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Supplemental Information

Profiling the Mismatch Tolerance of Argonaute 2 through Deep Sequencing of Sliced Polymorphic Viral RNAs

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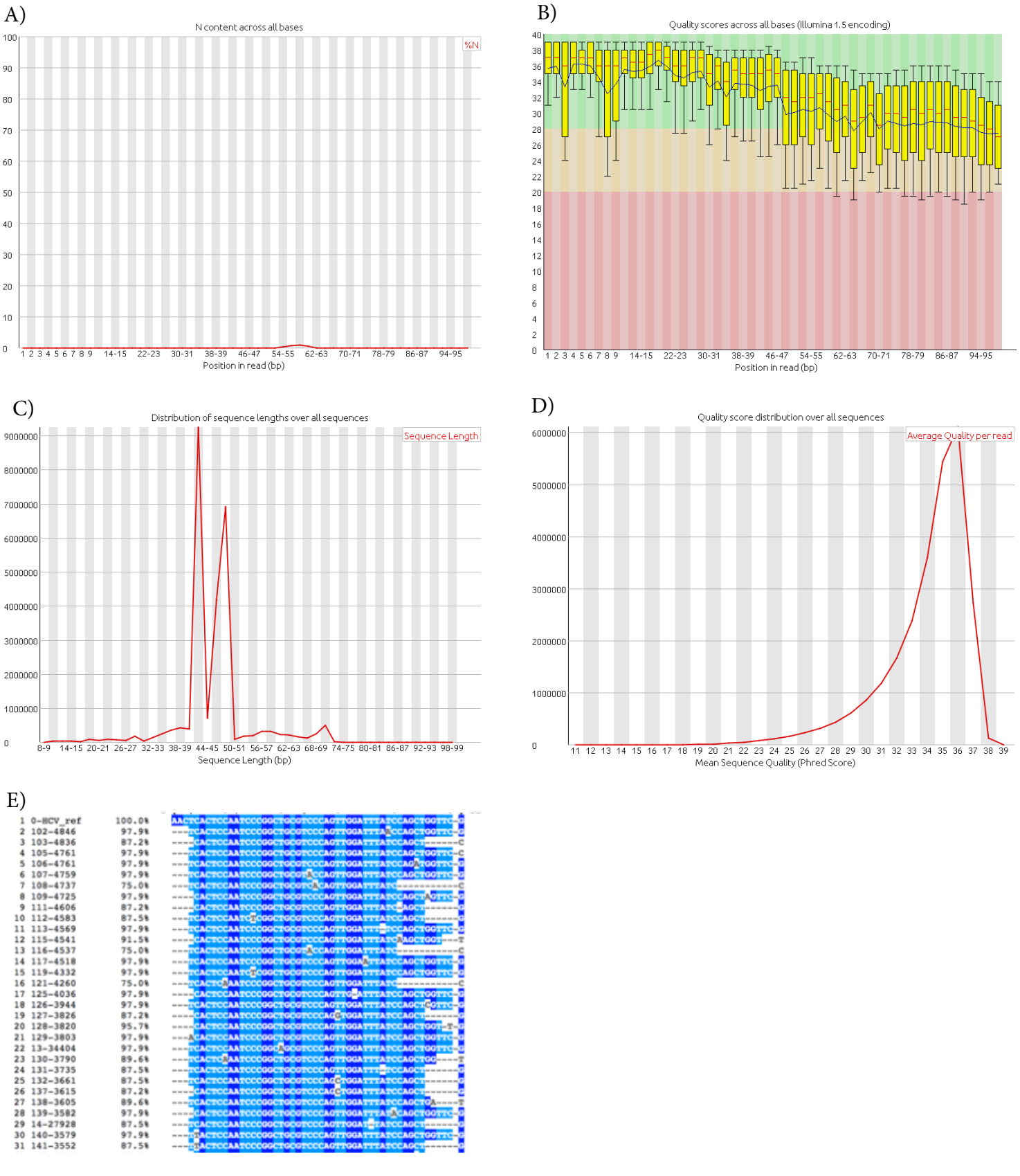

Supplemental Figure 1. FastQC Quality Control report output excerpt after 5' RACE adapter trimming using Cutadapt and visualisation of the nature of unaligned reads. A) Limited incidence of unresolved bases (N content) across the RACE-SEQ reads. B) Per base PHRED Quality score distribution does not fall under Q20 in 5' RACE adapter-treamed reads. C) Trimmed read size distribution profile. D) Read PHREAD score distribution across all reads after RNA adapter trimming. E) Visualising in MView a T-Coffee multiple sequence alignment of collapsed 5' RACE SEQ reads indicates that a large fraction of reads might be arising from HCV quasi-species featuring insertions, deletions and mutations (Purines in dark blue, pyrimidines in light blue)

Supplemental Table 1. Extended Cutadapt summary report following RACE adapter removal.

