

**TABLE S1. Primers used for amplification and sequencing of HRV region.**

Region	Primer	Polarity	Position	Primer Length	Sequence (5'-3')	Reference
VP3-VP1-2A	92378	Sense	2060-2084 <sup>a</sup>	25	ATGITIGGIACICAYGTNGTNTGGG	(1)
	187	Sense	2435-2454 <sup>a</sup>	20	ACIGCIGYIGARACIGGNCA	(2)
	VP1F	Sense	2645-2669 <sup>a</sup>	25	GARATGGCICARATYAGRIGIAAAT	(3)
	VP1F78	Sense	2650-2674 <sup>a</sup>	25	GAGATGGCCCAGATTAGAAGAAAG	(3)
	VP1FB	Sense	2690-2712 <sup>a</sup>	23	TATGTIAGRRTTGTAYTCWGARTA	(3)
	VP1FA	Sense	2766-2787 <sup>a</sup>	22	TW GTIATGCARTAYATGT AT GT	(3)
	92379	Antisense	2768-2790 <sup>a</sup>	23	GGIGCICIGGIGGIGGNACATACAT	(1)
	VP1R	Antisense	3077-3100 <sup>a</sup>	24	IGCYCTIGGIGGICKICRCACCA	(3)
	PRPP	Sense	3086-3108 <sup>a</sup>	23	TGGTGYCCIMGISCICIMGTGC	(3)
	92383	Antisense	3498-3518 <sup>a</sup>	21	CCICCICAITCWCWGGTTTC	(1)
VP1	92380	Sense	2645-2669 <sup>b</sup>	25	GAI ATG GTICAIATYAGR AGRAAA T	(1)
	92580	Sense	2435-2454 <sup>b</sup>	20	ACI GCI GYI GAR ACI GGN CA	(2)
VP1(HRV-A)	W.F	Sense	1995-2017 <sup>c</sup>	23	MGHTTYAGYTTYATGTTYTGTTGG	(4)
	X.F	Sense	2430-2448 <sup>c</sup>	19	TRGAYGCWGCWGARACWGG	(4)
	X.R	Antisense	3333-3358 <sup>c</sup>	26	GTRTTTGTKCGGTADATGAYTARRTC	(4)
	W.R	Antisense	3525-3547 <sup>c</sup>	23	CCACARTCWCCWGGYTACADGG	(4)
VP4-VP2 <sup>d</sup>	Y.F	Sense	458-478 <sup>c</sup>	21	CCGGCCCCTGAATGYGGCTAA	(4)
	Z.F	Sense	547-569 <sup>c</sup>	23	ACCRACACTTTGGGTGTCCGTG	(4)
	Z.R	Antisense	1087-1109 <sup>c</sup>	23	TCWGGHARYTTCCAMCACCANCC	(4)
	Y.R	Antisense	1125-1149 <sup>c</sup>	25	ACATRTTYTSNCCAAANAYDCCCAT	(4)

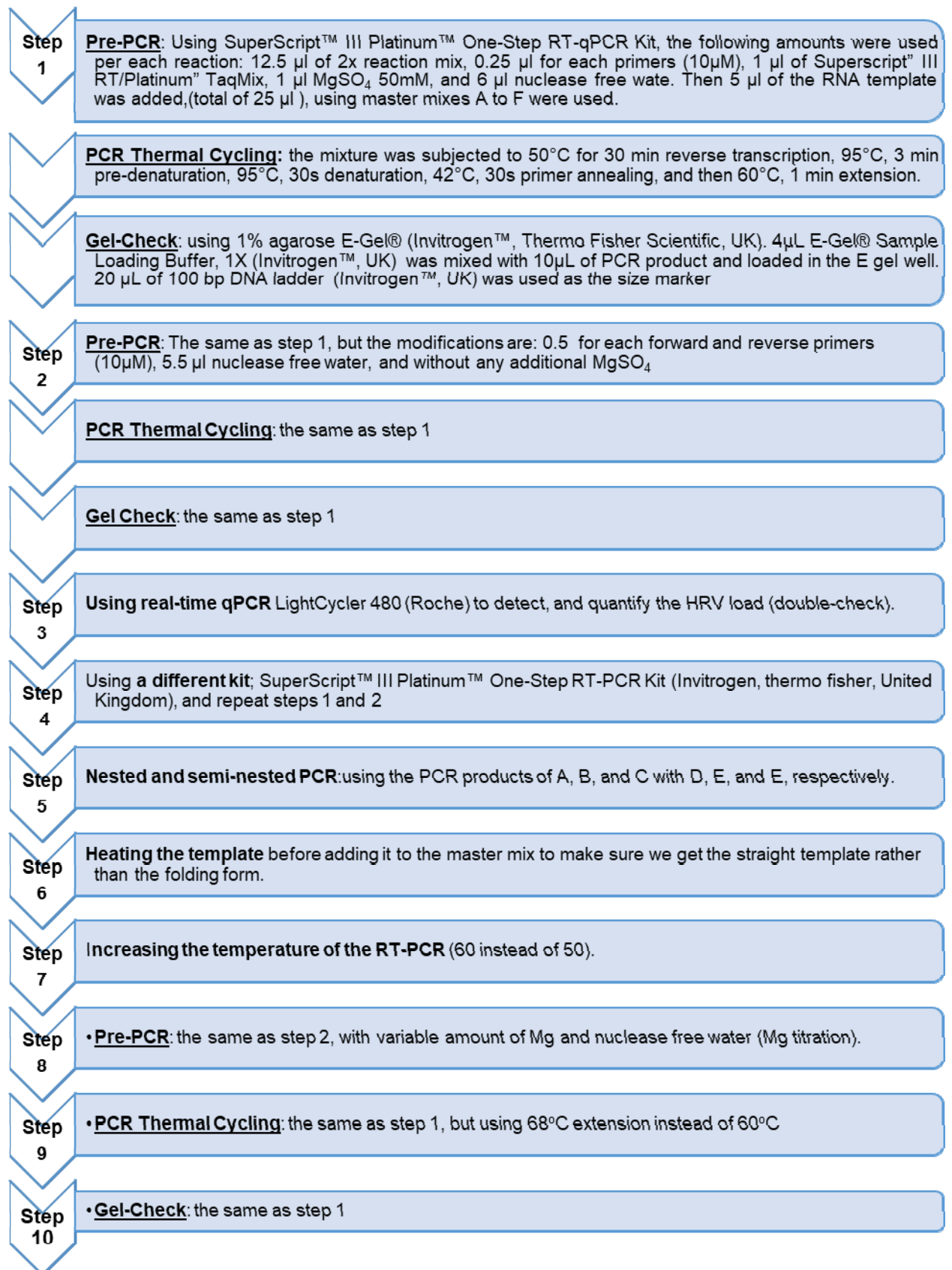
<sup>a</sup> The primer positions were numbered according to the complete HRV14 genome sequence (5).

