

Text S1. Nucleotide sequences of mutant HCV segments. In the following section, the substituted nucleotides are shown in Red (non-conserved change) and Green (conserved change). The nucleotides in the wobble positions of codons were substituted. Hepatitis C viruses containing each mutant segment were tested for replication kinetics in cell culture condition. nt: nucleotide

1) Segment 1 (nt 3872-4156) (285 bp, Black:170, Green:85, Red:30)

GGGCAUGUGGUGGGCUUGUUUAGGGCCGCCGUCUGUAGCAGGGGGGUCGCUAAGAGCAUUGACUUUAUAC
CAGUGGAAACCUUGGAUGUGGUGACCCGGAGCCCAACUUUCCGAUAAUUCACCCCCCGCCGUC
ACAACGUACCAAGUGGGCUAUCUCCACGCCCCACCGGUCGGCAAUCCACGAAAGUGCCCGUGGCC
UACGCGGCUCAAGGCUAUAAGGUGUUGUCUUGAAUCCAAGCGUGGCCGCUACGUUGGGCUUCGGCGCCU
AUUUG

2) Segment 2 (nt 4157-4441) (285 bp, Black:178, Green:88, Red:19)

AGCAAAGCCACGGGAUUAACCCAAAUAUCCGCACCGCGUGCGGACGGUCAUGACGGGCGAAGCGAUUA
CCUAUAGCACCUACGGGAAGUUUUGGUGACGGCGGGUGGCCUCGGGGCUUACGAUUAUUAUUAUCUG
UGACGAGUGUCAUGCCGUCGACGCCACGAGCAUCUUGGGGAUAGGCACCGUGUUGGACCAGGCCGAAACC
GCGGGCGUGCGGUUGACCGUCUUGGCCACCGCGACCCACCAGGCAGCGUCACCACGCCACACCAGACA
UCGAG

3) Segment 3 (nt 4442-4726) (305 bp, Black:200, Green:85, Red:20)

GAAGUGGGGUUGGGCAGGGAAGGCGAUAUACCAUUUACGGCCGGGCCAUCCC AUUGAGCUGUAUAAAAG
GCGGCCCGGAUUUGAUUUUGUCAUAGCAA AAGAAUGCGAUGAAUUGGCCGCCGCGUUGAGGGGGAU
GGGGCUGAAUGCCGUAGCCUACUACUCAACGCGUGUCGCCUAUUAACGGGGCCUCGAUGUGAGCAUCAUC
CCGCCCAAGGCGACGUCGUGCGUGGCUACGGAU GCGUUGAUGACCGGCUAUACCGGCGAUUUCGAUAG
CGUCAUUGAUUGUAACGUGGCCGUG

4) Segment 4 (nt 4727-5011) (285 bp, Black:171, Green:86, Red:28)

ACGCAGGCCGUGGAUUUUCUCCUCGAUCCAACGUUJACCAUCACGACCCAAACCGUGCCCCAGGAUGCCG
UGAGCAGGUCCAAAGGAGGGGCAGGACCGGCCGGGGCCGGCAAGGGACCUACCGGUACGUGAGCACC GG
CGAGAGGGCUAGCGGCAUGUUCGAUUCGUGGUCUUGUGCGAAUGUUAUGAUGCCGGCGCCGCCUGGUAU
GACUUGACCCCGCCGAACGACGUGCGGUUGCGGGCCUACUUUAUACCCAGGGUUGCCAGUCUGCC
AGGAU

5) Segment 5 (nt 5012-5299) (288 bp, Black:186, Green:87, Red:15)

CACUUGGAGUUCUGGGAAGCCGUGUUUACGGGGUUGACCCAUAUCGAUGC CAUUUUUUGAGCCAGACCA
AACAGGCCCGCGAAAAUUUUGCCUAUJUGGUGGCGUAUCAGGCCACCGUCUGUGCGCGGGCGAAAGGCC
CCCACCAGCUGGGAUGCGAUGUGGAAUGUUUGGCUAGGUUGAAACCCACCUUGGCCGGGCCAACCC
UUUGUUGUAUAGGUCUGGGCCCAUCACGAACGAAGUGACGUUGACCCAUCCGGCACCAAUAUUAUGCUA
CAUGCAUG