```
biolinux@E-Laptop-38[NEW_old] cutadapt -gGGACACTGACATGGACTGAAGGAGTAGAAA -e0 --no-
indels -m10 --discard-untrimmed -o trimmed_009.fastq.gz siRNA_009.fastq.gz
This is cutadapt 1.10 with Python 2.7.6
Command line parameters: -gGGACACTGACATGGACTGAAGGAGTAGAAA -e0 --no-indels -m10 --
discard-untrimmed -o trimmed_009.fastq.gz siRNA_009.fastq.gz
Trimming 1 adapter with at most 0.0% errors in single-end mode ...
Finished in 1351.46 s (22 us/read; 2.77 M reads/minute).

=== Summary ===

Total reads processed:              62,334,988
Reads with adapters:                26,412,283 (42.4%)
Reads that were too short:             609,109 (1.0%)
Reads written (passing filters):    26,238,713 (42.1%)

Total basepairs processed: 4,598,424,045 bp
Total written (filtered):  1,203,983,899 bp (26.2%)

=== Adapter 1 ===

Sequence: GGACACTGACATGGACTGAAGGAGTAGAAA; Type: regular 5'; Length: 30; Trimmed:
26412283 times.

No. of allowed errors:
0-30 bp: 0

Overview of removed sequences
length     count expect     max.err    error counts
3     18777 973984.2   0     18777
4     7404  243496.0   0     7404
5     2050  60874.0    0     2050
6     593   15218.5    0     593
7     380   3804.6     0     380
8     290   951.2 0     290
9     372   237.8 0     372
10    603   59.4  0     603
11    453   14.9  0     453
12    640   3.7   0     640
13    124   0.9   0     124
14    307   0.2   0     307
15    1484  0.1   0     1484
16    1131  0.0   0     1131
17    1419  0.0   0     1419
18    653   0.0   0     653
19    3507  0.0   0     3507
20    1922  0.0   0     1922
21    7440  0.0   0     7440