<sup>b</sup> The primer positions were numbered according to HRV1B complete genome sequence (6).

<sup>c</sup> the 5' base position was numbered in accordance with the HRV-B serotype 14 genome (GenBank accession number NC\_001490).

<sup>d</sup> these primers targets both human rhinovirus (HRV) and human enterovirus (HEV).

Abbreviations for the variable nucleotide (letters) in the sequence column: "I", Inosine; "K", G or T; "R", A or G; "Y", C or T; "M", C or A; "W", A or T; "V", A or C or G; "S", G or C; "N", A or C or G or T.



**FIGURE S1. Stage A in PCR optimisation.**

This flow chart summarises the PCR optimisation steps using DST-purified primers targeting VP3/VP1 and VP1 in the HRV region. RT-qPCR: reverse-transcriptase quantitative polymerase chain reaction; VP: viral-capsid protein.

**TABLE S2. Master mixes and primer combinations were used to detect the variable regions of the HRV genome.**

Master mix	Primers	PCR product length (bp)	1 <sup>st</sup> and/or 2 <sup>nd</sup> round PCR use(s)
A	F 92378 R 92379	730	1 <sup>st</sup> PCR
B	92580 = F187 R 92383	1100	1 <sup>st</sup> PCR
C	F 92380 R 92383	873	1 <sup>st</sup> PCR
C.2	<i>F VP1F</i> R 92383	873	1 <sup>st</sup> PCR
D	F 92580 = F 187 R 92379	355	1 <sup>st</sup> PCR & 2 <sup>nd</sup> PCR
E	F VP1F R VP1R	455	1 <sup>st</sup> PCR & 2 <sup>nd</sup> PCR
F	F PRPP R 92383	432	1 <sup>st</sup> PCR
W	W.F W.R	1530	1 <sup>st</sup> PCR
X	X.F X.R	929	2 <sup>nd</sup> PCR
Y	Y.F Y.R	692	1 <sup>st</sup> PCR
Z	Z.F Z.R	563	2 <sup>nd</sup> PCR

**TABLE S3. The preparation of the first-round PCR master mixes.**

	Component	Amount for 1 reaction (in $\mu$ L)
1.	2X Reaction Mix <sup>1</sup>	12.5
2	Sense primer (10 $\mu$ M)	0.5
3	Anti-sense primer (10 $\mu$ M)	0.5
4	Superscript <sup>®</sup> III RT/Platinum <sup>®</sup> TaqMix	1
5	MgSO <sub>4</sub>	-
6	Nuclease-free water	5.5
	<b>The total volume of master mix</b>	<b>20 <math>\mu</math>L</b>

<sup>1</sup> The Superscript III One-step RT-PCR kit contains the 2x Reaction Mix with 0.4 mM of each dNTP and 3.2 mM MgSO<sub>4</sub> as active ingredients.

**TABLE S4. The preparation of the second-round PCR reactions using the LightCycler® Multiplex DNA master mix.**

	<b>component</b>	<b>The amount for one reaction (in <math>\mu\text{L}</math>)</b>
1	5X Reaction Mix	5
2	Sense primer (10 $\mu\text{M}$ )	1
3	Anti-sense primer (10 $\mu\text{M}$ )	1
4	Nuclease-free water	17
	<b>The total volume of the master mix</b>	<b>24 <math>\mu\text{L}</math></b>

**TABLE S5. The preparation of the second-round PCR reactions using the Platinum® SYBR® Green qPCR SuperMix-UDG.**

	<b>component</b>	<b>The amount for one reaction (in <math>\mu\text{L}</math>)</b>
1	Platinum® SYBR® Green qPCR SuperMix-UDG	12.5
2	Sense primer (10 $\mu\text{M}$ )	1
3	Anti-sense primer (10 $\mu\text{M}$ )	1
4	Nuclease-free water	9.5
	<b>The total volume of the master mix</b>	<b>24 <math>\mu\text{L}</math></b>

**TABLE S6. Thermal Cycling conditions used in PCR reactions.**

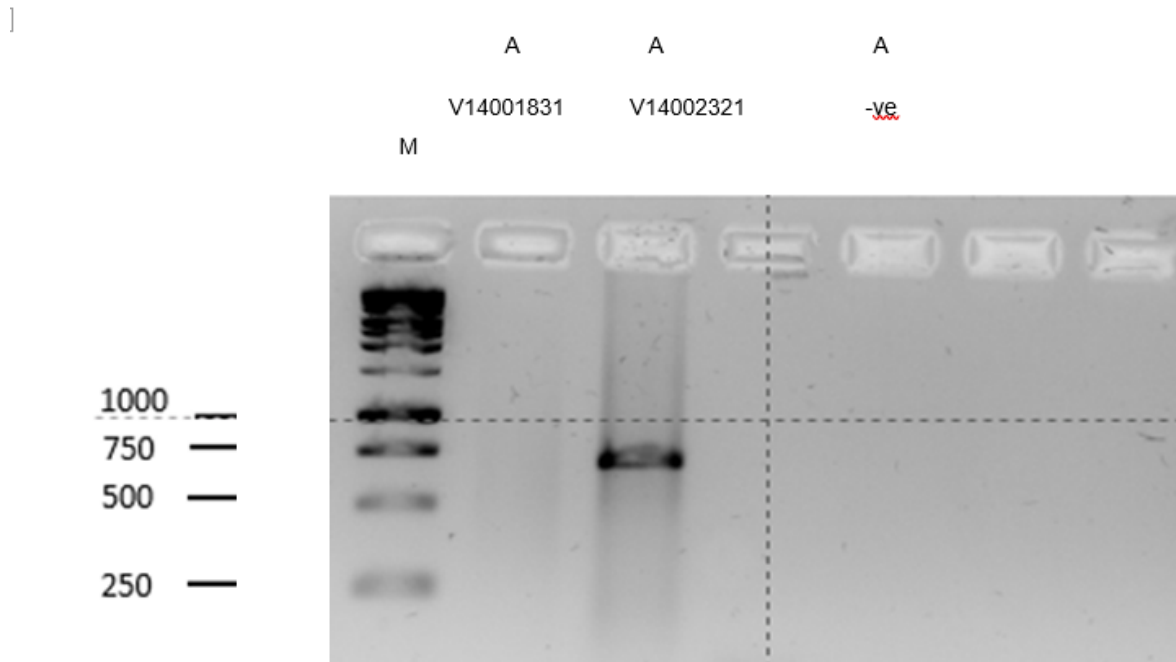
<b>Steps</b>	<b>cDNA synthesis &amp; pre-denaturation</b>		<b>Denature</b>	<b>Anneal</b>	<b>Extend</b>	<b>Final extension (optional)</b>
<b>No. of cycles</b>	1 cycle			40 cycles		1 cycle
<b>Temperature</b>	45°C	95°C	95°C	42°C	68°C	68°C
<b>Time</b>	30 min	3 min	30 s	30 s	1 min	5 min

