

SUPPLEMENTAL MATERIAL TO

Hepatitis E virus genotype 3 genomes from RNA positive but serologically negative plasma donors have CUG as start codon for ORF3

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Supplemental Table S1

Sets of primers used to amplify different HEV genome fragments

Primer Set	Primer name	Nucleotide sequence
1	1 F1	TCGATGCCATGGACGCCAYCA
	1 F2	TCGATGCCATGGAGGCCAYCA
	391 R1	GCAAAGCGGAGCGGCGCAGTT
	391 R2	GCAAAGCRGARCGGCGCAATT
2	3G-22 F1	TCGATGCCATGGAGGCCAYCAATTYAT
	3G-22 F2	TCGATGCCATGGAGGCCAYCAGTTYAT
	3G-1272 R1	GGATGTARTCRCGRCTGACTTCTCAA
	3G-1272 R2	GGATGTARTCRCGRCTGACTTCTCGAA
	3G-1272 R3	GGATGTARTCRCGRCCGATTTCTCAA
	3G-1272 R4	GGATGTARTCRCGRCCGATTTCTCGAA
	3G-1272 R5	GGATGTARTCRCGRCCGACTTCTCAA
	3G-1272 R6	GGATGTARTCRCGRCCGACTTCTCGAA
	3G-68 F1	TACTGCCATTGAGCAGGCTGCTCTG
	3G-68 F2	TACTGCCATTGAGCAAGCTGCTCTG
	3G-68 F3	TACTGCCATCGAGCAGGCTGCTCTG
	3G-68 F4	TACTGCCATCGAGCAAGCTGCTCTG
	gt3-3253 S1	GAGCTTGCCCCGACCAGTTGGTG
	gt3-3253 S2	GAGCTTGCGCCCACCAGTTGGTG
	gt3-3253 S3	GAGCTTGACCCACCAGCTGGTG
	gt3-3253 S4	GAGCTAGCACCTACCAGTTGGTG
	gt3-3253 S5	GAGCTTGCCCCAACCAGCTGGTG
	gt3-3253 S6	GAGCTTGACCRACGAGTTGGTG
	gt3-3253 S7	GAGCTTGACCAACAAGTTGGTG
	gt3-3253 S8	GAGCTYGCCCCAACTAGTTGGTG
	gt3-3253 S9	GAGCTWGCYCCTACTAGTTGGTG
	gt3-3253 S10	GAGCTTGCBCCACTAGTTGGTG
	gt3-3253 S12	GAGCTTGCDCCAACGAGCTGGTG
	gt3-3253 S13	GAGCTTGCGCCGACGAGCTGGTG
	gt3-3253 S14	GAGCTTGCCCTCACTAGCTGGTG
	gt3-3253 S15	GAGCTCGCCCCTACTAGCTGGTG
	gt3-3253 S16	GAGCTTGCVCCTAACTAGCTGGTG
	gt3-3253 S17	GAGCTTGCGCCAACGCGCTGGTG
	gt3-3253 S18	GAGCTTGACCAACAAGCTGGTG
	gt3-3253 S19	GAGCTTGACCAACGACCTGGTG
	gt3-3253 S20	GAGCTTGCCCCAACCACCTGGTG
	gt3-3253 S21	GAGCTCGCCCCAACCGCTGGTG
	gt3-3253 S22	GAACTTGTCCCAACAAAGTGGTG
3	gt3-4466 AS1	ACTGGGGCATGCCGCACTCTCCATGA
	gt3-4466 AS2	ACTGGGGCATGCCACACTCTCCATGA
	gt3-4466 AS3	ACTGGGGCATGCCACATTCCTCCATGA
	gt3-4466 AS4	ACTGAGGCATACCGCATTCCTCCATGA

