

**Table S3 - Selective pressure in rupintrivir contacts.****Data S3 – Consensus structures HRVA and HRVB 5’NCR elements R1-R7.**

Consensus secondary structure and sequence of HRVA and HRVB 5’NCR elements identified in Witwer, C et al., 2001). Circled residues, positions where compensatory substitutions are detected in the alignment. Gray residues indicate positions where substitutions that disrupt basepairing potential are detected in the alignment. (PDF)

**Data S4 – Consensus structures HRVA and HRVB 3’NCR element.**

Consensus secondary structure and sequence of HRVA and HRVB 3’NCR element identified in Witwer, C et al., 2001). Circled residues, positions where compensatory substitutions are detected in the alignment. Gray residues indicate positions where substitutions that disrupt basepairing potential are detected in the alignment. (PDF)

**Figure S8 – Analysis of HRVA and HRVB cis-replicating elements (CREs).**

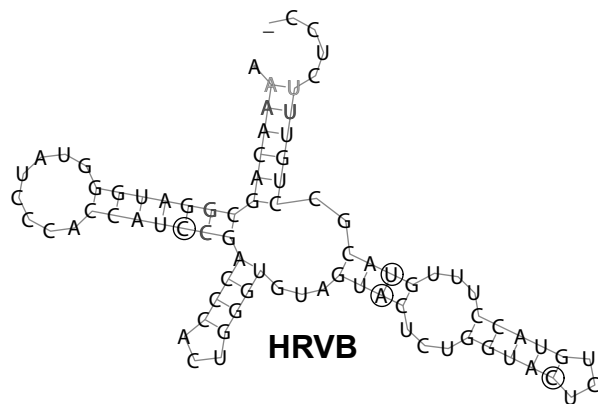
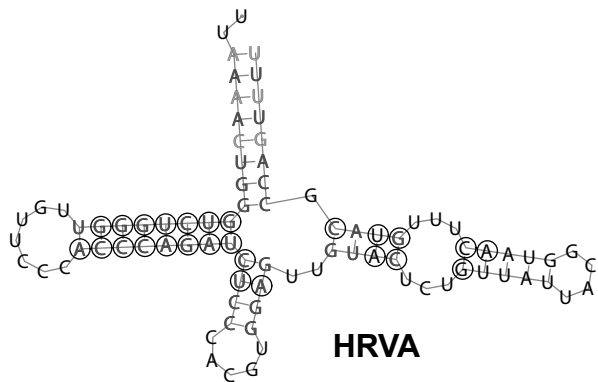
ClustalW alignment of HRVA (A) and HRVB (B) region of the P2A gene (HRV002 nucleotides 3268 – 3302) in which the minimal functional CRE has been identified for a member of the HRVA subgroup (Gerber, K. et al., 2001). ClustalX alignment of HRVA (A) and HRVB (B) region of the VP1 gene (HRV014 nucleotides 2353 to 2386) in which the minimal functional CRE has been identified for a member of the HRVB subgroup (McKnight, KL and Lemon, SM, 1998; Yang, Y et al., 2002). Shorthand for consensus secondary structures deduced from each of these alignments (Hofacker, I et al., 2004) are depicted above with parentheses above the clustalx alignment indicate base-paired nucleotides, while dots or commas indicate unpaired nucleotides.. (PDF)

Table S3: Selective pressure in ruprintrivir contacts<sup>a</sup>

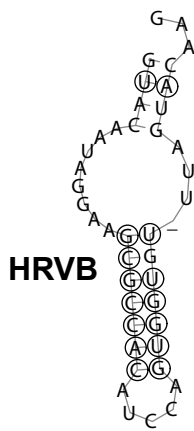
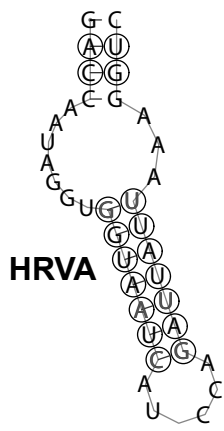
Residue number <sup>b</sup>	dN/dS value
25	0.05
40	0.05
71	0.05
125	0.05
127	0.05
128	0.05
130	0.05
142	0.05
147	0.05
161	0.05
163	0.05
164	0.05
165	0.05