**TABLE S7. Reagents used in sequencing reactions.**

Reagent	Volume
Sequencing primer (1 $\mu$ M in TE 0.1M)	1.6 $\mu$ l
Big Dye Reaction v3.1 (ABI Part No. 4336911)	0.16 $\mu$ l
ddH <sub>2</sub> O	4.84 $\mu$ l
<b>Total volume of master mix</b>	<b>8.6<math>\mu</math>l</b>
PCR product	1.4 $\mu$ l

**TABLE S8. Cycling conditions used in sequencing reactions.**

Number of cycles	Temperature	Time
1	96°C	20s
25	96°C	10s
	50°C	5s
	60°C	4 min
4°C soak until required		



**FIGURE S2. PCR product representation of master mix A.**

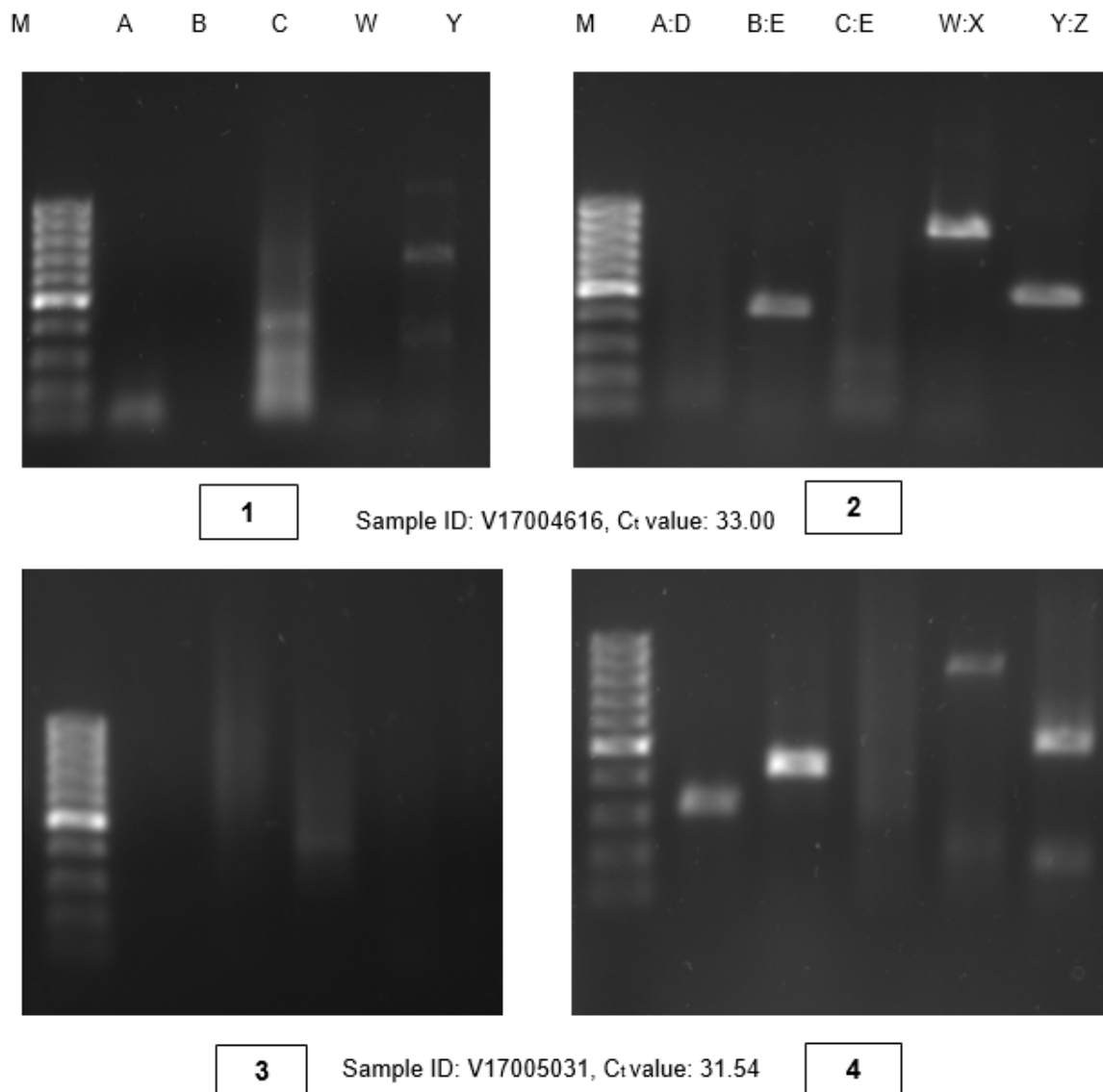
This gel picture is showing a single, brighter product band on the 1 % agarose gel, of the right size (~730 bp) with the sample V14002321, but not with sample V14001831 although both samples have almost the same HRV load (the same  $C_t$  value), and this reflects the high variation in the targeted region between HRV species. The “A” assay (master mix) is targeting a highly variable region of VP3/VP1 in HRV. GeneRuler 1 kb DNA Ladder (Thermo Scientific, UK) was used.