22    5714  0.0   0     5714
23    12998 0.0   0     12998
24    50732 0.0   0     50732
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25    117156     0.0   0     117156
26    155802     0.0   0     155802
27    289944     0.0   0     289944
28    264827     0.0   0     264827
29    419351     0.0   0     419351
30    24098522   0.0   0     24098522
31    877221     0.0   0     877221
32    58377 0.0   0     58377
33    3779  0.0   0     3779
34    468   0.0   0     468
35    265   0.0   0     265
36    212   0.0   0     212
37    332   0.0   0     332
38    253   0.0   0     253
39    489   0.0   0     489
40    332   0.0   0     332
41    334   0.0   0     334
42    805   0.0   0     805
43    260   0.0   0     260
44    386   0.0   0     386
45    634   0.0   0     634
46    355   0.0   0     355
47    534   0.0   0     534
48    334   0.0   0     334
49    526   0.0   0     526
50    335   0.0   0     335
51    133   0.0   0     133
52    149   0.0   0     149
53    249   0.0   0     249
54    118   0.0   0     118
55    654   0.0   0     654
56    103   0.0   0     103
57    11    0.0   0     11
58    5     0.0   0     5
59    3     0.0   0     3
60    2     0.0   0     2
62    2     0.0   0     2
64    11    0.0   0     11
65    1     0.0   0     1
66    7     0.0   0     7
67    1     0.0   0     1
68    3     0.0   0     3
72    1     0.0   0     1
73    1     0.0   0     1
80    1     0.0   0     1
82    1     0.0   0     1
95    1     0.0   0     1

biolinux@E-Laptop-38[NEW_old]                                    [ 2:47AM]