	gt3-4466 AS5	ACTGGGGCATGCCGCACTCCTCCATGA
	gt3-4466 AS6	ACTGGGGCATGCCRCATTCTCCATAA
	gt3-4466 AS7	ATTGGGGCATRCCGCACTCCTCCATAA
	gt3-4466 AS8	ACTGGGGCATRCCACACTCCTCCATAA
	gt3-4466 AS9	ACTGGGGCATGCCRCAYTCTTCCATAA
	gt3-4466 AS10	ACTGGGGCATACCACACTCTTCCATAA
	gt3-3294 S1	CCCGGCCGATGTATGCGAGCTCATTCG
	gt3-3294 S2	TCCAGCTGATGTCTGTGAGTTAATTCG
	gt3-3294 S3	CCCGGCCGATGTATGTGAGCTTATCCG
	gt3-3294 S4	TCCTGCCGAYGMTGTGAGCTCATACG
	gt3-3294 S5	CCCGGCCGAYGTATGYGAGCTCATACG
	gt3-3294 S6	CCCGGCCGAYRTGTGCGAGCTCATACG
	gt3-3294 S7	CCCGGCCGATGTGTGYGARCTCATACG
	gt3-3294 S8	CCCGGCCGATGTNTGTGAACTCATACG
	gt3-3294 S9	CCCGCTGAYGTTTGTGAGCTCATACG
	gt3-3294 S10	CCCAGCCGAYGTGTGTGAGCTTATACG
	gt3-3294 S11	CCCGGCCGAYGTATGTGAGCTTATACG
	gt3-3294 S12	CCCAGCTGATGTATGTGAACTTATACG
	gt3-3294 S13	CCCGCTGAYGTGTGCGAGCTTATACG
4	gt3-4095 S1	GTYTTTGARCTYACGGACATAGT
	gt3-4095 S2	GTYTTTGARCTCACAGATATAGT
	gt3-4095 S3	GTYTTTGARCTYACGGATATAGT
	gt3-4893 AS1	CTACARAGRACYACYGAATCATC
	gt3-4893 AS2	CTACARAGRACCACYGAGTCATC
	gt3-4893 AS3	CTACARAGRACCACYGAATCGTC
	gt3-4829 AS1	AATGYGCTATRATTGCCATGTTC
	gt3-4829 AS2	AATGYGCTATRATCGCCATATTC
	gt3-4829 AS3	AATGYGCTATRATCGCCATGTTC
	4532 F1	GGCCTGGATCCTACAGGCCCCGAA
	4532 F2	AGCCTGGACTCTACAGGCCCCGAA
	4532 F3	GGCCTGGAYTCTGCAGGCCCCGAA
	4532 F4	GGCCTGGACCCTGCAGGCCCCGAA
	4532 F5	GGCCTGGATMCTACAGGCGCCGAA
	4532 F6	TGCCTGGATTTTACAGGCGCCGAA
	4532 F7	CGCCTGGATTTTGCAAGCGCCGAA
	4532 F8	GGCCTGGATCCTACAGGCACCGAA
	4532 F9	GGCTTGGACTTTGCAGGCTCCGAA
5	5563 R1	GACGTAAGCGGGGACGTGGACAGATT
	5563 R2	GACGTGAGAGGGGACGTAGACAGATT
	5563 R3	GATGTAAGYGGGGATGTGGATAAATT
	5563 R4	GATGTGAGCGGGGATGTGGATAAATT
	5563 R5	GACGTAAGCGGGGACGTGGACAAATT
	5563 R6	GAAGTCAGAGGTGAAGTAGACAAATT
	5563 R7	GATGTAAGCGGGGACGTGGACAAGTT
	gt3-5320 S1	ACAGCGGBTAGAATGAATAACATGT
6		

gt3-5320 S2 ATATCGGGTGGGAATGAATAACATGT
gt3-5320 S3 ACAGAGGYTGGGAATGAATAACATGT

gt3-6559 AS1 GCCATTGGCYGAGACAAC
gt3-6559 AS2 GCCATTGGCYGAGACGAC

gt3-5508 S1 CTTGCGCCTCCCCTATATTC
gt3-5508 S2 CTTGCGCACTCCCCTATATTC
gt3-5508 S3 CTTGCGCCATCCCCTATATTC

6074 F1 AGTTCAGGAACCTGACACCCGGAATAC
6074 F2 AGTTTAGGAATTTGACACCCGGAAATAC
6074 F3 AATTTAGAAATCTGACACCCGGAATAC
6074 F4 AGTTTAGAAACCTGACACCCGGAACAC
6074 F5 AGTTTAGGAACCTGACGCCCCGGAACAC
6074 F6 AGTTTAGGAATCTGACACCCGGAACAC
6074 F7 AATTTAGGAATCTGACCCCCGGAACAC