6) Segment 6 (nt 5300-5650) (351 bp, Black:225, Green:97, Red:29)

CAGGCCGAUUUGGAAAGUGAUGACGUCACCUGGGUGUUGGCCGGCGGCGUGUUGGCCGCGGGUGGCGGCCU
ACUGUUUGGCCACCGGCUGUGUGAGCAUUAUAGGGAGGCUCCAUGUGAAUCAAGGGUGGUGGGUGGCCCC
CGACAAAGAAGUGUUGUACGAAGCCUUCGACGAAUUGGAAGAGUGUGCGAGCCGGCCGCCUUGAUAGAG
GAAGGCCAAAGGAUCGUGAAAUGCUCAAAGCAAUAACAGGGGCUUUGCAACAAGCGAGCAAACAAG
CUCAAGAUAUCCAGCCAGCAUGCAAGCCAGCUGGCCAAAGGUCGAGCAGUUCUGGGCGCGGCAUAUGUG
G

7) Segment 7 (nt 5651-6010) (360 bp, Black:218, Green:108, Red:34)

AAUUUUAUCUCGGGAUUCAGUAUUUGGCCGGCCUCAGCACCUUGCCCGGCAAUCCAGCCGUCGCCAGCA
UGAUGGCCUUUUCCGCUGCGUUGACGUCCCCCUCAGCACGUCCACGACGAUUUUGUUGAAUAUUAUGGG
CGGGUGGCUCGCGCAGCCAAUAGCCCCCAGCCGGCGCGACGGGUUCGUGGGUUCGGGUUGGUCCGC
GCCGCUUGUCGGGUCCAUCGGGUUGGGCAAAGUCUUGGUCGAUUUUUGGCCGGCUACGGCGCCGGGAUCA
GCGGCGCGUUGGUGGCCUUUAAAUUAUGAGCGGGGAAAACCAAGCAUGGAGGACGUGAUAAACUUGUU
GCCCGGAUC

8) Segment 8 (nt 6011-6301) (291 bp, Black:173, Green:92, Red:26)

CUCAGCCCCGGCGCGUUGGUUGUCGGCGUGAUUUGCGCGUAUCUUGAGGAGGCAUGUCGGCCCCGGCG
AAGGGCCGUGCAGUGGAUGAAUCGGUUGAUCGCUUUCGCGAGCCGGGCAAUCAUGUGGCGCCACCCA
UUAUGUCACCGAAAGCGACGCGCAGCCAAAGGGUCACGCAGUUGUUGGGGAGCUUGACCAUCACGUCCUUG
UUGCGGCGGUUGCAUAAACUGGAUCACCGAAGAUUGUCCAAUUCUUGUAGCGGCAGCUGGUUGAGGGAUG
UCUGGGAUUGG

9) Segment 9 (nt 6302-6592) (291 bp, Black:187, Green:83, Red: 21)

GUGUGUACGAUACUCACCGAUUUUAAGAACUGGUUGACGAGCAAGCUCUUUCCAAAUAUGCCAGGGUUGC
CAUUUAUAAGCUGCCAGAAAGGCUAUAAAGGCGUCUGGGCUGGGACCGGGAUUAUGACGACCAGGUGJCC
CUGUGGGGCUAAUAUJAGCGGGAACGUGAGGUUGGGGAGCAUGCGGAUAACCGGCCCAAGACGUGUAUG
AAUACGUGGCAAGGCACGUUCCCCAUUAAACUGUUAUACCGAAGGGCAUUGUCGCCCAAGCCACCAACCA
AUUAUAAAACG

10) Segment 10 (nt 6593-6880) (288 bp, Black:178, Green:78, Red:32)

GCGAUUUGGCCGGGUCGCCGCGAGCGAAUAUGCCGAAGUCACCCAACACGGCAGCUAUAGCUACGUGACCG
GCUUGACGACCAGUAACUUGAAGAUCCCCUGUCAGUUGCCAGCCCCGAGUUCUUUAGCUGGGUCGAUJGG
CGUCCAAUAACACCGGUUCGCCCAACCCCAAACCCUUCUUUAGGGACGAAGUGAGCUUUUGUGUGGGC
UUGAAAGCUACGCGCGUGGGCAGCCAUUGCCAUJCGAGCCCGAAACAGAUJCCGAUJUGUCUCGGAGCA
UGUUGACC