**FIGURE S3. The second-round PCR product representation of A:D and C:E assays.**

Using 1% agarose gel, this figure showing a product of ~355 bp, and ~455 bp for A:D and C:E assays, respectively. the Platinum® SYBR® Green qPCR SuperMix-UDG (Thermo Fisher, cat. no. 11733038) was used to prepare D and E master mixes. M: GeneRuler™ 100 bp DNA Ladder (marker) (Thermo Scientific, UK), Lane 1: A:D with V14000746 specimen, Lane 2: A:D with V14001545 specimen, Lane 3: A:D with V14001831 specimen, Lane 4: A:D with V14002321

specimen, Lane 5 is a replicate of lane 3 (both gave no product), Lane 6 is a replicate for lane 4 (both gave the same positive product with the same length), Lane 7: C:E with V14002321 specimen.



**FIGURE S4. Gel electrophoresis of first-round and second-round PCR products of 10 different assays.**

The representative 1 % agarose gel pictures show PCR amplification products for 5 different first-round assays (pictures 1 and 3) and 5 related second-round PCR assays (pictures 2 and 4) for two different specimens with the corresponding HRV  $C_t$  value. Figures 2 and 4 show a product of ~455 bp, ~929 bp, and ~563 bp for B:E, W:X, and Y:Z assays, respectively for the two different respiratory samples, in addition to ~355 bp for A:D assay for the second sample (picture 4). M, GeneRuler™ 100 bp DNA marker.

**TABLE S9. The PCR product positivity rate of HRV-VP assays using the fifteen respiratory samples.**

<b>Assay</b>	<b>Assay's target</b>	<b>Positivity rate</b>
<b>A</b>	VP3/VP1	6/15 (40 %)
<b>B</b>	VP1	1/15 (7 %)
<b>C</b>	VP1	2/15 (13 %)
<b>W</b>	VP1	0/15 (0 %)
<b>Y</b>	VP4/VP2	8/15 (53 %)
<b>A:D</b>	VP1	6/15 (40 %)
<b>B:E</b>	VP1	5/15 (33 %)
<b>C:E</b>	VP1	3/15 (20 %)
<b>W:X</b>	VP1	4/15 (27 %)
<b>Y:Z</b>	VP4/VP2	13/15 (87 %)

**TABLE S10. The successful representation of the Sanger sequencing primers.**

<b>Primer</b>	<b>Primer Failure No.</b>	<b>Total reactions</b>
F92378	0	6
R92379	7	25
F92580	4	13
Y.F	0	7
Y.R	0	7
F VP1F	0	6
VP1R	1	6
X.F	3	6
X.R	2	6
Z.F	5	22
Z.R	2	15
Total	24	119



**TABLE S11. A summary of gel-check, sequencing successful, and sequences assembly results of the clinical HRV tested samples.**

Specimen ID	C <sub>t</sub> value	PCR product on gel (+, -)						Total successful seq. ratio	Contigs no.		Contig length*	No. of seq.	No. (top, bottom)	Avg Coverage
		A	Y	A:D	B:E	W:X	Y:Z							
V14002321	18.06	+	+	+	+	-	+	14/16	2	Contig 1	671	4	(2,2)	2.93
										Contig 2	686	5	(3,2)	2.22
V14001831	18.10	-	+	+	+	-	+	7/7	1	Contig 1	618	5	(3,2)	3.06
V14001545	19.08	+	+	-	-	-	+	6/8	1	Contig 1	643	4	(2,2)	2.83
V14000746	19.12	+	-	+	+	-	+	11/16	3	Contig 1	465	3	(1,2)	2.01
										Contig 2	703	4	(2,2)	2.26
										Contig 3	574	3	(1,2)	2.46
V17004616	33.00	-	+	-	+	+	+	9/12	1	Contig 1	62	2	(1,1)	1.76
V17004470	31.10	-	-	-	-	+	+	3/5	0	-	-	-	-	-
V17005728	26.69	+	+	+	-	+	+	12/13	2	Contig 1	634	2	(1,1)	1.49
										Contig 2	600	5	(3,2)	2.95
V17006129	31.90	+	+	-	-	-	+	4/5	1	Contig 1	665	2	(1,1)	1.91
V17006131	29.91	+	-	-	-	-	-	6/10	0	-	-	-	-	-
V17006286	33.07	-	+	+	-	-	+	3/3	0	-	-	-	-	-
V17004189	28.88	-	-	+	-	-	+	2/3	0	-	-	-	-	-
V17003665	30.09	-	-	-	-	-	+	2/2	0	-	-	-	-	-
V17004870	21.09	-	+	-	-	-	-	4/7	1	Contig 1	229	2	(1,1)	1.53
V17005031	31.54	-	-	+	+	+	+	5/8	0	-	-	-	-	-
V17004381	28.75	-	-	-	-	-	+	2/2	0	-	-	-	-	-