3G-7127 R ACTCCCGGGTCTTACCTACCTTCATTTTAA
7103 R1 ACTCCCGGGTTTTACCTACCTTCATTTTAA
7103 R2 ACTCCCGGGTYTTGCCTACCTTCATTTTAA
7103 R3 ACTCCCKGGTCTTACCTACCTTCATTTTAA
7103 R4 ACTCCCGGGTTTTGCCTACCTCCRTTTTAA
7103 R5 ACTCCCGGGTTTTRCCTACCTTCGTTTTAA
7103 R6 ACTCCCGGGTTTTACCTACCTTCATCTTAA
7103 R7 ACTCCCGGGTTTTACCCACCTTCACCTTAA
7103 R8 ACTCCCGGGTCTTACCTACCTTCATTYTAA
7103 R9 ACTCCCGGGTTTTACCTAACTTCATTTTAA
7103 R10 ACTCCCGGGTTTTACCTACCTTCATTTTAA
7103 R11 ACTCCCGGGTCTTACCTACCTTCATTTTGA

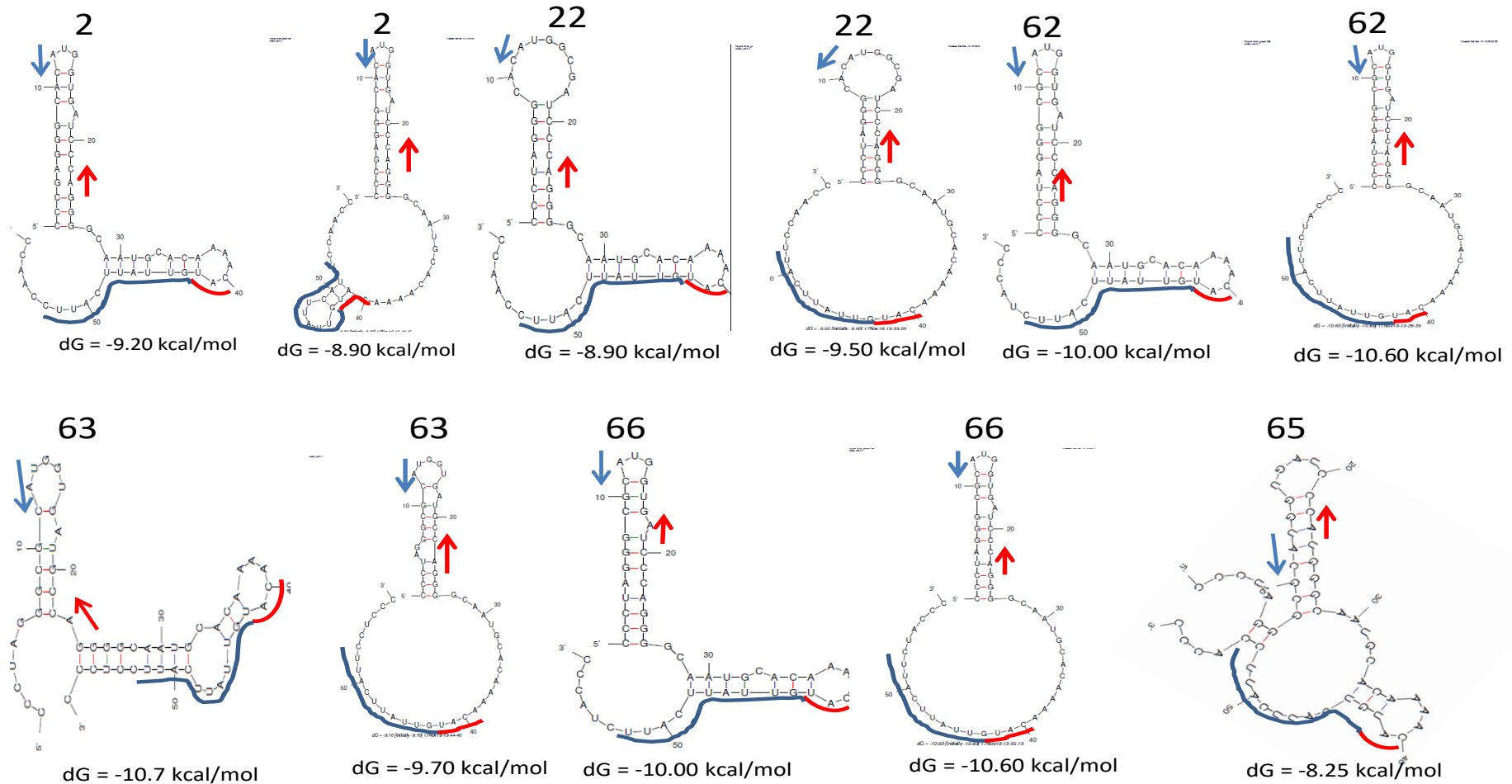
Supplemental Table S2. Characterization of the 152 complete HEV genomes used in the analysis.