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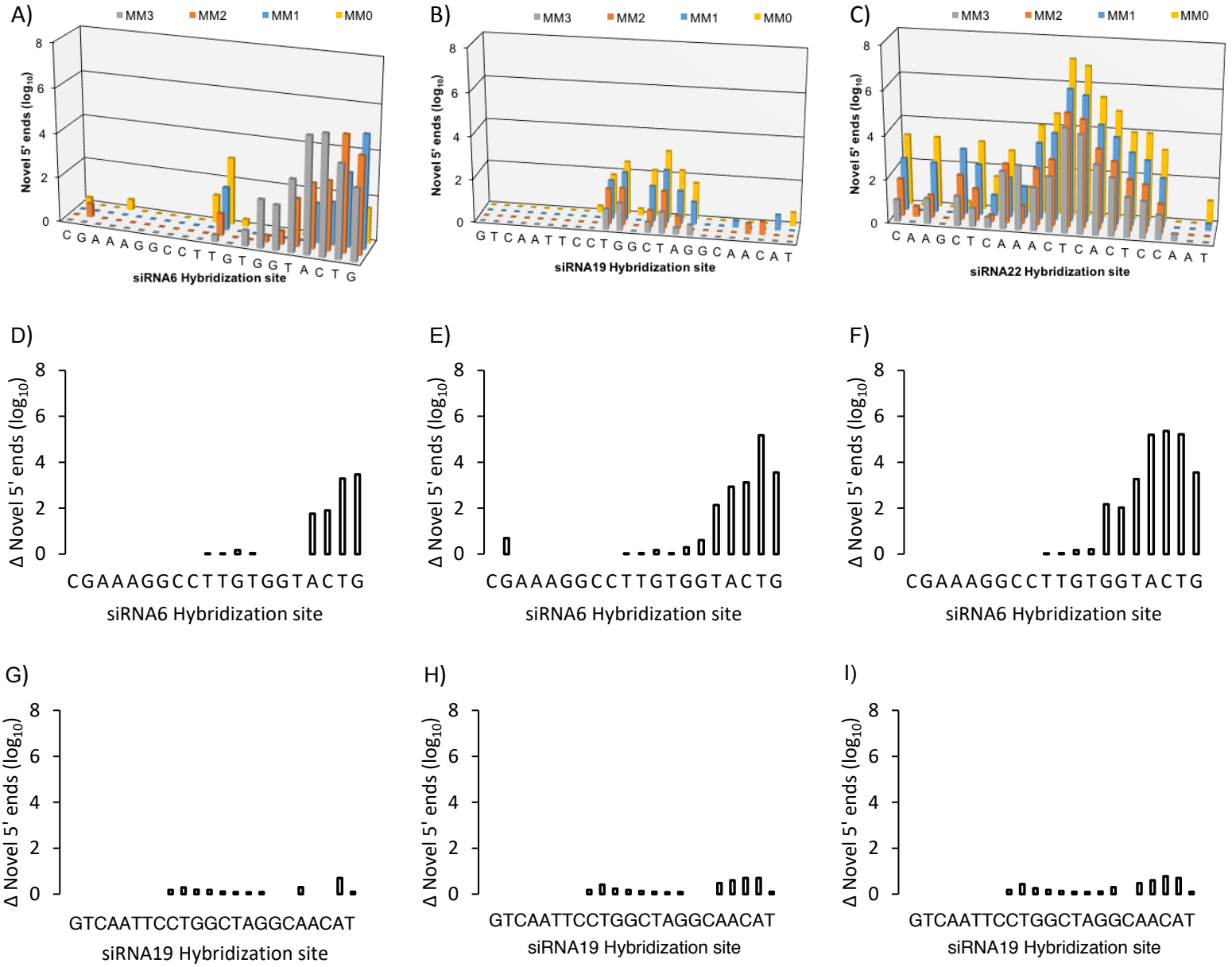
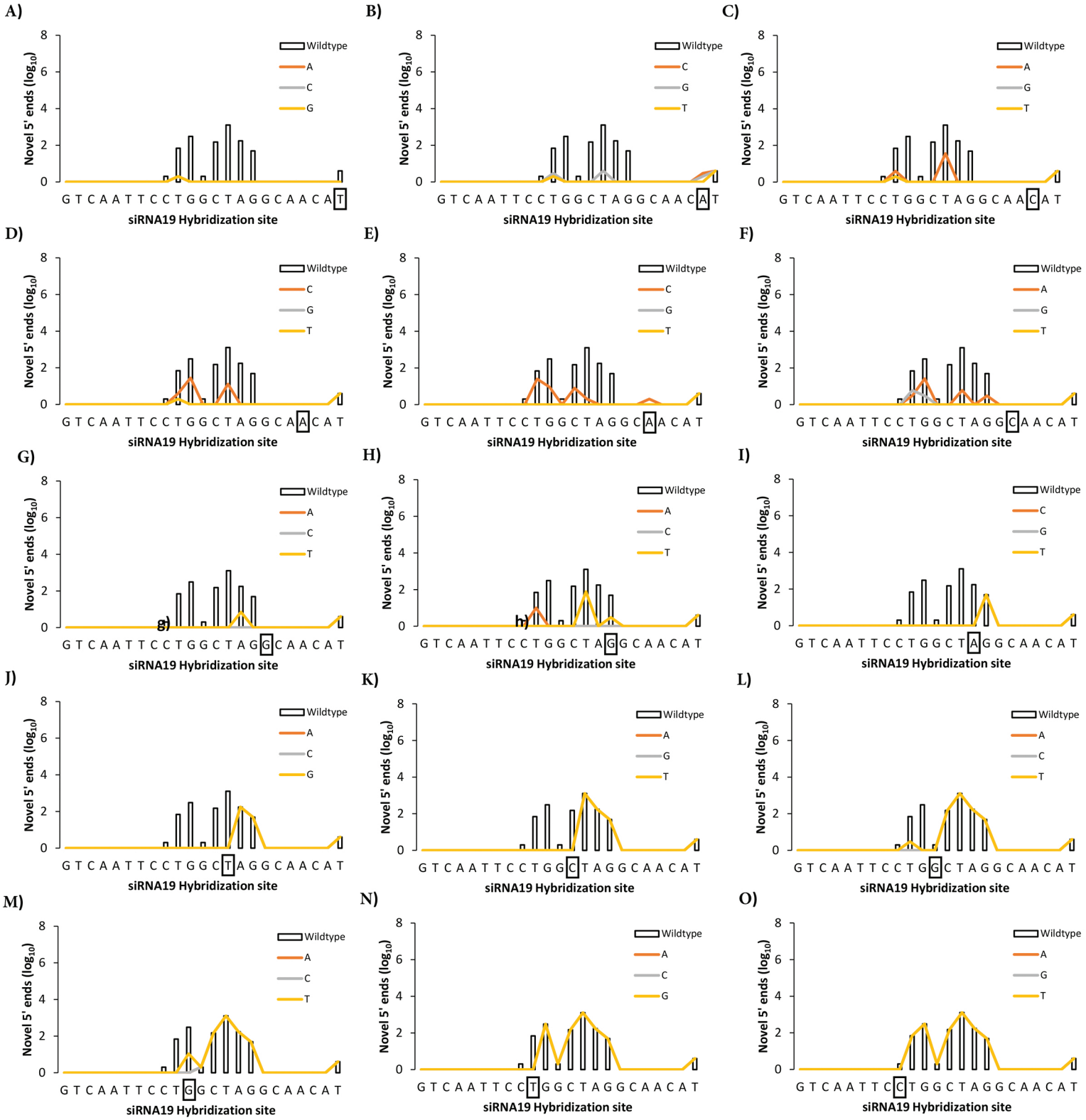
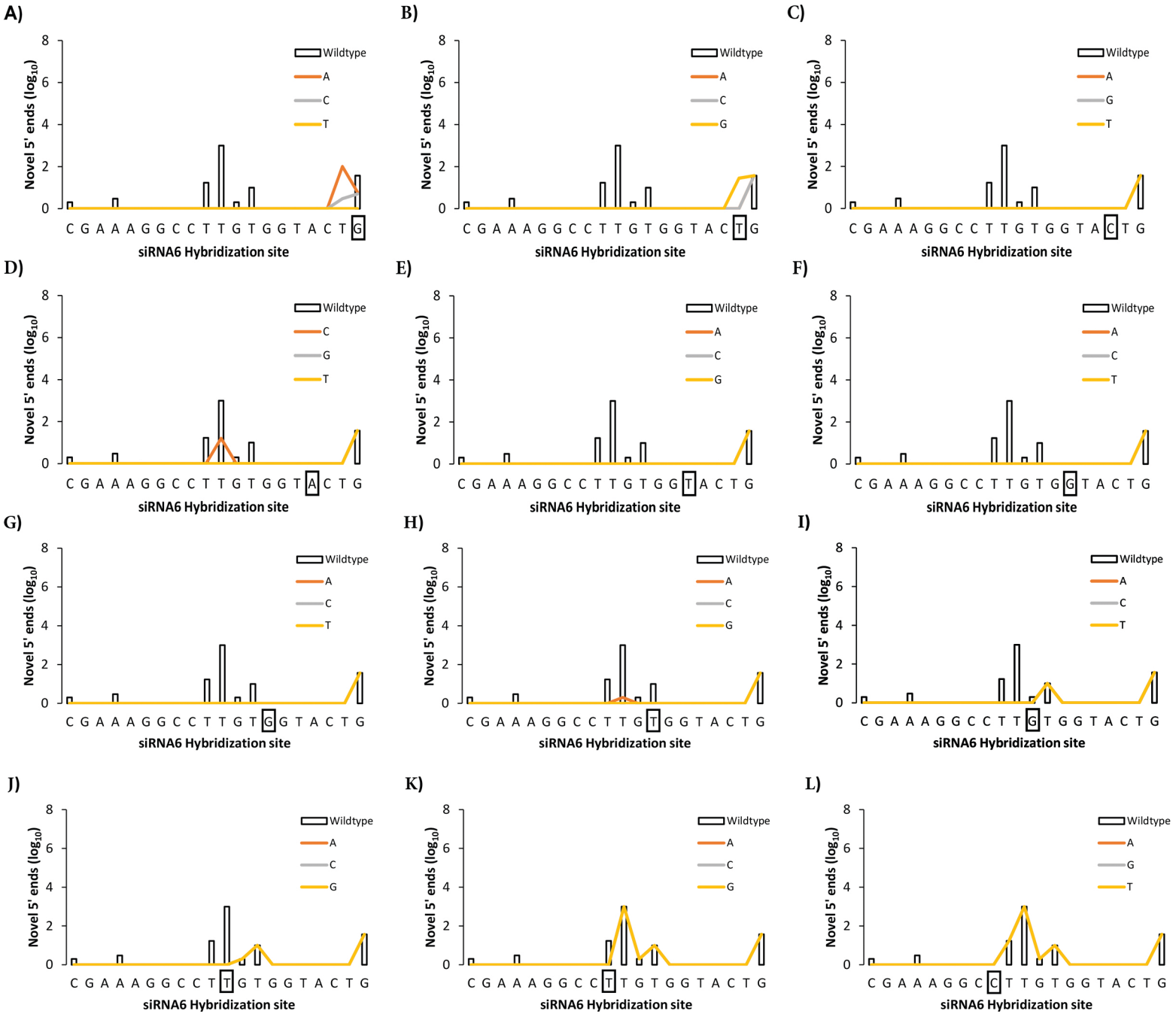


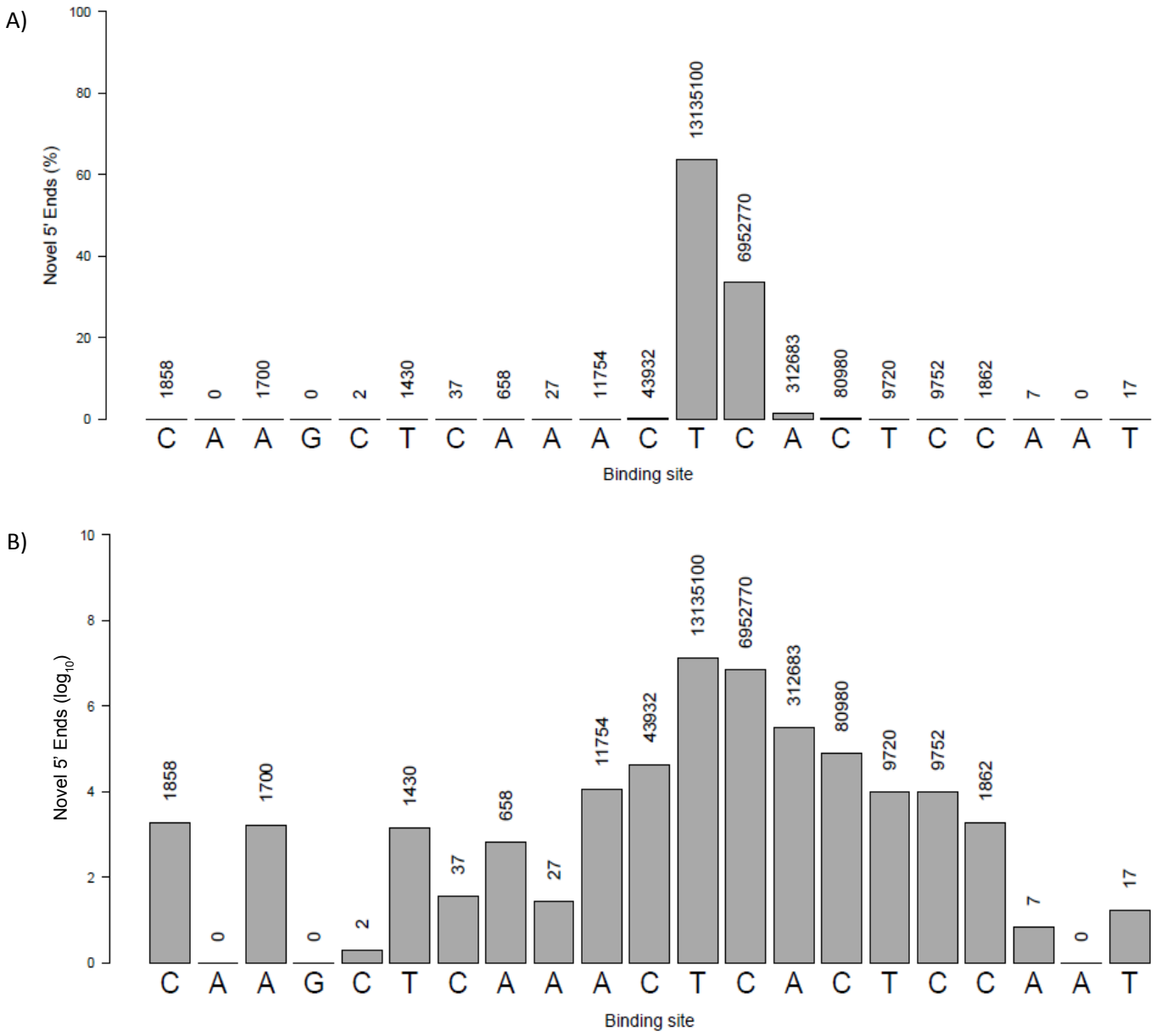
Supplemental Figure 2: Changes in 5'-RACE-SEQ product profiles at increasing levels of random mismatch tolerance during data alignment to the corresponding target sequence. The incremental increases in novel 5' end counts when accepting progressively more (MM0-MM3) mismatches are plotted for siRNA6 (A), siRNA19 (B) and siRNA22 (C), synthetic siRNA analogues to the anti-HCV shRNA encoded in TT-034.The log-scale changes in novel 5' profiles generated under 1 (D, G), 2 (E, H) or 3 (F, I) maximum random mismatches are displayed for the synthetic analogs of the anti-HCV-encoded TT-034 siRNA6 (A-C) and siRNA19 (D-F).



