2	0.991
3	3-10	97.6	0.966
Mean		97.5333333	0.98606667
Std Dev		5.61969034	0.01873211

Dataset S2. Analytical sensitivity data. qPCR assay analytical sensitivity was evaluated by applying qPCR to 42 qPCR replicates of low gene copy number (1, 5, 50, and 500 copies) plasmid DNA. Based on a 50% certainty cutoff (OIE 2009), results show a minimum limit of detection of 50 gene copies per reaction.

qPCR run #	CFPHV copy #	Replicate #	Cq
1	1	1	32.54
2	1	2	33.64
3	1	3	No Cq
4	1	4	No Cq
5	1	5	36.66
6	1	6	No Cq
7	1	7	No Cq
8	1	8	No Cq
9	1	9	No Cq
10	1	10	No Cq
11	1	11	No Cq
12	1	12	No Cq
13	1	13	No Cq
14	1	14	No Cq
15	1	15	No Cq
16	1	16	No Cq
17	1	17	No Cq
18	1	18	No Cq
19	1	19	No Cq
20	1	20	No Cq
21	1	21	No Cq
22	1	22	No Cq
23	1	23	No Cq
24	1	24	No Cq
25	1	25	No Cq
26	1	26	No Cq
27	1	27	39.51
28	1	28	No Cq
29	1	29	38.42
30	1	30	No Cq
31	1	31	No Cq
32	1	32	No Cq
33	1	33	38.78
34	1	34	No Cq
35	1	35	No Cq
36	1	36	No Cq
37	1	37	No Cq
38	1	38	No Cq
39	1	39	No Cq
40	1	40	39.6
41	1	41	No Cq
42	1	42	No Cq

Total # positive by qPCR: 7

qPCR run #	CFPHV copy #	Replicate #	Cq
1	5	1	33.29
2	5	2	30.12
3	5	3	37.83
4	5	4	36.17
5	5	5	38.79
6	5	6	No Cq
7	5	7	39.63
8	5	8	No Cq
9	5	9	No Cq
10	5	10	No Cq
11	5	11	No Cq
12	5	12	39.43
13	5	13	No Cq
14	5	14	No Cq
15	5	15	No Cq
16	5	16	No Cq
17	5	17	No Cq
18	5	18	No Cq
19	5	19	No Cq
20	5	20	No Cq
21	5	21	39.27
22	5	22	38.32
23	5	23	No Cq
24	5	24	No Cq
25	5	25	38.14
26	5	26	No Cq
27	5	27	38.27
28	5	28	37.23
29	5	29	36.36
30	5	30	36.16
31	5	31	No Cq
32	5	32	39.07
33	5	33	39.03
34	5	34	No Cq
35	5	35	No Cq
36	5	36	38.17
37	5	37	No Cq
38	5	38	No Cq
39	5	39	35.87
40	5	40	36.07
41	5	41	36.47
42	5	42	36.05

Total # positive by qPCR: 21

qPCR run #	CFPHV copy #	Replicate #	Cq
1	50	1	31.87
2	50	2	No Cq
3	50	3	36.49
4	50	4	37.25
5	50	5	39.46
6	50	6	35.86
7	50	7	37.85
8	50	8	38.14
9	50	9	37.28
10	50	10	36.65
11	50	11	38.79
12	50	12	No Cq
13	50	13	39.13
14	50	14	38.94
15	50	15	36.47
16	50	16	38.91
17	50	17	38.49
18	50	18	No Cq
19	50	19	35.36
20	50	20	39.39
21	50	21	37.36
22	50	22	37.49
23	50	23	36.39
24	50	24	36.56
25	50	25	34.25
26	50	26	33.46
27	50	27	32.49
28	50	28	No Cq
29	50	29	32.62
30	50	30	32.09
31	50	31	37.93
32	50	32	37.8
33	50	33	37.3
34	50	34	No Cq
35	50	35	No Cq
36	50	36	31.85
37	50	37	32.56
38	50	38	38.54
39	50	39	32.2
40	50	40	32.75
41	50	41	32.74
42	50	42	32.88