\*All without gaps

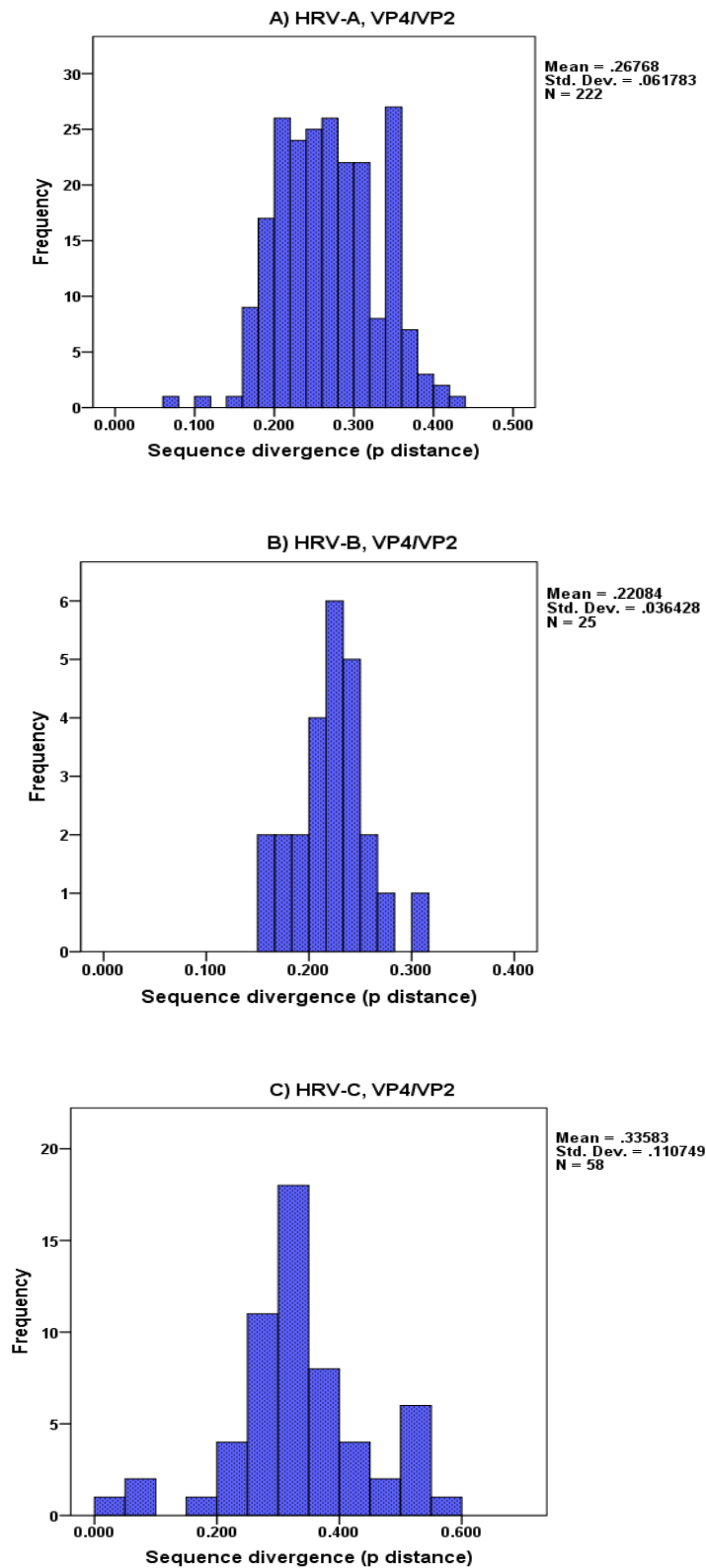
Pro Assembly algorithm parameters used for contigs assembly using SeqMan Pro (DNASTAR Lasergene version 15) are as the following: match size = 25, minimum match percentage = 70, minimum sequence length = 50, gap penalty = 0.00, gap length penalty = 0.00, match spacing = 150, maximum mismatch end bases = 15.

**TABLE S12. Description of the VP regions' sequences assembly.**

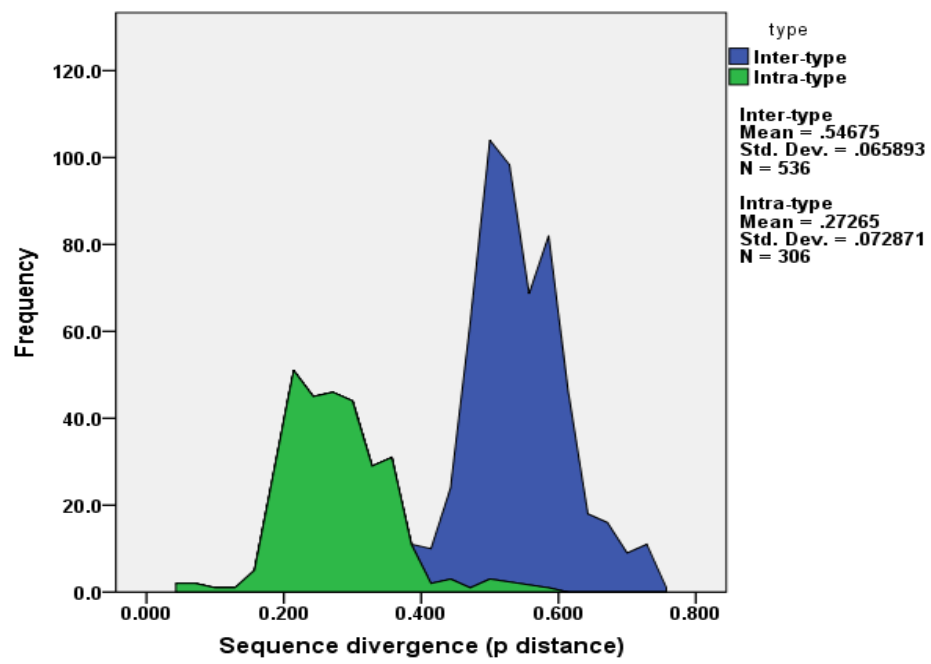
No.	Reaction name	Pre-trim length	Trimmed length	Sequence range	Contig	Average quality
<b>Sample V14002321</b>						
1.	11-V14002321-Y_ZF	647	541	(1>541)	Contig 1	30
2.	20-V14002321-AD_F92580	283	34	(24>57)	-	55
3.	20-V14002321-AD_R92379	326	68	(1>68)	Contig 2	19
4.	32-V14002321-WX_R92379	144	72	(16>87)	Contig 2	18
5.	45-V14002321-YZ_ZF	320	39	(28>66)	Contig 1	14
6.	45-V14002321-YZ	473	153	(1>153)	Contig 1	26
7.	V14002321-A_F92580	326	67	(3>69)	Contig 2	23
8.	4-V14002321-A_F92378	694	682	(1>682)	Contig 2	27
9.	4-V14002321-A_R92379	698	603	(1>603)	-	35
10.	11-V14002321-Y_YF	895	624	(1>624)	-	32
11.	11-V14002321-Y_YR	638	629	(4>632)	Contig 1	38
12.	32-V14002321-WX_XR	203	26	(22>47)	-	23
13.	25-V14002321-BE_FVP1F	386	75	(34>108)	-	14
14.	25-V14002321-BE_VP1R	411	92	(38>129)	-	12
<b>Average</b>		<b>460</b>	<b>265 (57%)</b>			<b>27</b>
<b>Sample V14001831</b>						
1.	12-V14001831-Y_ZF	670	502	(1>502)	Contig 1	28
2.	26-V14001831-BE_FVP1F	427	160	(23>182)	-	17
3.	26-V14001831-BE_R92379	426	219	(33>251)	-	10
4.	46-V14001831-YZ_ZF	280	57	(30>86)	Contig 1	38
5.	46-V14001831-YZ_ZR	436	108	(1>108)	Contig 1	27
6.	12-V14001831-Y_YF	722	598	(1>598)	Contig 1	21
7.	12-V14001831-Y_YR	682	610	(2>611)	Contig 1	26
<b>Average</b>		<b>520</b>	<b>322 (62%)</b>			<b>24</b>
<b>Sample V14001545</b>						
1.	13-V14001545-Y_YF	794	610	(1>610)	Contig 1	30
2.	13-V14001545-Y_YR	631	618	(1>618)	Contig 1	38
3.	13-V14001545-Y_ZF	544	500	(1>500)	Contig 1	26
4.	47-V14001545-YZ_ZR	398	72	(11>82)	Contig 1	22
5.	5-V14001545-A_F92378	408	286	(32>317)	-	17
6.	5-V14001545-A_R92379	362	332	(31>362)	-	16
<b>Average</b>		<b>523</b>	<b>403 (77%)</b>			<b>25</b>
<b>Sample V14000746</b>						
1.	14-V14000746-Y_YF	1000	548	(23>570)	Contig 3	30
2.	14-V14000746-Y_YR	641	493	(12>504)	Contig 3	25
3.	14-V14000746-Y_ZF	754	360	(1>360)	Contig 3	30
4.	21-V14000746-AD_F92580	463	321	(1>321)	Contig 2	47
5.	21-V14000746-AD_R92379	516	315	(2>316)	Contig 2	40
6.	27-V14000746-BE_FVP1F	907	417	(3>419)	Contig 1	39
7.	27-V14000746-BE_VP1R	628	418	(1>418)	Contig 1	37
8.	48-V14000746-YZ_ZR	276	68	(1>68)	-	16
9.	V14000746-BE_R92379	424	93	(22>114)	Contig 1	25
10.	6-V14000746-A_F92378	696	673	(2>674)	Contig 2	26
11.	6-V14000746-A_R92379	673	267	(26>292)	Contig 2	26
<b>Average</b>		<b>634</b>	<b>361 (57%)</b>			<b>31</b>
<b>Sample V17004616</b>						
1.	7-V17004616-Y_ZF	694	524	(36>559)	-	24
2.	22-V17004616-BE_R92379	438	394	(22>415)	-	15
3.	22-V17004616-BE_VP1R	239	48	(20>67)	-	10
4.	28-V17004616-WX_R92379	131	52	(20>71)	Contig 1	14
5.	28-V17004616-WX_XF	245	17	(25>41)	-	15
6.	34-V17004616-YZ_ZR	189	64	(1>64)	-	29
7.	7-V17004616-Y_YF	316	41	(27>67)	-	10
8.	7-V17004616-Y_YR	665	116	(23>138)	-	63
9.	22-V17004616-BE_FVP1F	353	53	(20>72)	Contig 1	18
<b>Average</b>		<b>363</b>	<b>146 (40%)</b>			<b>22</b>
<b>Sample V17004470</b>						
1.	29-V17004470-WX_XR	135	9	(42>50)	-	23
2.	35-V17004470-YZ_ZR	154	69	(1>69)	-	26
3.	29-V17004470-WX_XF	211	81	(15>95)	-	17
<b>Average</b>		<b>167</b>	<b>53 (32%)</b>			<b>22</b>
<b>Sample V17005728</b>						