<sup>a</sup>HRV2 contacts with ruprintrivir, a protease inhibitor targeted to the 3C protease active site (Binford, SL et al., 2005. Antimicrobial Agents Chemother. 49, 619-26). <sup>b</sup>Residue number indicates residue within the 3C protease relative to N-terminal proteolytic cleavage site.

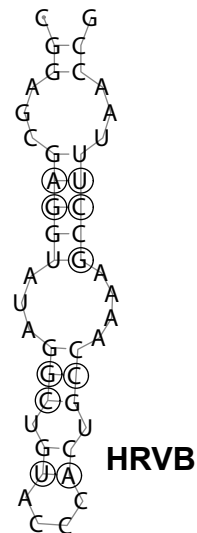
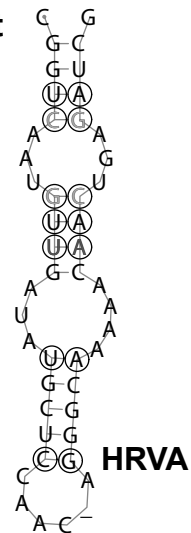
## A. R1 cloverleaf element



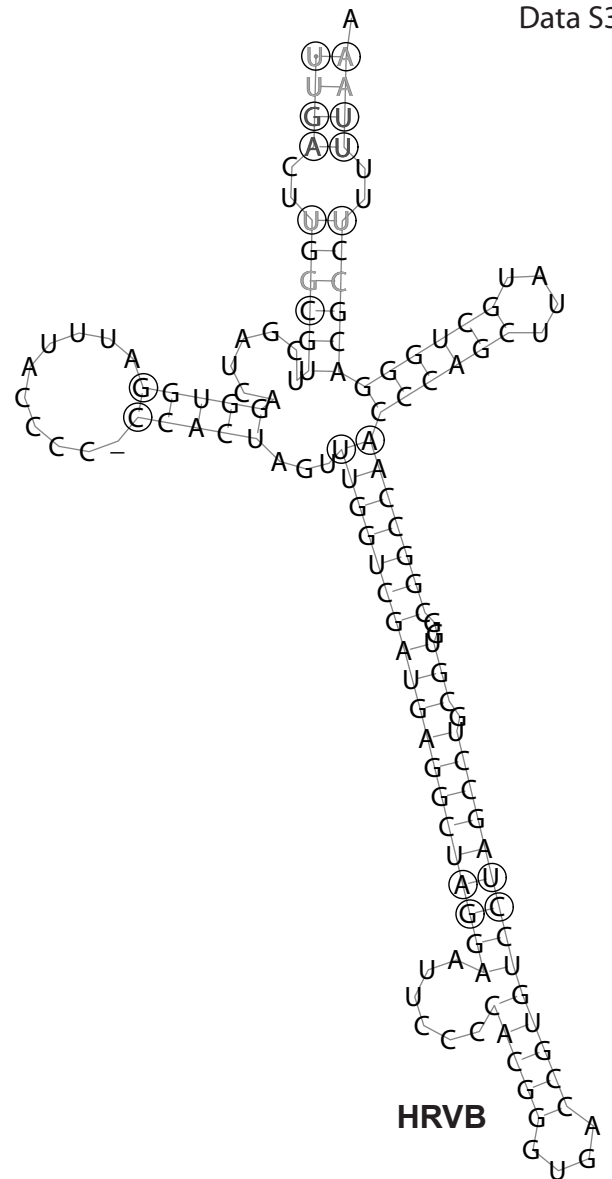