Total # positive by qPCR: 36

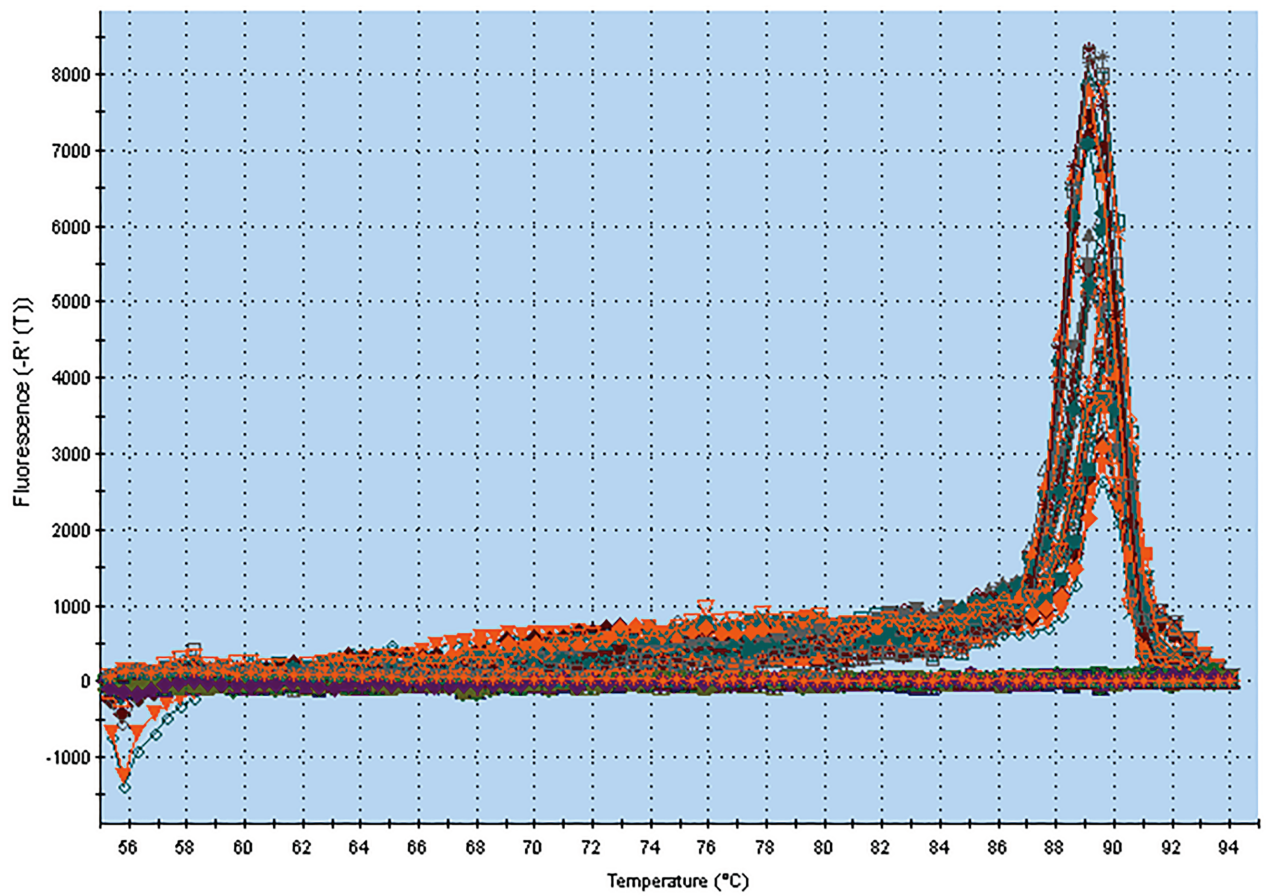
qPCR run #	CFPHV copy #	Replicate #	Cq
1	500	1	27.22
2	500	2	26.93
3	500	3	35.06
4	500	4	34.35
5	500	5	34.05
6	500	6	32.9
7	500	7	34.14
8	500	8	35
9	500	9	32.6
10	500	10	34.14
11	500	11	36.15
12	500	12	35.32
13	500	13	35.37
14	500	14	35.77
15	500	15	33.5
16	500	16	35.73
17	500	17	36.51
18	500	18	36.89
19	500	19	32.94
20	500	20	32.44
21	500	21	32.75
22	500	22	33.33
23	500	23	32.54
24	500	24	32.93
25	500	25	31.45
26	500	26	30.25
27	500	27	32.96
28	500	28	28.16
29	500	29	28.4
30	500	30	30.4
31	500	31	34.53
32	500	32	34.77
33	500	33	34.4
34	500	34	30.09
35	500	35	31.96
36	500	36	31.23
37	500	37	30.39
38	500	38	35.06
39	500	39	28.95
40	500	40	28.99
41	500	41	28.79
42	500	42	28.85