11) Segment 11 (nt 6881-7168) (288 bp, Black:168, Green:73, Red:47)

GACCCCCACAUAUAACCGCCGAAACCGCCGCAGGAGGCUCGCAGGGGCAGCCCCCAGCGAAGCCU
CCAGCAGCGUCUCCAAUUGAGCGCCCCAGCUUGAGGGCGACGUGUACGACGCAUCCAAUACAUCGA
UGUCGAUAUGGUGGACGCGAAUUUGUUGAUGGAAGGGGGCGUCGCCAAACCGAACCCGAAAGCCGGGUC
CCAGUGUUGGAUUUCUUGGAACCCAUGGCUGAAGAGGAAUCCGAUUUGGAACCAAGCAUCCCCAGCGAAU
GUAUGUUG

12) Segment 12 (7169-7456) (288 bp, Black:174, Green:93, Red:21)

CCACGCUCCGGCUUCCCCAGGGCUCUCCCCGCCUGGGCCAGGCCCGAUUAUAAUCCCCCUUGGUCGAGA
GCUGGCGGCGGCCGACUAUCAGCCCCAACGGUGGCCGGCUGCGCUUGCCACCACCAAAAAAGCUCC
CACCCCCCACC CGCGGAGGAGACCGUCGGCUUGUCCGAUCCACGAUCAGCGAGGCUUUGCAACAG
UUGGCGAUUAAAACGUUCGGCAACCAACAGCUCCGGCGACGCCGGGAGCAGCACCGGC GC CGGGCUG
CGGAGAGC

13) Segment 13 (nt 7457-7786) (330 bp, Black:199, Green:73, Red:58)

GGCGGACCGAC CAGCCCCGGCGAACCCGCUCCAAGCGAAACCGGCAGCGCGAGCAGCAUGCCACCAUUGG
AAGGCGAACCCGGCGACCCCGAUUUGGAAAGCGACCAAGUGGAAUUGCAGCCCCACCAACAAGGCGGCGG
CGUGGCCCCAGGCAGCGGGAGCGGCAGCUGGAGCACCUGUAGCGAAGAAGAUACACGACGGUCUGUUGU
AGCAUGAGCUAUAGCUGGACGGGCGCCUUGAUCACCCAUGCUCCCCAGAGGAAGAGAAACUCCCCAUAA
AUCCCCUCUCAAUAGCUUGCUCAGGUAUCACAAUAAAGUCUAUUGUACA

14) Segment 14 (nt 7787-8113) (327 bp, Black:193, Green:94, Red: 40)

ACCAGCAAUCCGCUAGCCAACGGGCCAAGAAAGUGACCUUCGAUCGGACCAGGUCUUGGAUUGCACUC
ACGAUAGCGUGCUCAAAGAUAUAAAUUGGCCGCCAGCAAAGUUCGCCCGGUUGUUGACGCUCGAAGA
AGCCUGUCAACUCACCCCCCACAGCGCCGGAGCAAUAACGGCUUUGGCGGAAAGAAUGAGGUCC
CUCAGCGGCCGGGCGGUGAAUCAUAUAAAAGCGUCUGGAAAGAUUUGUUGGAAGA UCCCAGACCCCA
UCCCAACCACGAUUAUGGCGAAGAACGAAGUCUUUUGUGUCGAUCCA

15) Segment 15 (nt 8114-8440) (327 bp, black:202, green:91, red:34)

GCUAAAGGCGGCAA AAAGCCCGCCAGGUUGAUUGUGUAUCCCGAUUUGGGGGUGAGGGUGUGUGAAAGA
UGGCGUUGUACGAUAUCACCCAGAAAUUGCCCCAAGCCGUGAUGGGCGCCAGCUACGGUUUCAUAUAG
CCCCGCU CAGAGGGUCGAUAUCUUGCUC AAGGCCUGGGCCGAGAAAAGAUCCA AUGGGCUUCAGCUAC
GACACGAGGUGUUUGAUAGCACGGUGACCGAACGGGAUAUUCGGACGGAAGAAAGCAUCUAUCAAGCGU
GUAGCUUGCCAGAAAGAAGCGAGGACCGCUAUCCAUAGCUUGACCGAA

