Supplementary Information

Single-molecular real-time deep sequencing reveals the dynamics of multi-drug resistant haplotypes and structural variations in the hepatitis C virus genome

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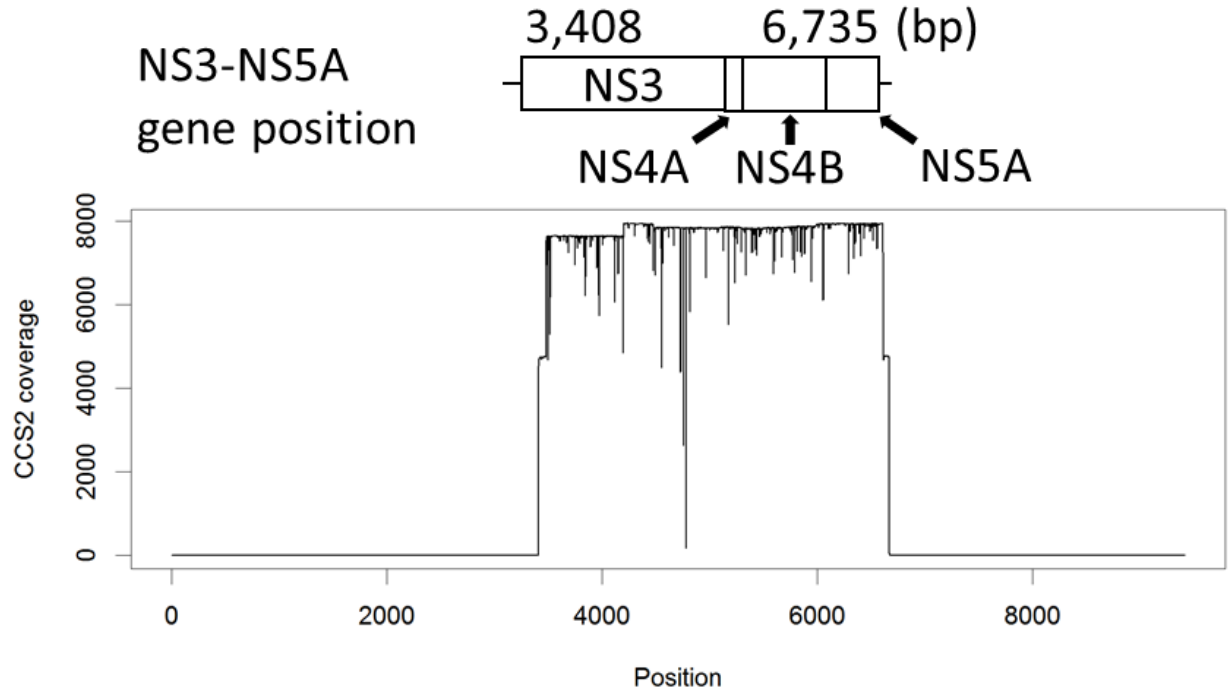
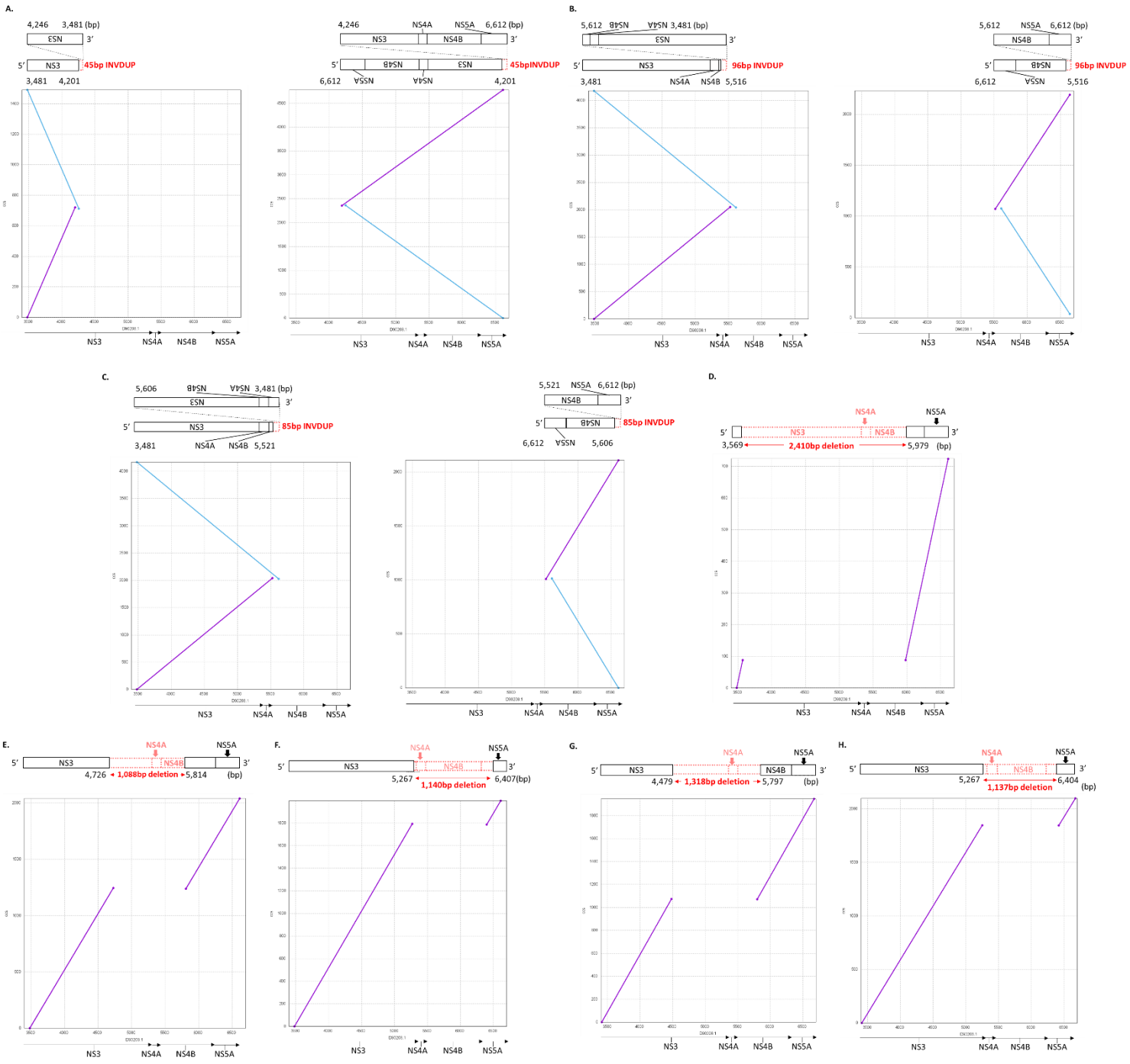