1.	8-V17005728-Y_YF	882	595	(1>595)	Contig 2	29
2.	8-V17005728-Y_YR	840	508	(1>508)	Contig 2	23
3.	8-V17005728-Y_ZF	499	487	(2>488)	Contig 2	26
4.	15-V17005728-AD_R92379	287	59	(13>71)	-	13
5.	30-V17005728-WX_R92379	169	68	(20>87)	-	27
6.	36_V17005728-YZ_ZF	315	75	(21>95)	Contig 2	16
7.	36-V17005728-YZ_ZR	235	84	(5>88)	Contig 2	18
8.	1-V17005728-A_F92378	645	632	(1>632)	Contig 1	32
9.	1-V17005728-A_R92379	687	307	(2>308)	Contig 1	27
10.	V17005728-A_F92580	586	336	(111>446)	-	14
11.	30-V17005728-WX_XF	167	54	(25>78)	-	15
12.	30-V17005728-WX_XR	346	57	(25>81)	-	24
<b>Average</b>		<b>472</b>	<b>272 (58%)</b>			<b>22</b>
<b>Sample V17006129</b>						
1.	2-V17006129-A_R92379	634	634	(1>634)	Contig 1	32
2.	37-V17006129-YZ_ZR	291	115	(3>117)	-	23
3.	2-V17006129-A_F92378	637	626	(1>626)	Contig 1	30
4.	2-V17006129-A_F92580	290	183	(7>189)	-	15
<b>Average</b>		<b>463</b>	<b>390 (85%)</b>			<b>25</b>
<b>Sample V17006131</b>						
1.	3-V17006131-A_F92580	163	136	(28>163)	-	17
2.	9-V17006131-Y_YF	704	265	(19>283)	-	29
3..	9-V17006131-Y_YR	661	157	(17>173)	-	18
4.	9-V17006131-Y_ZF	519	466	(1>466)	-	28
5.	3-V17006131-A_F92378	640	286	(162>447)	-	17
6.	16-V17006131-AD_F92580	245	25	(28>52)	-	33
<b>Average</b>		<b>489</b>	<b>223 (46%)</b>			<b>24</b>
<b>Sample V17006286</b>						
1.	39-V17006286-YZ_ZF	213	8	(32>39)	-	73
2.	17-V17006286-AD_F92580	153	19	(15>33)	-	17
3.	17-V17006286-AD_R92379	156	47	(4>50)	-	15
<b>Average</b>		<b>174</b>	<b>25 (14%)</b>			<b>35</b>
<b>Sample V17004189</b>						
1.	40-V17004189-YZ_ZR	20	20	(61>80)	-	9
2.	18-V17004189-AD_R92379	46	46	(25>70)	-	28
<b>Average</b>		<b>33</b>	<b>33 (100%)</b>			<b>19</b>
<b>Sample V17003665</b>						
1.	41-V17003665-YZ_ZF	159	8	(17>24)	-	21
2.	41-V17003665-YZ_ZR	148	45	(25>69)	-	10
<b>Average</b>		<b>154</b>	<b>27 (18%)</b>			<b>16</b>
<b>Sample V17004870</b>						
1.	10-V17004870-Y_YR	320	176	(7>182)	Contig 1	31
2.	10-V17004870-Y_ZF	285	208	(17>224)	-	15
3.	10-V17004870-Y_YF	291	168	(19>186)	Contig 1	15
4.	23-V17004870-BE_FVP1F	139	52	(34>85)	-	12
<b>Average</b>		<b>259</b>	<b>151 (59%)</b>			<b>19</b>
<b>Sample V17005031</b>						
1.	24-V17005031-BE_FVP1F	243	47	(25>71)		27
2.	24-V17005031-BE_R92379	167	74	(94>167)		10
3.	24-V17005031-BE_VP1R	310	61	(20>80)		10
4.	43-V17005031-YZ_ZR	198	59	(1>59)		14
5.	19-V17005031-AD_F92580	159	57	(5>61)		15
<b>Average</b>		<b>215</b>	<b>60 (28%)</b>			<b>16</b>
<b>Sample V17004381</b>						
1.	44-V17004381-YZ_ZF	139	31	(45>75)	-	42
2.	44-V17004381-YZ_ZR	246	99	(6>104)	-	21
<b>Average</b>		<b>193</b>	<b>65</b>			<b>32</b>