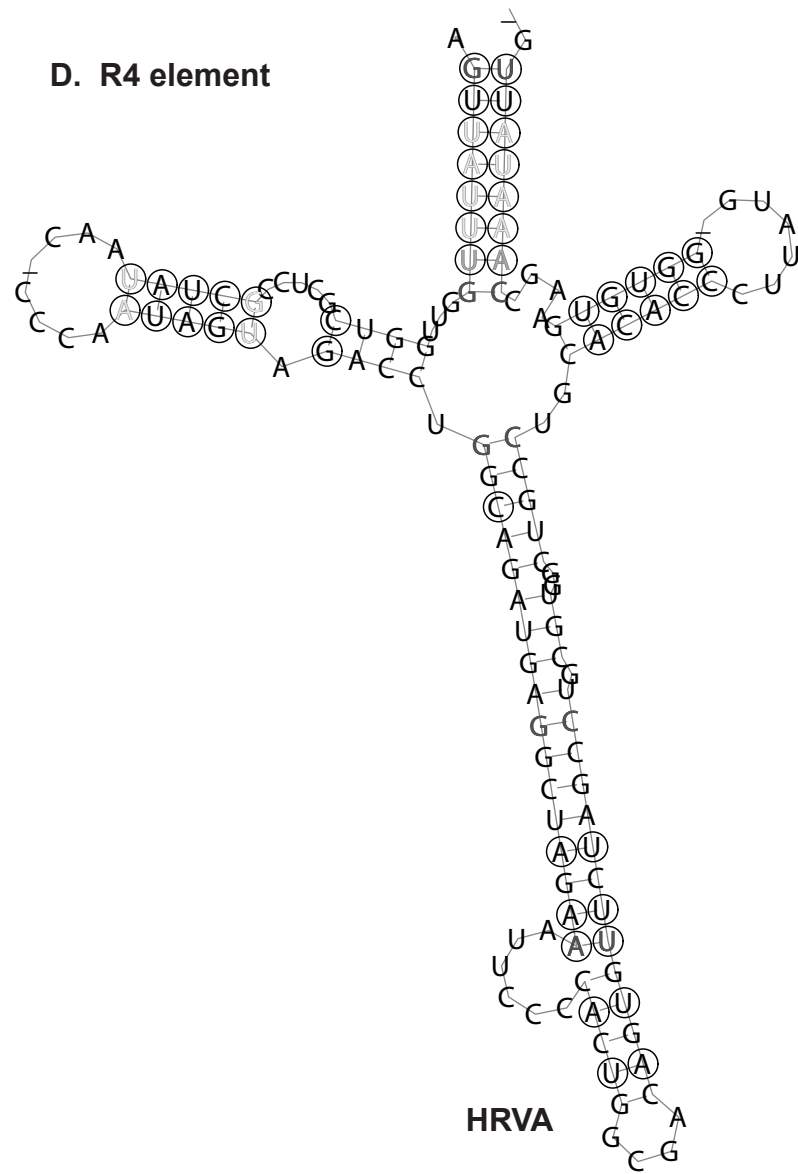
## B. R2 element



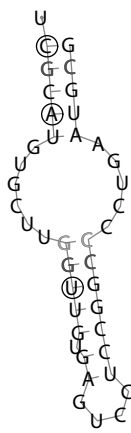
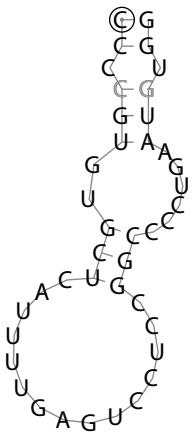
## C. R3 element



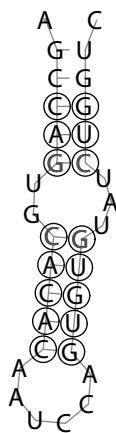
## D. R4 element



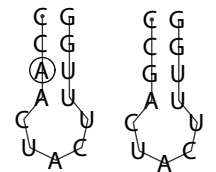
## E. R5 element



## F. R6 element



## G. R7 element



HRVA

HRVB

HRVA

HRVB



Fig. S8AB

.((((...(((.....))))...))).