16) Segment 16 (nt 8441-8767)(327 bp, black:200, green:91, red:36)

CGGUUGUAUGUGGGCGGCCCAAUGUUUAAUUCCAAAGGCCAGACGUGUGGCUAUCGGAGGUGUAGGGCUU
CCGGCGUCUUGACGACCUCCAUGGGCAAUACGAUAACCUUUACGUCAAGGCGUUGGCCGCUUGUAAAGC
CGCCGGCAUCGUGGCCCCAACCAUGUUGGUGUGUGGGGACGAUUUGGUGGUAUAGCGAGUCCCAAGGC
ACCGAAGAAGAUGAAAGGAAUUUGCGGGCUUUUACCGAAGCGAUGACCGGUUAUAGCGCGCCCCCGGCG
ACCCACCACGGCCCGAGUACGAUUUGGAAUUGAUCACCAGCUGCAGC

17) Segment 17 (nt 8768-9097) (330 bp, black:201, green:91, red:38)

AGCAACGUCAGCGUCGCCUCGGGCCAGGGGGAGGAGCGGUUAUUAUUUGACCGCGGAUCCACGACCC
CCUUGGCUAGGGCCGCGUGGGAGACCGUGCGGCAUAGCCCCAUAAACAGCUGGUUGGGCAAUAUAAUACA
AUACGCCCCACGAUCUGGGUGAGGAUGGUGUUGAUGACC AUUUUUUAGCAUCUUGAUGGUGCAGGAU
ACGUUGGAUCAAAAUUUGAAUUUCGAAUGUACGGCAGCGUGUAUAGCGUCAACCCUCGAUUUGCCCG
CUAUCAUCGAACGGCUCCAUGGCUUGGAUGCGUUCAGCAUGCACACCUAU

Text S2. Sequence alignment of wild-type and mutant sub-segments in the JFH-1 NS5B coding region. Nucleotide changes introduced in the 28 sub-segments are shown below. JFH-1 genome positions and total number of silent mutations introduced in each sub-segment are given. Asterisks indicate unmodified nucleotides. CLUSTAL 2.0.12 multiple sequence alignment program was used.

1. Sub-segment 7787-7837 (total substitutions = 22)

wildtype	ACAUCAAGAGCGCCUCACAGAGGGCUAAAAAGGUAACUUUUGACAGGACG
mutant	ACCAGCAAUCCGCUAGCCAACGGGCCAAGAAAGUGACCUUCGAUCGGACC
	** ** *** ** ***** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** **

2. Sub-segment 7838-7888 (total substitutions = 22)

wildtype	CAAGUGCUCGACGCCAUUAUGACUCAGUCUAAAAGGACAUCAAGCUAGCG
mutant	CAGGUCUUGGAUGCGCACUACGAUAGCGUGCUCAAAGAUUAAAAUUGGCC
	** ** * ** ** ** ** ** ** ** ** * ** ** ** ** ** ** * **

3. Sub-segment 7889-7939 (total substitutions = 23)

wildtype	GCUUCCAAGGUCAGCGCAAGGCUCACCUUGGAGGAGGCGUGCCAGUUG
mutant	GCCAGCAAAGUGUCCGCCCGGUUGUUGACGCUCGAAGAAGCCUGUCAACUC
	** *** ** *** ** * * ** * ** ** ** ** ** ** ** ** ** *

4. Sub-segment 7940-7990 (total substitutions = 21)

wildtype	ACUCCACCCCAUUCUGCAAGAUCCAAGUAUGGAUUCGGGGCCAAGGAGGUC
mutant	ACCCCCCACACAGCGCCCGGAGCAAUACGGCUUUGGCGGAAAGAAGUC
	** ** ** ** ** ** * *** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** **