Sample no	Acession number	Strain designation	host	Geographical origin	clade
1	AF455784	Osh 205	Swine	Kyrgyzstan	3II
2	This study	BD15-75	Blood donor	Sweden	3II
3	KF922359	rt93100008	Chronically infected patient	France	3II
4	KP698919	SwHEVE2IT12	Swine	Italy	3II
5	JQ013795	TR02	Human	France	3II
6	HM055578	HEV072/sw/HUN-05	Swine	Hungary	3II
7	FJ998015	SA21	Wild boar	Germany	3II
8	AB248522	swJ12-4	swine	Japan	3II
9	AB481226	swJB-E10	swine	Japan	3II
10	AB248521	swJ8-5	swine	Japan	3II
11	JQ953665	FR-SHEV3e	swine	France	3II
12	AB291958	JNH-Ehi04L	Human	Japan	3II
13	AB248520	HE-JA04-1911	Human	Japan	3II
14	AB290313	swMN06-C1056	swine	Mongolia	3II
15	AB780450	JBOAR100-Mie10	Wild boar	Japan	3II
16	AB780451	JBOAR107-Mie11	Wild boar	Japan	3II
17	AB780452	JBOAR111-Mie11	Wild boar	Japan	3II
18	AB780453	JBOAR124-Mie11	Wild boar	Japan	3II
19	KT447527	Sing-HEV20	Human	Singapore	3II
21	EU360977	swX07-E1	Swine	Sweden	3II
22	This study	BD15-84	Blood donor	Sweden	3II
23	EU723512	SW626	Swine	Spain	3II
24	EU723513	SW627	Swine	Spain	3II
25	JQ953666	FR-SHEV3f	Swine	France	3II
26	FJ956757	HEV_RKI	Plasma donor	Germany	3II
27	KT591532	FR-HEV-GD1	Chronically infected patient	France	3II
28	KT591533	FR-HEV-GD2	Chronically infected patient	France	3II
29	KT591534	FR-HEV-GD3	Chronically infected patient	France	3II
30	EU375463	Thai-swHEV07	Swine	Thailand	3II
31	AB291961	JMH-Osa04C	Human	Japan	3II
32	FJ653660	CU001	Human	Thailand	3II
33	AB369687	E116-YKH98C	Human	Japan	3II
34	EU723514	SWP6	Swine	Spain	3II
35	EU723515	SWP7	Swine	Spain	3II
36	EU723516	SWP8	Swine	Spain	3II
37	LC055973	JMH-Sai14	Human	Japan	3II
38	AB850879	HE-JA12-0725	Human	Japan	3II
40	EU495148	TLS25	Human	France	3II
41	LC055972	JAO-SpaTok12	Human	Japan	3II
42	KC166967	TLS09-0	Immunocompromised human	France	3II
43	KC166968	TLS09-1	Immunocompromised human	France	3II

