

Supplementary material for Hillung J, Cuevas JM, Elena SF, 2015, Estimating the within-host fitness effects of mutations fixed during virus adaptation to different ecotypes of a new host, *Phil. Trans. R. Soc. B.* doi: 10.1098/rstb.2015.0292

Mutation	Forward primer	Reverse primer
C795U	5' CGTCACATGTATGGT <u>G</u> GAGAGGAAAAGGGTG 3'	5' CACCCTTTTCCTCTC <u>A</u> CCATACATGTGACG 3'
G1272U	5' GTTGAGGGTGAGTC <u>I</u> GGAGACAGTGTCAC 3'	5' GTGACACTGTCTCC <u>A</u> GACTCACCTCAAC 3'
C2116U	5' CTTTTCTTIGCTCTA <u>T</u> TAGTGAATGTCAAGG 3'	5' CCTTGACATTCACTA <u>A</u> TAGAGCAAAGAAAATG 3'
G3639A	5' CATCTACATGCAGAGTTT <u>A</u> GATGATTACGTTACAACC 3'	5' GGTTGTAACGTAATCATC <u>T</u> IAAACTCTGCATGTAGATG 3'
G6420A	5' GAAGAAATAATGGAACACT <u>A</u> TGGTCCAATCACTACATG 3'	5' CATGTAGTGATTGGACCA <u>A</u> TAGTGTTCCATTATTTCTTC 3'
C8636U	5' CTGGAAC TTCAGGAACATTCT <u>I</u> AGTTCCACGAATAAATGC 3'	5' GCATTTATTTCGTGGA <u>A</u> CTAAGAATGTTCCCTGAAGTTCCAG 3'
A9240G	5' GTACTGCAGAGG <u>A</u> GACTGAACGG 3'	5' CCGTTCAGTGTC <u>C</u> TCCTCTGCAGTAC 3'

**Supplementary Table 1:** Pairs of PCR primers used for mutagenesis experiments. The mutagenized site is underlined.