5. Sub-segment 7991-8041 (total substitutions = 21)

wildtype	CGCAGCUUGUCCGGGAGGGCCGUUAACCACAUCAAGUCCGUGUGGAAGGAC
mutant	AGGUCCCUCAGCGGCCGGGCGUGAAUCAUAAAAAGCGUCUGGAAAGAU
	* * * *** ***** ** ** ** ** ** ** ** ** ** ** *** ***** **

6. Sub-segment 8042-8092 (total substitutions = 16)

wildtype	CUCCUGGAAGACCCACAAACACCAAUCCCAACCAUCAUGGCCAAAAAU
mutant	UUGUUGGAAGAUCCCAGACCCCAUCCCAACCACGAUUAUGGCGAAGAAC
	* ***** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** * ** **

7. Sub-segment 8093-8143 (total substitutions = 19)

wildtype	GAGGUGUUCUGCGUGGACCCCGCCAAGGGGGGUAAGAAACCAGCUCGCCUC
mutant	GAAGUCUUUUGUGUCGAUCCAGCUAAAGGCGGCAAAAAGCCCGCCAGGUUG
	** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** ** * *

8. Sub-segment 8144-8194 (total substitutions = 18)

wildtype	AUCGUUUACCCUGACCUCGGCGUCCGGGUCUGCGAGAAAUGGCCCUUAU
mutant	AUUGUGUAUCCCGAUUUGGGGGUGAGGGUGUGUGAAAAGAUGGCGUUGUAC
	** ** ** ** ** * ** ** ***** ** ** ** ***** * **

9. Sub-segment 8195-8245 (total substitutions = 18)

wildtype	GACAUUACACAAAAGCUUCCUCAGGCGGUAUUGGGAGCUUCCUAUGGCUUC
mutant	GAUAUCACCCAGAAUUGCCCCAAGCCGUGAUGGGCGCCAGCUACGGGUUU
	** ** ** ** * ** ** ** ** ** ***** ** *** ** **

10. Sub-segment 8246-8296 (total substitutions = 19)

wildtype	CAGUACUCCCCUGCCCAACGGGUGGAGUAUCUCUUGAAAGCAUGGGCGGAA
mutant	CAAUUAGCCCCGUCAGAGGGUCGAAUACUUGCUCUAAAGCCUGGGCCGAG
	** ** *** ** ** ***** ** ** * * ** ** ***** **

11. Sub-segment 8297-8347(total substitutions = 21)
wildtype AAGAAGGACCCCAUGGGUUUUUCGUAUGAUACCCGAUGCUUCGACUCAACC
mutant AAAAAAGAUCCAAUGGGCUUCAGCUACGACACGAGGUGUUUUGAUAGCACG
** ** *

12. Sub-segment 8348-8398(total substitutions = 20)
wildtype GUCACUGAGAGAGACAUCAGGACCGAGGAGUCCAUAUACCAGGCCUGCUCC
mutant GUGACCGAACGGGAUAUUCGGACGGAAGAAAGCAUCUAUCAAGCGUGUAGC
** ** *

13. Sub-segment 8399-8449(total substitutions = 22)
wildtype CUGCCCGAGGAGGCCCGCACUGCCAUAACACUCGCGUGACUGAGAGACUUUAC
mutant UUGCCAGAAGAAGCGAGGACCGCUAUCCAUAAGCUUGACCGAACGGUUGUAU
**** *

14. Sub-segment 8450-8500(total substitutions = 19)
wildtype GUAGGAGGGCCCAUGUUCAACAGCAAGGGUCAAAACCUGCGGUUACAGACGU
mutant GUGGGCGGCCCAUGUUUAAUUCCAAAGGCCAGACGUGUGGCUAUCGGAGG
** ** *

15. Sub-segment 8501-8551(total substitutions = 20)
wildtype UGCCGCGCCAGCGGGGUGCUAACCACUAGCAUGGGUAAACCAUCAUAUGC
mutant UGUAGGGCUUCCGGCGUCUUGACGACCUCCAUGGGCAAUACGAUAACCUGU
** *

16. Sub-segment 8552-8602(total substitutions = 18)
wildtype UAUGUGAAAGCCCUAGCGGCCUGCAAGGCUGCGGGGAUAGUUGCGCCACACA
mutant UACGUCAAGGCGUUGGCCGCUUGUAAAGCCGCGGCAUCGUGGCCCAAC
** ** *