Total # positive by qPCR: 42

Dataset S3. Analytical specificity data. qPCR assay analytical specificity was evaluated by applying qPCR to various related samples and evaluating for cross-reactivity with our qPCR primers and probe. Results show no cross-reactivity with BHV-3, PhHV-1, PhHV-2, samples from a captive-raised green turtle from a collection in which FP has not been documented, or samples from 2 adult female freshwater turtles (*T. scripta elegans*).

Sample ID	Sample Type	CFPHV UL30 Cq
BHV-3	Purified plasmid	No Cq
PhHV-1	Purified plasmid	No Cq
PhHV-2	Purified plasmid	No Cq
<i>T. scripta elegans</i> 1	Blood	No Cq
<i>T. scripta elegans</i> 1	Plasma	No Cq
<i>T. scripta elegans</i> 1	Skin	No Cq
<i>T. scripta elegans</i> 1	Oral swab	No Cq
<i>T. scripta elegans</i> 1	Cloacal swab	No Cq
<i>T. scripta elegans</i> 1	Feces	No Cq
<i>T. scripta elegans</i> 2	Blood	No Cq
<i>T. scripta elegans</i> 2	Plasma	No Cq
<i>T. scripta elegans</i> 2	Skin	No Cq
<i>T. scripta elegans</i> 2	Oral swab	No Cq
<i>T. scripta elegans</i> 2	Cloacal swab	No Cq
<i>T. scripta elegans</i> 2	Feces	No Cq
<i>C. mydas</i> neg control	Blood	No Cq
<i>C. mydas</i> neg control	Plasma	No Cq
<i>C. mydas</i> neg control	Skin	No Cq
<i>C. mydas</i> neg control	Oral swab	No Cq
<i>C. mydas</i> neg control	Cloacal swab	No Cq
<i>C. mydas</i> neg control	Feces	No Cq
<i>C. mydas</i> neg control	Urine	No Cq

Figure S2. ChHV5 qPCR melt curve analysis. Melt curve analysis showing a single peak of amplified qPCR product, illustrating assay specificity.



Dataset S4. Repeatability data. Repeatability was evaluated by applying qPCR to 3 ChHV5 DNA-positive samples each of FP, non-tumored skin, blood, plasma, urine, and cloacal swab samples spanning the qPCR assay linear operating range. Samples were tested in replicates of 8. Intra-assay CV values ranging between 0 to 20% were considered acceptable.

Sample Type: FP tumors		
Sample	Replicate #	CFPHV Copy #
Low copy #	1	23294
Low copy #	2	21176
Low copy #	3	22668
Low copy #	4	23937
Low copy #	5	17740
Low copy #	6	18230
Low copy #	7	29967
Low copy #	8	19382
Mean		22049.25
Std Dev		3950.040027
CV(%)		0.179146231
Med copy #	1	1413211
Med copy #	2	1293505
Med copy #	3	1403622
Med copy #	4	1413211
Med copy #	5	945701
Med copy #	6	808625
Med copy #	7	1347439
Med copy #	8	1320197
Mean		1243188.875
Std Dev		233063.0458
CV(%)		18.74719525
High copy #	1	244640017
High copy #	2	226987722
High copy #	3	231671631
High copy #	4	238067536
High copy #	5	222398511
High copy #	6	223917845
High copy #	7	169378649
High copy #	8	325622473
Mean		235335548
Std Dev		43056308
CV(%)		18.29570941