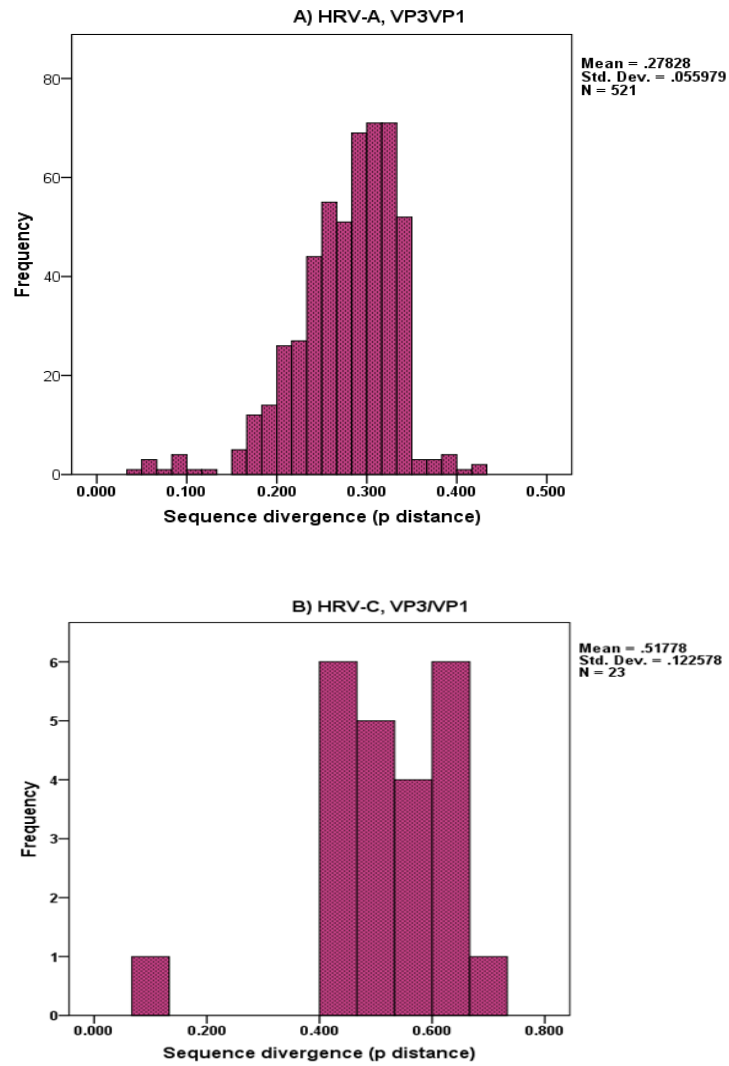
Trim ends parameters used: trace threshold = 8, quality threshold = 10, non-trace window size = 30.



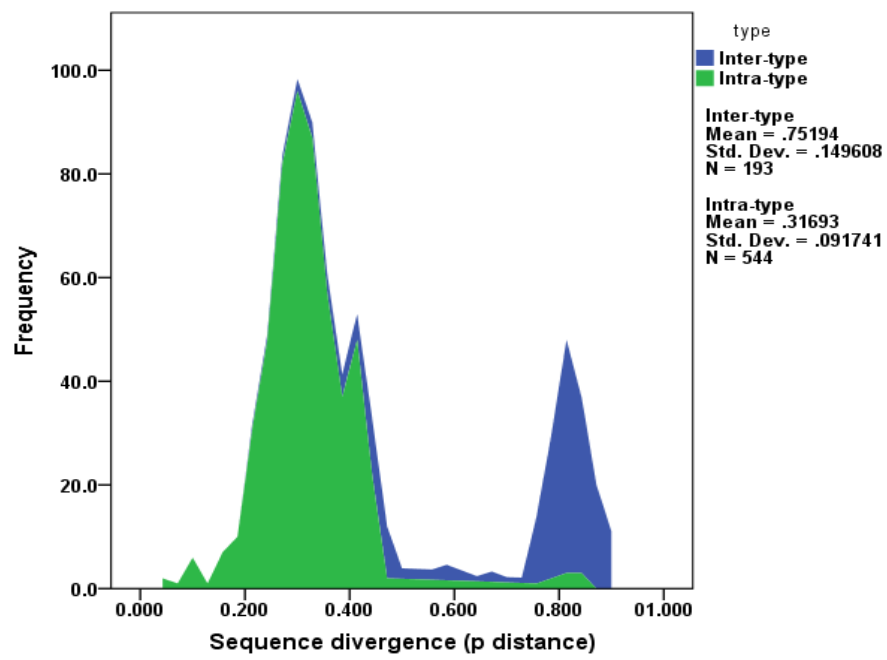
**FIGURE S5.** Distribution of nucleotide p-distances for HRV-A (A), HRV-B (B), and HRV-C (C) detected in the study compared with sequences from reference strains based on the nucleotide sequences of the VP4/VP2 region.