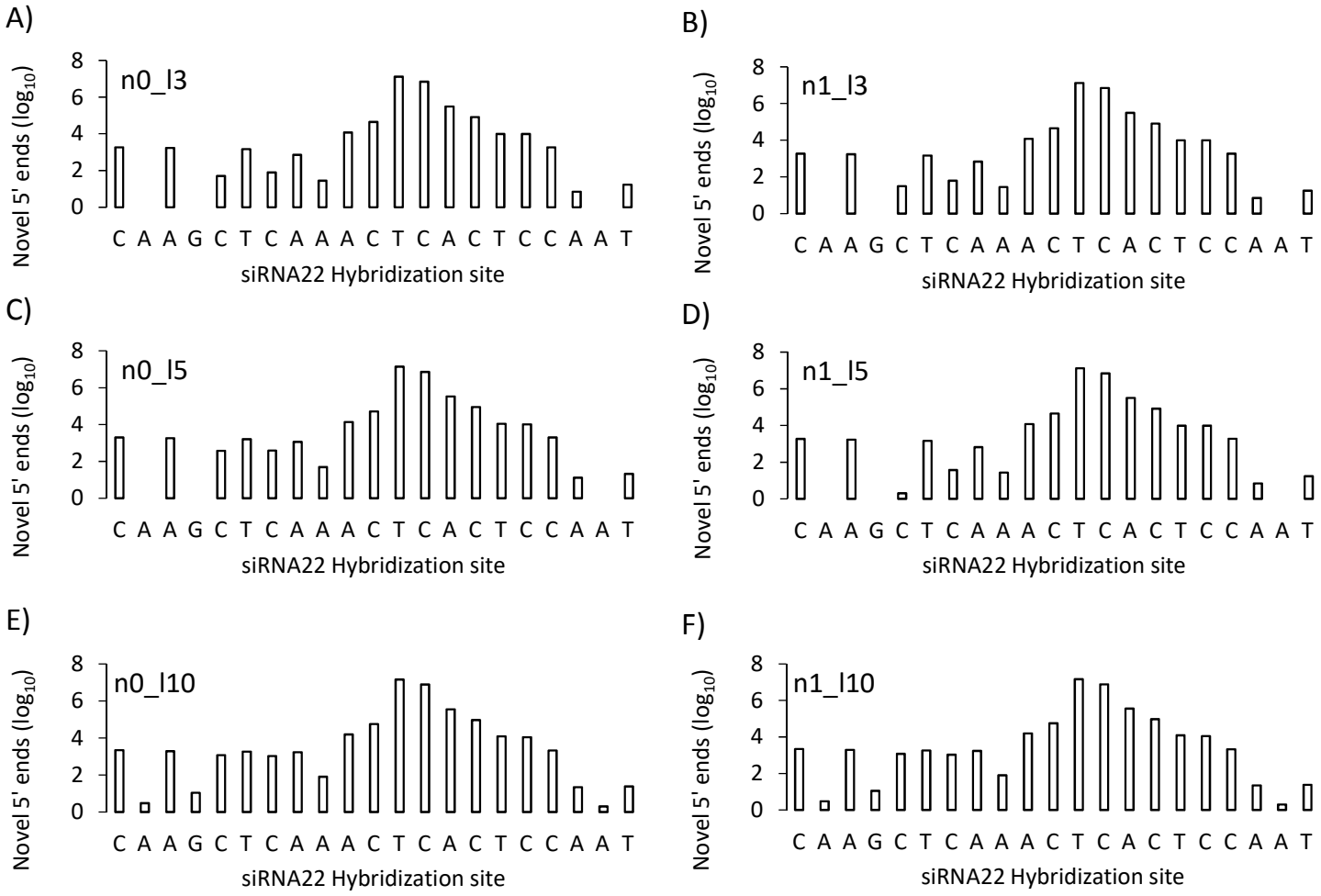
Supplemental Figure 3. Effect of specific nucleotide substitutions on RACE-SEQ profiles across the 15 Watson-Crick pairing positions from the 5' end of the siRNA 19 guide strand directed against the Con1B HCV replicon. A boxed nucleotide on the X axis highlights the base substituted within each panel. A differentially coloured line represents the effect of each possible base substitution within the boxed base.