Sample Type: Non-FP skin		
Sample	Replicate #	CFPHV Copy #
Low copy #	1	177
Low copy #	2	200
Low copy #	3	218
Low copy #	4	197
Low copy #	5	189
Low copy #	6	220
Low copy #	7	226
Low copy #	8	197
Mean		203
Std Dev		16.88617355
CV(%)		8.318312093
Med copy #	1	23243
Med copy #	2	21087
Med copy #	3	25497
Med copy #	4	23504
Med copy #	5	24150
Med copy #	6	19047
Med copy #	7	15860
Med copy #	8	27658
Mean		22505.75
Std Dev		3739.340116
CV(%)		16.61504334
High copy #	1	41549
High copy #	2	35069
High copy #	3	46626
High copy #	4	49898
High copy #	5	51271
High copy #	6	33899
High copy #	7	54498
High copy #	8	41831
Mean		44330.125
Std Dev		7525.108342
CV(%)		16.97515706

Mean CV(%) 12.40735

Mean CV(%) 13.9695042

Sample Type: Blood		
Sample	Replicate #	CFPHV Copy #
Low copy #	1	326
Low copy #	2	315
Low copy #	3	360
Low copy #	4	280
Low copy #	5	375
Low copy #	6	288
Low copy #	7	269
Low copy #	8	260
Mean		309.125
Std Dev		42.36722959
CV(%)		13.70553323
Med copy #	1	2515
Med copy #	2	2358
Med copy #	3	3495
Med copy #	4	3052
Med copy #	5	2576
Med copy #	6	3356
Med copy #	7	2102
Med copy #	8	3157
Mean		2826.375
Std Dev		505.896358
CV(%)		17.89912372
High copy #	1	10785
High copy #	2	10702
High copy #	3	11299
High copy #	4	9344
High copy #	5	12340
High copy #	6	13569
High copy #	7	12257
High copy #	8	10486
Mean		11347.75
Std Dev		1322.277878
CV(%)		11.65233529

Sample Type: Plasma		
Sample	Replicate #	CFPHV Copy #
Low copy #	1	640
Low copy #	2	471
Low copy #	3	397
Low copy #	4	623
Low copy #	5	586
Low copy #	6	555
Low copy #	7	431
Low copy #	8	422
Mean		515.625
Std Dev		96.72632601
CV(%)		18.75904504
Med copy #	1	1157
Med copy #	2	1081
Med copy #	3	1381
Med copy #	4	1096
Med copy #	5	1419
Med copy #	6	1118
Med copy #	7	834
Med copy #	8	924
Mean		1126.25
Std Dev		200.3181398
CV(%)		17.78629432
High copy #	1	1550
High copy #	2	1400
High copy #	3	1438
High copy #	4	1498
High copy #	5	1540
High copy #	6	1529
High copy #	7	1478
High copy #	8	1615
Mean		1506
Std Dev		67.83383058
CV(%)		4.504238418

Mean CV(%) 14.4189974

Mean CV(%) 13.6831926

Sample Type: Urine		
Sample	Replicate #	CFPHV Copy #
Low copy #	1	220
Low copy #	2	319
Low copy #	3	224
Low copy #	4	227
Low copy #	5	271
Low copy #	6	235
Low copy #	7	278
Low copy #	8	209
Mean		247.875
Std Dev		37.72243554
CV(%)		15.21833002
Med copy #	1	1574
Med copy #	2	1715
Med copy #	3	1141
Med copy #	4	1045
Med copy #	5	1602
Med copy #	6	1418
Med copy #	7	1726
Med copy #	8	1096
Mean		1414.625
Std Dev		283.0234405
CV(%)		20.00695877
High copy #	1	7512
High copy #	2	7076
High copy #	3	7521
High copy #	4	4710
High copy #	5	6220
High copy #	6	5075
High copy #	7	4742
High copy #	8	6567
Mean		6177.875
Std Dev		1193.818775
CV(%)		19.32410052

Mean CV(%) 18.1831298

Sample Type: Cloacal swabs		
Sample	Replicate #	CFPHV Copy #
Low copy #	1	543
Low copy #	2	411
Low copy #	3	606
Low copy #	4	484
Low copy #	5	515
Low copy #	6	558
Low copy #	7	518
Low copy #	8	574
Mean		526.125
Std Dev		59.98913592
CV(%)		11.40206908
Med copy #	1	2822
Med copy #	2	2940
Med copy #	3	3169
Med copy #	4	3042
Med copy #	5	3212
Med copy #	6	3105
Med copy #	7	4161
Med copy #	8	3042
Mean		3186.625
Std Dev		412.7662561
CV(%)		12.95308535
High copy #	1	6092
High copy #	2	7731
High copy #	3	8108
High copy #	4	6610
High copy #	5	8220
High copy #	6	5848
High copy #	7	9291
High copy #	8	9103
Mean		7625.375
Std Dev		1314.568472
CV(%)		17.23939442