A

HRV075	AATCATTTC	CCGAACAAAC	CAAAAGGT	GATGATT
HRV088	AATCATTTC	CCGAACAAAC	CAAAAGGC	GATGATG
HRV030	AATCATTTC	CCGAACAAAC	ACTGTAGGT	GATGATT
HRV002	AATCATTTC	CCGAACAAAC	ACTGTAGGT	GATGATT
HRV023	AATCATTTC	CCGAACAAAC	ACTATAGGT	GATGATT
HRV049	AATCATTTC	CCGAACAAAC	ACCATAGGT	GATGATT
HRV-Hanks	AGTCATTTC	CCGAACAAAC	ACCACAGG	GATGACT
HRV060	AGTCATTTC	CCGAACAAAC	ACCACAGG	GATGACT
HRV021	AGTCATCTA	CCGAACAAAC	ACCACAGG	GATGACT
HRV082	AGTCATCTA	CCGAACAAAC	ACCACAGG	GATGACT
HRV038	AGTCATTTC	CCGAACAAAC	ACAACAGG	GATGACT
HRV062	AGTCATTTC	CCGAACAAAC	ACAACAGG	GATGACT
HRV031	AGTCATTTC	CCGCACAAAC	ACTACAGG	CAATGACT
HRV066	AGTCATTTC	CCGCACAAAC	ACTACAGG	CAATGACT
HRV047	AGTCATCTA	CCGCACAAAC	ACTACAGG	CAATGACT
HRV056	AGTCATTTC	CCGCACAAAC	ACTACAGG	GATGACT
HRV044	AGTCATTTC	CCGAACAAAC	ACCACAGG	TAATGACT
HRV029	AGTCATTTC	CCGAACAAAC	ACCACAGG	TAATGACT
HRV025	AGTCATTTC	CCGAACAAAC	ACAACAGG	TAATGACT
HRV100	AGTCATTTC	CCGAACAAAC	ACTACAGG	TAATGACT
HRV010	AGTCATTTC	CCGAACAAAC	ACTATAGG	TAATGACT
HRV020	TGTCATTTC	CCGAACAAAC	ACAACAGG	GATGACA
HRV068	TGTCATCTA	CCGAACAAAC	ACAACAGG	GATGACA
HRV046	TGTCATTTC	CCGCACAAAC	ACTACAGG	GATGACA
HRV053	TGTCATTTC	CCGCACAAAC	ACTACAGG	GATGACA
HRV028	TGTCATTTC	CCGAACAAAC	ACGACAGG	TAATGATG
HRV057	AGTTATCTA	CCGAACAAAC	ACTACAGG	GATGACT
HRV022	AGTCATCTA	CCGAACAAAC	ACTACAGG	GATGATT
HRV013	AGTCATCTA	CCGAACAAAC	ACTATAGG	GATGACT
HRV041	AGTCATCTA	CCGAACAAAC	ACTATAGG	GATGACT
HRV015	AGTCATTTC	CCGAACAAAC	ACTAAAGG	GATGACT
HRV073	AGTCATTTC	CCGAACAAAC	ACTATAGG	GATGACT
HRV033	AGTCATATA	CCGAACAAAC	ACTACAGG	GATGACT
HRV076	AGTCATATA	CCGAACAAAC	ACTACAGG	GATGACT
HRV018	AGTCATATA	CCGAACAAAC	ACAACAGG	GATGACT
HRV074	AGTCATATA	CCGAACAAAC	ACCAAGG	GATGACT
HRV039	AGTCATATA	CCGAACAAAC	ACCCAAGG	GATGACT
HRV071	AGTCATATA	CCGAACAAAC	ACGGAAGG	GATGACT