**FIGURE S6.** Distribution of pairwise nucleotide p-distances for the VP4/VP2 region of all HRV sequences.



**FIGURE S7. Distribution of nucleotide p-distances for HRV-A (A), and HRV-C (B) detected in the study compared with sequences from reference strains based on the nucleotide sequences of the VP3/VP1 region.**



**FIGURE S8.** Distribution of pairwise nucleotide p-distances for the VP3/VP1 region of all HRV sequences.

**TABLE S13. The multiple sequence alignments (MSAs) data and genotype identification.**

MSA no.	VP region	MSA length (bps)	Samples	Genotype	Bootstrap	Distance
MSA 1	VP4/VP2	512	V14001831_Contig_1	HRV-C_32	100	0.047
			V17005728_Contig_2	HRV-C_45	73	0.289
MSA 2	VP4/VP2	545	V14002321_Contig_1	HRV-A_21	100	0.065
			V14001831_Contig_1	HRV-C_32	100	0.052
			V14001545_Contig_1	HRV-B_79	55	0.150
			V14000746_Contig_3	HRV-A_1	N	0.196
MSA 3	VP4/VP2	142	R_41-V17003665-YZ_ZR	HRV-C_24	69	0.268
MSA 4	VP4/VP2	427	V17006131-Y_ZF	HRV-C_45	N	0.296
			V17004870-Y_ZF	HRV-C_32	N	0.927
MSA 5	VP4/VP2	310	V14002321-YZ_ZF	HRV-A_21	95	0.104
			V17006286-YZ_ZF	HRV-A_50	50	0.264
MSA 6	VP4/VP2	177	R_V17004616-YZ_ZR	HRV-A_98	N	0.173
			R_V14000746-YZ_ZR	HRV-A_1	N	0.211
			R_V17004470-YZ_ZR	HRV-A_13	N	0.236
MSA 7	VP4/VP2	189	R_V17006129-YZ_ZR	HRV-C_3	79	0.181
			R_V17004381-YZ_ZR	HRV-C_36	100	0.066
MSA 8	VP4/VP2	170	V17004870-Y_ZF	HRV-C	99	0.476
MSA 9	VP3/VP1	478	V14002321_Contig_2	HRV-A_21	99	0.096
			V14000746_Contig_2	HRV-A_40	99	0.089
			17005728_Contig_1	HRV-C_7	99	0.506
			V17006129_Contig_1	HRV-C_7	99	0.098
MSA 10	VP3/VP1	335	V14002321_Contig_2	HRV-A_21	97	0.046
			V14002321-AD_F92580	HRV-A_45	86	1.883
			V14000746_Contig_2	HRV-A_40	96	0.055
			V17005031-AD_F92580	HRV-C_7	86	2.191
MSA 11	VP3/VP1	81	V14002321-AD_F92580	HRV-A_21	80	0.120
			V17005031-AD_F92580	HRV-A_47	52	0.087
MSA 12	VP3/VP1	104	R_V14001545_A_R92379	HRV-A_40	63	0.112
			R_V17005728-WX_R92379	HRV-A_78	83	0.133
MSA 13	VP3/VP1	101	V14002321_Contig_2	HRV-A_21	99	0.053
			R_V17005728-WX_R92379	HRV-A_78	82	0.133
MSA 14	VP3/VP1	261	V14002321_Contig_2	HRV-A_21	99	0.072
			V17004616-WX_XF	HRV-A_59	58	0.208
			V17004470-WX_XF	HRV-A_41	N	0.634
MSA 15	VP3/VP1	364	V14002321_Contig_2	HRV-A_21	100	0.059
			R_V14001545_A_R92379	HRV-A_21	100	0.179
MSA 16	VP3/VP1	410	V14000746_Contig_1	HRV-A_21	99	0.114
			R_V14001831_BE_R92379	HRV-A_21	99	0.118

MSA: Multiple Sequence Alignment, N: not significant bootstrap (< 50%).



**TABLE S14. A summary of recombination events detected in the multiple sequence alignments.**

MSA no.	Overall Rec. no.	Study Sample	Major Parent	Minor Parent	R	B	M	C	S	T
MSA 1	6	-	-	-	-	-	-	-	-	-
MSA 2	6	V14000746_Contig_3	A_11	B_37	$2.18 \times 10^{-7}$	$1.30 \times 10^{-2}$	$1.16 \times 10^{-3}$	$1.71 \times 10^{-4}$	$7.08 \times 10^{-3}$	$7.91 \times 10^{-4}$
MSA 3	-	-	-	-	-	-	-	-	-	-
MSA 4	1	-	-	-	-	-	-	-	-	-
MSA 5	2	V17006286-YZ_ZF	A_50	Unknown	$1.64 \times 10^{-3}$	-	-	-	-	$1.24 \times 10^{-3}$
MSA 6	1	R_V17004470-YZ_ZR	Unknown	A_19	$3.80 \times 10^{-2}$	-	-	-	-	-
MSA 7	-	-	-	-	-	-	-	-	-	-
MSA 8	-	-	-	-	-	-	-	-	-	-
MSA 9	1	-	-	-	-	-	-	-	-	-
MSA 10	1	-	-	-	-	-	-	-	-	-
MSA 11	-	-	-	-	-	-	-	-	-	-
MSA 12	-	-	-	-	-	-	-	-	-	-
MSA 13	-	-	-	-	-	-	-	-	-	-
MSA 14	1	V17004616-WX_XF	A_100	Unknown	$1.71 \times 10^{-2}$	-	$4.93 \times 10^{-2}$	-	-	$2.88 \times 10^{-2}$
MSA 15	-	-	-	-	-	-	-	-	-	-
MSA 16	-	-	-	-	-	-	-	-	-	-

The recombination detection programmes used: RDP (R), Bootscan (B), Maximum X2 (M), Chimaera (C), SiScan (S) and 3Seq (T).

The recombination events were assessed by the average p values of recombination events with  $<1.00 \times 10^{-5}$  considered significant. None of the recombinant sequences was detected by more than two programmes with the average p values of recombination events of  $< 1.00 \times 10^{-5}$ .

**Abbreviations:** MSA: multiple sequence alignment; Rec: recombination.

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