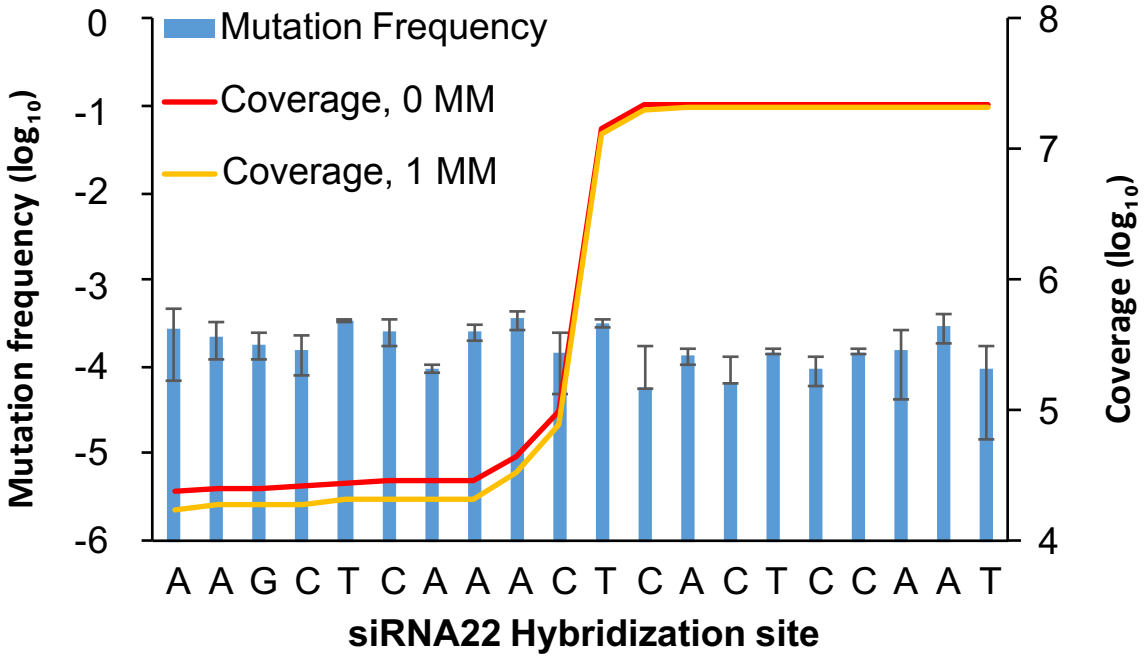
Supplemental Figure 4. Effect of specific nucleotide substitutions on RACE-SEQ profiles across the 12 Watson-Crick pairing positions from the 5' end of the siRNA 6 guide strand directed against the Con1B HCV replicon. A boxed nucleotide on the X axis highlights the base substituted within each panel. A differentially coloured line represents the effect of each possible base substitution within the boxed base.



Supplemental Figure 5. Sample output of the RACE-SEQ lite pipeline processing 5'-RACE SEQ data.



Supplemental Figure 6. Impact of Bowtie2 seed substring length parameter adjustment on RACE-SEQ data outputs highlights the incompatibility of this aligner with RACE-SEQ data processing. As Bowtie2 uses substrings of a read to map faster reads onto a reference genome, we explored the effect of substringth length of 3 (A, B), 5 (C, D) and 10 (E, F) nucleotides with no (A, C, E) or one (B, D, F) mismatch permitted during alignment for the RACE-SEQ products of the TT-034-encoded siRNA22. The parameter specification is represented in each panel with -n identifying the number of mismatches and -l the seed length.



Supplemental Figure 7: Per base coverage in replicon systems defines RACE-SEQ sensitivity to quasi-species susceptibility to slicer cleavage. The per-base mutation frequency of HCV replicons (primary Y axis) across the siRNA22 target site (X axis) was reported as 1:1,000 to 1:10,000 by Geller *et al*,[44] setting a 4 log per base coverage threshold for effective detection of HCV quasi-species. Whilst coverage of the siRNA22 hybridization site exceeds this threshold, the 5' sensitivity of 5' RACE-SEQ results in greater coverage (secondary Y axis) downstream from the primary slicer cleavage point (scissors). Coverage is also not substantially increased by permitting 1 mismatch (MM) in 5' RACE-SEQ read alignment to the reference genome; together with the 8.4×10^{-8} per base error rate in duplex sequencing used to generate this data, these mismatches are likely the outcome of quasi species sequencing and not PCR/SEQ error propagation.