B

HRV006	GGCAGTTGT	GTTTACTGG	AGCACATGG	CGCAGAGA
HRV079	AGCCATAGT	GTTTACAGG	TGCACATGG	GGGCTGAGA
HRV072	AGCAATTGT	TTC AACAGG	AGCACATGG	TGCAGAGA
HRV014	AGCAATAGT	CTCAACAGG	AGGACATGG	TGCAGAAA
HRV037	AGCAATTGT	ATACACAGG	TGCACATGG	TGCGGAAA
HRV035	AGCCATTGT	AGCAACAGG	TGCTCATGG	TGCAGAAA
HRV092	AGCAGTAGT	ATCAACTGG	TGCTCATGG	AGCAGAGA
HRV003	AGCAGTAGT	TGCTACTGG	AGCACATGG	AGCAGAAA
HRV070	AGCAGTAGT	AGCAACAGG	AGCACAGGG	CTCAGAAA
HRV052	AGCAGTTGT	AGCAACCGG	AGCACAGGG	CTCAGAAA
HRV017	GGCAGTAGT	GGCAACAGG	AGCGCTGGG	TT CAGAAA
HRV048	GGCAGTTGT	GGCAACAGG	AGCGCAAGG	CTCAGAAA
HRV091	GGCAGTGGT	AGCAACAGG	TGCACAAGG	CTCAGAAA
HRV069	GGCAGTGGT	AGCAACGGG	GGCACAAGG	TCAGAGA
HRV083	GGCCGTGGT	GTC AACAGG	TGCTCATGG	TGCTGAAA
HRV042	TGCCATCGT	GAGCACGGG	TGCTCACGG	TT CAGAGA
HRV093	AGCAGTGGT	TAAACATTA	ATGCCCATGG	ATCAGAGA
HRV097	GGCAGTAGT	CAACATAAA	TGCTCATGG	TT CCGAGA
HRV027	AGCAGTAAAT	TAGTATAAA	TGCTCATGG	TT CAGAAA
HRV084	AGCAATAAT	CAACCTAAAT	TGGGCATGG	TT CAGAAA
HRV005	AGCCATTAT	CAACATAGG	TGCTCATGG	CTCAGAAA
HRV026	GGCTGTAGT	TGGGGTGGG	TGCCCATGG	TT CAGAAA
HRV004	GGCAATAGT	TGATGCAAGG	GCTCACGG	TTCTGAAA