17. Sub-segment 8603-8653(total substitutions = 20)
wildtype AUGCUGGUAUGCGGCGAUGACCUAGUAGUCAUCUCAGAAAGCCAGGGGACU
mutant AUGUUGGUGUGUGGGGACGAUUUGGUGGUGAUUAGCGAGUCCCAAGGCACC
*** ** *

18. Sub-segment 8654-8704(total substitutions = 17)
wildtype GAGGAGGACGAGCGGAACCUGAGAGCCUUCACGGAGGCCAUGACCAGGUAC
mutant GAAGAAGAUGAAAGGAAUUUGCGGGCUUUUACCGAAGCGAUGACGCGGUAU
** ** *

19. Sub-segment 8705-8755(total substitutions = 21)
wildtype UCUGCCCCUCCUGGUGAUCCCCCAGACCGGAAUAUGACCUGGAGCUAAUA
mutant AGCGCGCCCCCGGCGACCCACCACGGCCCCGAGUACGAUUUGGAAUUGAUC
** ** *

20. Sub-segment 8756-8806(total substitutions = 26)
wildtype ACAUCCUGUUCCUCAAAUGUGUCUGUGGCGUUGGGCCCGGGGCCCGCCGC
mutant ACCAGCUGCAGCAGCAACGUCAGCGUCGCCUCGGGCCAGGGGGAGGAGG
** ** *

21. Sub-segment 8807-8857(total substitutions = 19)
wildtype AGAUACUACCUGACCAGAGACCCAACCACUCCACUCGCCCGGGCUGCCUGG
mutant CGGUUUUUUUGACGCGGAUCCACGACCCCUUGGCUAGGGCCGCGUGG
* *

22. Sub-segment 8858-8908(total substitutions = 20)

wildtype	GAAACAGUUAGACACUCCCCUAUCAAUUCAUGGCUGGGAAACAUCAUCCAG
mutant	GAGACCGUGCGGCAUAGCCCCAUA AACAGCUGGUUGGGCAAUAUAUACAA
	** ** *

23. Sub-segment 8909-8959(total substitutions = 17)

wildtype	UAUGCUGCAACCAUAUGGGUUCGCAUGGUCCUAAUGACACACUUCUUCUCC
mutant	UACGCCCCCACGAUCUGGGUGAGGAUGGUGUUGAUGACCCAUUUUUUUAGC
	** *

24. Sub-segment 8960-9010(total substitutions = 17)

wildtype	AUUCUCAUGGUCCAAGACACCCUGGACCAGAACCUCAACUUUGAGAUGUAU
mutant	AUCUUGAUGGUGCAGGAUACGUUGGAUCAAAAUUUGAAUUUCGAAAUGUAC
	** *

25. Sub-segment 9011-9061(total substitutions = 22)

wildtype	GGAUCAGUAUACUCCGUGAAUCCUUUGGACCUCCAGCCAUAUUUGAGAGG
mutant	GGCAGCGUGUAUAGCGUCAACCCCCUCGAUUUGCCCGCUAUCGAACGG
	** *

26. Sub-segment 9062-9097(total substitutions = 14)

wildtype	UUACACGGGCUUGACGCCUUUUCUAUGCACACAUAC
mutant	CUCCAUGGCUUGGAUGCGUUCAGCAUGCACACCUAU
	* *

27. Sub-segment 9170-9226(total substitutions = 26)

wildtype	AGUCGGGCUCGCGCAGUCAGGGCGUCCUCAUCUCCCGUGGAGGGAAAGCGCCGUU
mutant	UCCAGGGCCAGGGCCGUGCGGGCCAGCUUGAUAAGCAGGGGCGGCAAGGCCGCGGUG
	**** *

28. Sub-segment 9227-9289(total substitutions = 25)

wildtype:	UGC GCC GAUAUCUCUCAAUUGGGCGGUGAAGACCAAGCUCAAACUCACUCCAUUGCCGGAG
mutant:	UGUGGGAGGUACUUGUUUAACUGGGCCGUCAAAACGAAAUUGAAGUUGACCCCCUCCCCGAG
	** ** *