

**Table S2.** Oligonucleotides used to perform the site-directed mutagenesis, qRT-PCR, control of basal amino acid sequencing error, and control of PCR recombination.

Primer Name	Sense	Sequence (5'-3')	Position <sup>a</sup>
Jc1-NS5A-F2	Fw	ACTACCTTCTCCAGAGTTTTTC	6697
Bch-0-3 <sup>b</sup>	Rv	AGCAGGTTGGCATCGACCATGTCCACGTCA TAGGTATGGCTGTGGGTGGTGCAGGTGG	7047
Bch-0-4 <sup>c</sup>	Fw	TGCCAACCTGCTCATGGAGGGCGGTGTGGCT CAGACA <u>AA</u> ACCTGAGTCCAGGGTGCCCGT	7036
Bch-0-21 <sup>d</sup>	Rv	AGGCGTCGGA <u>AC</u> CTTCTTGG	7315
Bch-0-6 <sup>d</sup>	Fw	AGAAGGTTCCGACGCCTCCCCCAAGGAGAC	7299
Jc1-NS5B-R1	Rv	TCTCGCAGACCCGGACGCCGAG	8180
HCV-5UTR-F2	Fw	TGAGGAACTACTGTCTTCACGCAGAAAG	47
HCV-5UTR-R2	Rv	TGCTCATGGTGCACGGTCTACGAG	347
Jc1NS5Au6521	Fw	CAGGGGACCTTTCCTATCAATTGCT	6521
Jc1NS5Ad7211	Rv	GCCGTGCCCAAGCCGGTA	7211
Jc1NS5AM13u6693 <sup>e</sup>	Fw	<u>GTTGTAAAACGACGGCCAGTGCCA</u> ACTACCTTCT CCAGAGTTTT	6693
Jc1NS5AM13d7131 <sup>e</sup>	Rv	<u>CACAGGAAACAGCTATGACCCTCTCTTCCTC</u> GGCCATTGGCT	7131

<sup>a</sup>According to JFH-1 accession number #AB047639

<sup>b</sup>Underlined nucleotides indicate modifications of the genomic sequence introduced to express NS5A with substitution N248H

<sup>c</sup>Underlined nucleotides indicate modifications of the genomic sequence introduced to express NS5A with substitution E269K

<sup>d</sup>Underlined nucleotides indicate modifications of the genomic sequence introduced to express NS5A with substitution A346V

<sup>e</sup>Underlined nucleotides indicate universal M13 oligonucleotide