Fig. S8CD

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HRV28 AGCACTACTTAAATGAAGTGCTTGTGGTCCAAAC

HRV53 GGCTATCTTAAATGAAATATTAGTAGTCCCAAAAC

HRV20 AGCTATTTTAAATGAAATCTTGTAGTCCCAAAAT

HRV68 AGCCGTCTTAAATGAGGTCTTGTGGTCCAAAC

HRV51 GGCTTTACTCAATGAAATGTTGGTGGTCCCAAC

HRV65 AGCTTACTTAAATGAAATGTTGGTGGTCCCAAAAT

HRV71 AGCAATACTCAATGAGGTCTTAGTTGTGCCAAAT

HRV80 AGCAATCCTCAATGAAGTCTTGTGTACCAAAAC

HRV46 AGCTGTCTTAAATGAGGTCTTGTAGTACCTAAC

HRV78 TCAGGTCTTAAATGAGGTCTTGTAGTTGTCCAAAC

HRV12 TGAGGTCTTAAATGAAATCTTAGTTGTCCAAAC

HRV95 GGCAGTATTAAACGAGGTCTTGTAGTTCCAAAT

HRV8 GGCAGTATTAAACGAGGTCTTGTAGTTCCAAAC

HRV45 AGCAGTCTTGAATGAAATATTAGTAGTCCAAAC

HRV88 TAACCTGTTAAACGAAATGCTAGTAGTCCCTAAC

HRV7 TAGCTTACTTAAATGAAGTATTAGTGGTACCTAAC

HRV36 TAATGTATTAAATGAAATCTTGTAGTCCCAAAAC

HRV089 TAGTGTATTAAATGAAATCTTGTGGTCCCAAAAT

HRV58 TAATGTGTTAAATGAAATCTTGTAGTTCCAAAT

HRV21 TGAAGTCTTAAATGAAGTCTTAGTAGTCCCAAAAT

HRVHanks TGAAGTCTTAAATGAGGTCTTAGTAGTCCCAAAAT

HRV55 TGAAGTCTTGAATGAAATGCTAGTAGTCCAAAT

HRV57 TGAGATCTTGAATGAAATGTTGGTGGTCCAGAT

HRV24 TGAAGTTTGAATGAAGTACTGGTTGTGCCCTAAC

HRV90 TGAAGTCTTAAATGAGGTATTGGTTGTCCCTAAC

HRV33 AGAGGTGTTGAATGAGGTCTTAGTAGTCCCTAAC

HRV76 TGAGATATTAAATGAGGTCTTGTAGTACCAAAAC

HRV11 TGGAACTCTTAAATGAGGTCTTAGTTGTACCTAAC

HRV34 TGAGGTACTTAAATGAGGTATTGGTTGTACCAAAAC

HRV50 TGAAGTATTAAATGAGGTATTAGTTGTCCCAAAAT

HRV18 TGAAGTACTTAAATGAAATGTTAGTGGTCCCAAAAT

HRV75 TGAAGTTTTAAACCAAGTCTTGGTAGTTCCAAAC

HRV43 TGAAGTTTTAAATCAAGTCTTGTAGTCCCAAAAC

HRV81 TGAAGTCTTAAATGAGGTATTGGTAGTCCCTAAC

HRV016 TGAAGTCTTAAATGAAATGTTAGTAGTGCCCAAT

HRV29 TGAGGTGCTTAAATGAAGTTTTAGTTGTGCCCTAAC

HRV44 TGAAGTACTTAAATGAAGTCTTAGTTGTGCCCTAAC

HRV25 TCAAGTACTTAAATGAGGTCTTAGTCTGACCAAAAT

HRV62 TCAAGTACTTAAATGAAGTTTTAGTTGTACCAAAAT

HRV31 AGAGGTCTTAAATGAAGTCTTAGTAGTACCTAAC

HRV47 AGAGGTACTTAAATGAGGTCTTAGTTGTACCAAAAT

HRV100 AGGTGTGCTGAAATGAAATACTAGTGGTACCTAAC

HRV10 TAATGTACTTAAATGAAGTCTTAGTAGTACCAAAAC

HRV77 TAATGTACTTAAATGAAGTCTTAGTAGTACCAAAAT

HRV66 GGGTGTTTTAAATGAAGTTTTAGTAGTACCAAAAC

HRV85 TGAAGTCTTAAATGAGGTCTTAGTGGTCCCAAAAT

HRV40 TGAGGTCTTAAATGAAGTCTTGGTAGTTCCCAAAAT

HRV56 TGATGTTTTAAATGAAGTTTTAGTTGTCCCAAAAT

HRV54 TGAAGTGTAAATGAAGTGTAGTTGTCCCAAAAC

HRV98 TGAAGTATTAAATGAAATGTTAGTTGTCCCAAAAC