Supplementary Figure S1. Mean coverage of ≥5pass CCS2 reads for HCV genome.

BLASR version 1.3.1 mapped ≥5pass CCS2 reads to HCV genome sequence (accession no. D90208.1) with default parameter and GATK version 3.2.2 calculated the coverage of ≥5pass CCS2 reads with parameter "-T DepthOfCoverage -DBQ 0). X-axis demonstrates the position based on HCV reference genome. Y-axis means the coverage of the CCS2 reads aligned to the position. The CCS2 reads covered the region between NS3 and NS5A genes at >7,000 coverage on average.


A.
4,246 3,481 (bp)
NS3
3′
5′
NS3
45bp INVDUP
3,481 4,201
NS3 NS4A NS4B NS5A
4,246 NS4A NS5A 6,612 (bp)
NS3 NS4B
3′
5′
NS4B NS3
45bpINVDUP
6,612 NS5A NS4A 4,201
NS3 NS4A NS4B NS5A
B.
5,612 NS4B NS4A 3,481 (bp)
NS3
3′
5′
NS3
96bp INVDUP
3,481 NS4A NS4B 5,516
NS3 NS4A NS4B NS5A
5,612 NS5A 6,612 (bp)
NS4B
3′
5′
NS4B
96bp INVDUP
6,612 NS5A 5,516
NS3 NS4A NS4B NS5A
C.
5,606 NS4B NS4A 3,481 (bp)
NS3
3′
5′
NS3
85bp INVDUP
3,481 NS4A NS4B 5,521
NS3 NS4A NS4B NS5A
5,521 NS5A 6,612 (bp)
NS4B
3′
5′
NS4B
85bp INVDUP
6,612 NS5A 5,606
NS3 NS4A NS4B NS5A
D.
NS4A NS5A
5′
NS3 NS4B
3′
3,569 2,410bp deletion 5,979 (bp)
NS3 NS4A NS4B NS5A
E.
NS4A NS5A
5′
NS3 NS4B
3′
4,726 1,088bp deletion 5,814 (bp)
NS3 NS4A NS4B NS5A
F.
NS4A NS5A
5′
NS3 NS4B
3′
5,267 1,140bp deletion 6,407(bp)
NS3 NS4A NS4B NS5A
G.
NS4A NS5A
5′
NS3 NS4B
3′
4,479 1,318bp deletion 5,797 (bp)
NS3 NS4A NS4B NS5A
H.
NS4A NS5A
5′
NS3 NS4B
3′
5,267 1,137bp deletion 6,404 (bp)
NS3 NS4A NS4B NS5A

Supplementary Figure S2. The mapping of CCS2 supporting ≥1% SVs to HCV genome.

The mapping software ngmlr version 0.2.6 aligned ≥5-pass CCS reads to HCV genome (accession no. D90208.1), and the structural variation (SV) calling software Sniffles detected ≥30-bp SVs. Of the called SVs, we selected the 10 examples existing at ≥1% in the samples, which were not shown in **Figure 4**. In each panel, the upper schema shows the HCV genome structure with SV at NS3 through NS5A genes. The red symbols stand for the region SV occurred in. Especially, the inverted gene symbols represent inversion occurring in the gene and the boxes with dash lines means deletion. The positions shown in the upper figure are based on the reference. The lower image demonstrates the alignment of the representative reads supporting the SVs in MUMmer program. X-axis shows the position of the HCV reference genome. Y-axis shows the position of the reads harbouring SVs. The purple solid line means the alignment for HCV reference genome without SVs. The blue solid line demonstrates the inverted alignment for HCV reference genome. (A). 45-bp INVDUP within the sample #2-DCV/ASV-pre. (B). 96-bp INVDUP within the sample #4-DCV/ASV-pre. (C). 85-bp INVDUP within #4-DCV/ASV-post. (D). 2 410-bp deletion within #5-DCV/ASV-pre. (E). 1 088-bp deletion within #6-DCV/ASV-pre. (F). 1 140-bp deletion within ##8-DCV/ASV-pre. (G). 1 318-bp deletion within #12-DCV/ASV-pre. (H). 1 137-bp deletion within #12-DCV/ASV-pre.

Supplementary Materials. The NS3-to-NS5A long amplicon RT-PCR amplification.

Total RNA was extracted from 140 mL of serum using a QIAamp Viral RNA Mini kit (QIAGEN, Valencia, CA). HCV sequences spanning NS3 and NS5A regions (3120 bp) of the HCV genome for serum samples were reverse-transcribed in a volume of 20 μL with the One step RT-PCR Kit AMV or the PrimeScript One Step RT-PCR kit ver.2 (Takara Bio, Otsu, Japan) according to the manufacturer's protocol. For nested PCR, the products of RT-PCR were amplified using Phusion High-Fidelity DNA polymerase (FINZYMES, Espoo, Finland) or the PrimeStar HS kit (Takara Bio) according to the manufacturer's protocol by using HCV-specific primers shown in **Supplementary Table S8**.

Supplementary Tables.

Supplementary Table S1. The summary of SMRT sequencing result.

Supplementary Table S2. The error rate of HCV sequencing calculated from HCV-containing plasmids.

Supplementary Table S3. The codon information of 7 RASs.

Supplementary Table S4. All of the coded haplotypes in each sample.

Supplementary Table S5. Summary of the CCS2 reads including SVs.

Supplementary Table S6. List of structural variations existing at ≥1% in the sample.

Supplementary Table S7. Patients' information.

Supplementary Table S8. PCR primer set for sequencing the region between NS3 and NS5A genes.