Sample no	Acession number	Strain designation	host	Geographical origin	clade
44	KC166969	TLS09-2	Immunocompromised human	France	3II
45	KC166970	TLS09-3	Immunocompromised human	France	3II
46	KC166971	TLS09-4	Immunocompromised human	France	3II
47	JN906974	FR-HuHEVF3f	Human	France	3II
49	JN906976	FR-SHEVB3f	Swine	France	3II
50	JQ953664	FR-SHEV3c-like	Swine	France	3I
51	AB290312	swMN06-A1288	Swine	Mongolia	3I
52	JQ013794	TR19	Human	France	3I
53	KU176132	TLS-S	Human	France	3I
54	KU176131	TLS-D	Human	France	3I
55	KY780957	SW/16-0282	Chronically infected patient	Switzerland	3I
56	MF346773	CH_17LA00702	Sausage	Switzerland	3I
57	MF346772	CH_16020189	Human	Switzerland	
58	KU513561	IC2011	Human	Spain	3c/i
59	KU176130	TLS-C	Human	France	3c/i
60	KP294371	MWP_2010	Wild boar	Germany	3c/i
61	FJ998008	BB02	Wild boar	Germany	3c/i
62	This study	BD15-71	Blood donor	Sweden	3c/i
63	This study	BD15-81	Blood donor	Sweden	3c/i
64	KJ701409	AB_7110	Chronically infected patient	France	3c/i
66	This study	BD15-88	Blood donor	Sweden	3c/i
67	KC618402	47832	Chronically infected patient	Germany	3c/i
68	FJ705359	wbGER27	Wild boar	Germany	3c/i
69	AB073912	swJ570	Swine	Japan	3I
70	AB074918	JKN-Sap	Human	Japan	3I
71	AB074920	JMY-Haw	Human	Japan	3I
72	AB089824	HE-JA10	Human	Japan	3I
73	AB091394	JJT-Kan	Human	Japan	3I
74	AB189070	JBOAR1-Hyo04	Wild boar	Japan	3I
75	AB189071	JDEER-Hyo03L	Deer	Japan	3I
76	AB189072	JMO-Hyo03L	Human	Japan	3I
77	AB189073	JSO-Hyo03L	Human	Japan	3I
78	AB189074	JTH-Hyo03L	Human	Japan	3I
79	AB189075	JYO-Hyo03L	Human	Japan	3I
80	AB222182	wbJSG1	Wild boar	Japan	3I
81	AB222183	wbJTS1	Wild boar	Japan	3I
82	AB222184	wbJYG1	Wild boar	Japan	3I
83	AB236320	JMNG-Ok02C	Mongoose	Japan	3I
84	AB246676	HEVN1	unknown	Japan	3I
85	AB291951	JIO-Sai97L	Human	Japan	3I
86	AB291952	Jiy-Tot05L	Human	Japan	3I
87	AB291953	JSO-Ok05L	Human	Japan	3I
88	AB291954	JSS-Oka04L	Human	Japan	3I
89	AB291955	JSW-Kyo-FH06L	Human	Japan	3I
90	AB291956	JYM-Tot04L	Human	Japan	3I