Mean CV(%) 13.8648496

Table S1. Summary of qPCR data for all samples taken from individual turtles. Summary of qPCR data for 351 biological samples taken from 67 tumored and tumor-free rehabilitating green turtles. Data is shown for all samples taken from each individual turtle. Turtles were divided into 3 study groups based on clinical signs at the time of sampling: tumored (Group A); history of tumors (Group B); and no known history of tumors (Group C). * denotes amplicon sequences matched to ChHV5 UL30 with $\geq 97\%$ identity via Sanger sequencing of PCR products.

Study Group	Turtle ID	Sample Type							
		FP tumor	Whole blood	Plasma	Non-tumored skin	Oral swab	Cloacal swab	Urine	Feces
Tumored (study group A)	1	290491257*			23242.8	--		7511.8	
	2	498969330.6			40608.6*	--	1311.6	480	
	3	736427473.3*	--	--		--	12864.3		
	4		180	--	14042.9*	--	--		
	5		--	--		--			
	6		1295.6	424.9		--	--		
	7		1243.7	938*		--	3536.4		
	8	8703736.8*	--	--					
	9	32467545.2	176.5	--	245.5	--	--	279.7*	--
	10	1626.3	--	--			--		
	11	63488097.5*	--	--	576.8	--	--		--
	12	53373143	--	--					
	13	72567.5	--	--					
	14	8883013.5	--	--		--	149.4*		--
	15	2653889.6*	--	--					
	16	2031752904	--	--	71414.4*	--	--	--	--
	17	605312608	--	--	--	--	--		--
	18	481119.4	--	--	4504.3	--	--	--	--
	19	794234.8*	--	--	65708.4	--	--	--	--
	20		--	--	--	--	--		--
	21	295668501.4	3607*	--		--	142.7		--
	22	1207452703	1730.4*	--	3482.3		497		--
	23	560786.2	3922	--	3392.7	--	--	5656.9*	
History of FP (study group B)	24		2514.9	--	2316.2*		--	361.9	
	25		1521.3	--		--	--		--
	26		--	--			--		
	27		--	--			--		
	28		--	--			--	--	
	29		--	--			--		
	30		--	--			--		
	31		--	--		--	62.53		
	32		14753.6	206.6			65.51*		--
	33		2134.1	--		--	--		
	34		--	--		--	--		
	35		8345.6	--		--	109	220.2*	--
	36		1573.6*	--		--	--	1513.5	
No history of FP (study group C)	37		--	--	--	--	--		
	38		--	--	--	--	--		
	39		5968.2	1306.1*		--	4439.4		
	40		--	--		--	--		
	41		10785.4*	--		--	--		--
	42		3654.6*	305.7	--	--	--		--
	43		--	--	--	--	--	--	--
	44		--	--	--	--	89.8	--	--

45	--	--	--	--	--	--	--
46	326	--	--	--	--	449*	--
47	--	--	--	--	--	--	--
48	--	--	--	--	--	--	--
49	--	--	--	--	--	--	--
50	--	--	--	--	--	--	--
51	--	--	648.9	--	--	--	--
52	--	--	--	--	--	--	--
53	--	--	--	--	--	--	--
54	--	--	--	--	3536.4*	--	--
55	--	--	--	--	--	--	--
56	607.1	--	8351.9	--	--	--	--
57	--	--	--	--	--	--	--
58	1938	--	603.5	--	157.3*	--	--
59	--	--	17374.8*	--	--	--	--
60	--	--	--	--	--	--	--
61	--	--	--	--	--	--	--
62	--	--	--	--	--	--	--
63	--	--	--	--	--	--	--
64	--	--	4738.6*	--	--	--	--
65	28382*	--	--	--	--	--	--
66	874.6*	--	1060.3	--	--	--	--
67	--	--	--	--	--	--	--

Table S2. qPCR product sequence data. Summary of sequence data obtained for 30 samples that were positive for ChHV5 DNA via qPCR. ChHV5 DNA-positive samples of FP tumors, non-tumored skin, blood, plasma, urine and cloacal swab samples aligned to the listed sequences with $\geq 97\%$ identity.