HRV59 TGATGTACTTAAATGAGGTCTTAGTAGTACCAAAAT

HRV63 TGATGTCTTAAATGAAGTGTAGTTGTCCCAAAAC

HRV39 TGAAGTATTAAATGAGGTATTAGTTGTCCCTAAC

HRV1A TGAAGTTTTAAATGAAGTTTTAGTAGTGCCGAAT

HRV001b TGAAGTTTTAAATGAAGTCTTAGTAGTACCAAAAT

HRV94 TGGTGTATTGAATGAAGTTTTAGTTGTCCCAAAAT

HRV64 TGGTGTGTTGAATGAAGTTTTGATTGTCCCAAAAC

HRV22 TGGTGTATTGAATGAAGTATTGGTTGTCCCAAAAC

HRV82 TAGTGTTTTAAACGAGGTATTAGTTGTCCCTAAC

HRV19 CACCACTCTGAATGAGGTGCTAGTTGTCCCAAAAT

HRV74 TGAAGTGTGAATGAAGTCTTAGTTGTCCCAAAAT

HRV15 TGAAGTGTAAATGAGGTCTTAGTAATCCAAAC

HRV38 TGGAGTCTTGAATGAGGTCTTGTGGTCCGAAC

HRV60 TGGGGTCTTAAACGAAGTCTTGTGGTCCCAAAAC

HRV32 TCAAGTTTTAAATGAAGTTTTGGTAGTTCCCAAAAC

HRV9 TCAGGTGCTTAAATGAGGTCTTGGTAGTCCCAAAAC

HRV67 TCAGGTATTGAATGAGGTCTTGGTGGTCCCAAAAT

HRV13 TGAAGTCTTGAATGAAGTCTTGTAGTACCAAAAT

HRV41 TGAGGTCTTGAATGAGGTCTTGTGGTACCAAAAT

HRV73 TGAAGTTTTGAATGAAGTCTTGTAGTACCAAAAT

HRV96 TGAAGTCTTGAATGAGGTCTTGTAGTCCCTAAC

HRV61 TGAAGTTTTAAATGAAGTCTTGTAGTCCCAAAAC

HRV30 TGAAGTCTTGTAGTGAAGTTTTAGTTGTCCCAAAAC

HRV23 TGAAGTCTTAAATGAAGTCTTAGTTGTCCCAAAAT

HRV2 TGAAGTCTTAAATGAAGTTTTAGTTGTCCCAAAAT

HRV49 TGAGGTCTTAAATGAGGTCTTAGTTGTCCCAAAAT

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HRV52 AGTTGTTGTCGACAAAATGAGACAGATAACAGCT

HRV48 AGTTGTTGTTGACAAAATGAAACAGATAACAGCT

HRV70 AGTTGTTATCGATAAAAATGAAACAGGTAAACAGCT

HRV17 AGTTGTTATGACAAAATGAAACAGGTAAACAGCT

HRV91 AGTTGTTATTGACAAAATGAAACAGGTAAACAGCT

HRV69 AGTTGTTATCGATAAAAATGAAACAGGTGACAGCT

HRV83 AGTTATTGTCGAGAAAACGAGACAGACTTAGCT

HRV92 AGTCATTGTTGAGAAAACGAAACAGACATTGGCT

HRV79 GGTCAATTGTCGAGAAAACGAGACAGACATTGGCC

HRV72 AGTCATCGTTCGAGAAAACGAAACAGACGTTGGCT

HRV014 AGTCATCGTTCGAGAAAACGAAACAGACGGTGGCC

HRV6 AGTTATTGTCGAGAAAACGAAACAGACACTAGCT

HRV37 AGTTATTGTTGAGAAAACGAAACAGACACTAGCT

HRV3 AGTCATTGTCGAGAAAACGAAACAGACATTGGCC

HRV35 AGTCATTGTCGAGAAAACGAAACAGACAGTGGCT

HRV86 AGTCATAGTCGAAAACGAAACAGACATTGGCT

HRV93 AGTAAATAGTCGACAAACGAAACAGACTATTGCT

HRV27 AGTGATAGTCGACAAACGAAACAGACTATTGCT

HRV97 AGTAAATAGTCGACAAACGAAACAGACTATTGCT

HRV84 AGTTATAGTTGATAAAGCAAAACAGACTATAGCT

HRV5 AGTCATTGTTGACAAACGCAACAAACATTGGCT

HRV42 AGTCATTGTTGACAAAACGAGCAAAACACTGGCT

HRV99 AGTCATTGTTGACAAAACGAGCAAAACACTGGCT

HRV26 AGTTATTGTTGACAAAACGAGCAAAACACTAGCT

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