Sample no	Acession number	Strain designation	host	Geographical origin	clade
91	AB291957	JYU-Ok04L	Human	Japan	3I
92	AB291960	JTK-Kag06C	Human	Japan	3I
93	AB291962	JHK-Toy04C	Human	Japan	3I
94	AB291963	JRM-Toy05C	Human	Japan	3I
95	AB301710	JE03-1760F	Cell culture	Japan	3I
96	AB362839	118d/Alex	Cell culture	Japan	3I
97	AB362840	114d/A549	Cell culture	Japan	3I
98	AB362841	JE03-1760F_p0	Cell culture	Japan	3I
99	ab362842	JE03-1760F_p5	Cell culture	Japan	3I
100	AB362843	JE03-1760F_p10	Cell culture	Japan	3I
101	AB369689	E088-STM04C	Human	Japan	3I
102	AB369691	E097-OSA05C	Human	Japan	3I
103	AB425830	JE03-1760F_p13-3	Cell culture	Japan	3I
104	AB425831	JE03-1760F_p5/B	Cell culture	Japan	3I
105	AB437316	pJE03-1760F/wt	Human	Japan	3I
106	AB437317	pJE03-1760F/fs	Cell culture	Japan	3I
109	AB443623	JIO-swJ19-1	Swine	Japan	3I
110	AB443624	JIO-swJ19-2	Swine	Japan	3I
111	AB443625	JIO-swJ19-5	Swine	Japan	3I
112	AB443627	JIO-swJ19-8	Swine	Japan	3I
113	AB481228	swJB-M8	Swine	Japan	3I
114	AB481229	swJR-P5	Swine	Japan	3I
115	AB591733	JMNG26-Ok08	Mongoose	Japan	3I
116	AB591734	JMNG36-Ok08	Mongoose	Japan	3I
118	AB593690	JE03-1760F_p10/B	Cell culture	Japan	3I
119	AB630970	HRC-HE104	Blood donor	Japan	3I
120	AB630971	JRC-HE3	Blood donor	Japan	3I
121	AB698071	Type III Highland/2000	Swine	Japan	3I
122	AB740232	G3-HEV83-2-27	Wild boar	Japan	3I
123	AF060668	HEV-US1	Human	USA	3I
124	AF060669	HEV-US2	Human	USA	3I
125	AF082843	Meng	Swine	USA	3I
126	AP003430	JRA1	Human	Japan	3I
127	AY115488	Arkell	Swine	Canada	3I
128	AY575857	pSHEV-1	Cell culture	USA	3I
129	AY575858	pSHEV-2	Cell culture	USA	3I
130	AY575859	pSHEV-3	Cell culture	USA	3I
131	FJ426403	swKOR-1	Swine	South Korea	3I
132	FJ426404	swKOR-2	Swine	South Korea	3I
133	FJ527832	SAAS-JDY5	Swine	Shanghai, China	3I
134	HQ389543	Kernow-C1	Cell culture	UK	3I
135	HQ389544	Kernow-C1	Cell culture	UK	3I
136	HQ709170	Kernow-C1	Cell culture	UK	3I
137	JN837481	US-C031008	Human	USA	3I
138	KF303502	GiSw	Swine	Germany	3I
139	KJ507955	swSTHY12-VAS19/2003/CA	Swine	Canada	3I
140	KJ507956	swSTHY42-VAS49/2003/CA	Swine	Canada	3I
141	KT447526	Sing-HEV01	Human	Singapore	3I
142	KT447528	Sing-HEV23	Human	Singapore	3I
143	KT633715	ZhJ-PJ050-3	Swine	China	3I
144	KT727028	CCST-517	Swine	China	3I
145	KX574712	HLJ-220	Swine	China	3I
146	KX981911	CCJD-517	Swine	China	3I

Sample no	Acesion number	Strain designation	host	Geographical origin	clade
147	LC126331	P5_pJE03-1760F_wt	Cell culture	Japan	3I
148	LC126332	P10_pJE03-1760F_wt	Cell culture	Japan	3I
149	LC131066	huJB-0414	Human	Japan	3I
150	LC176492	HE-JA16-0578	Human	Japan	3I
151	LC176493	HE-JA16-0579	Human	Japan	3I
152	KT581443	SW1_3-Uppsala	Swine	Sweden	3II
153	KT581444	SW3_6-Uppsala	Swine	Sweden	3II
154	KT581447	SW46_8-Dalarna	Swine	Sweden	3II
155	KT581448	SW8a24_Spain	Swine	Spain	3II
156	AB443626	JIO-swJ19-7	Swine	Japan	3I
157	JN906975	FR-SHEVF3f	Swine	France	3II



Supplemental Figure S1. Prediction of the structure of the junction region of the sequenced HEV genomes. The highly conserved CRE is marked with a blue line ending with the second AUG in frame with the start codon for ORF3, marked with a red line. The third AUG in frame which is the start codon for ORF3 is CUG in all strains from the seronegative plasma donors and AUG in strain 65 from a chronic carrier and are marked with a red arrow. The start codon for ORF2 is marked with a blue arrow. The calculated delta G (ΔG), the change in Gibbs Free Energy (in units of kcal/mole) is shown for each predicted structure.