Sequence identifier	GenBank accession number
Hawaiian green turtle herpesvirus thymidine kinase (UL23), membrane-associated protein (UL24), minor capsid protein (UL25), capsid maturation protease (UL26), virion scaffolding protein (UL26.5), virion membrane glycoprotein B (gB), DNA cleavage/packaging protein (UL28), single-stranded DNA-binding protein (UL29), DNA polymerase catalytic subunit (pol), nuclear phosphoprotein (UL31), DNA cleavage/ packaging (UL32), DNA cleavage/packaging protein (UL33), membrane-associated phosphoprotein (UL34), basic phosphorylated capsid protein (UL35) genes, complete cds; and very large tegument protein (UL36) gene	AF035003.2
Florida green turtle herpesvirus DNA polymerase (pol) gene, partial cds	AF035004.1
Green turtle herpesvirus polymerase gene, complete cds	AF239684.2
Chelonid herpesvirus 5 DNA polymerase gene, partial cds	AF299107.1
Chelonid herpesvirus 5 DNA polymerase gene, partial cds	AF299108.1
Chelonid herpesvirus 5 DNA polymerase gene, partial cds	AF299109.1
Chelonid herpesvirus 5 DNA polymerase gene, partial cds	AF299110.1
Fibropapilloma-associated turtle herpesvirus from Hawaii DNA polymerase (pol) gene	AY390420.1
Fibropapilloma-associated turtle herpesvirus from Puerto Rico DNA polymerase (pol) gene	AY390421.1
Fibropapilloma-associated turtle herpesvirus from California DNA polymerase (pol) gene	AY390422.1
Fibropapilloma-associated turtle herpesvirus DNA polymerase gene	AY395516.1
Fibropapilloma-associated turtle herpesvirus UL region containing UL9-UL30 genes	AY644454.1
Fibropapilloma-associated turtle herpesvirus strain FL var A polymerase (UL30) gene	AY646888.1
Fibropapilloma-associated turtle herpesvirus strain FL var C polymerase (UL30) gene	AY646889.1
Fibropapilloma-associated turtle herpesvirus strain HA variant polymerase (UL30) gene	AY646893.1
Fibropapilloma-associated turtle herpesvirus strain FL var C polymerase (UL30) gene	AY646894.1
Fibropapilloma-associated turtle herpesvirus isolate T1 polymerase gene	HM348895.1
Fibropapilloma-associated turtle herpesvirus isolate T3 polymerase gene	HM348896.1
Fibropapilloma-associated turtle herpesvirus isolate T4 polymerase gene	HM348897.1
Fibropapilloma-associated turtle herpesvirus isolate T8 polymerase gene	HM348898.1
Fibropapilloma-associated turtle herpesvirus isolate T2b polymerase (pol) gene	HQ000006.1
Fibropapilloma-associated turtle herpesvirus isolate T8b polymerase (pol) gene	HQ000007.1
Chelonid herpesvirus 5, partial genome	HQ878327.2
Fibropapilloma-associated turtle herpesvirus isolate PR2_cm_2009 DNA polymerase (UL30) gene	JN580279.1
Fibropapilloma-associated turtle herpesvirus isolate PR3_cm_2010 DNA polymerase (UL30) gene	JN580280.1
Fibropapilloma-associated turtle herpesvirus isolate PR6_cm_2006 DNA polymerase (UL30) gene	JN580283.1
Chelonid herpesvirus 5 isolate 14010SP DNA polymerase gene, partial cds	JN938584.1
Chelonid herpesvirus 5 isolate 12910SP DNA polymerase gene, partial cds	JN938585.1
Chelonid herpesvirus 5 isolate 02409ES DNA polymerase gene, partial cds	JN938586.1
Chelonid herpesvirus 5 isolate 13110SP DNA polymerase gene, partial cds	JN938587.1
Chelonid herpesvirus 5 isolate 03210BA DNA polymerase gene, partial cds	JN938588.1