Supplemental information

Simultaneous detection and mutation surveillance

of SARS-CoV-2 and multiple respiratory viruses

by rapid field-deployable sequencing

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Supplemental items for

Simultaneous Detection and Mutation Surveillance of SARS-CoV-2 and co-infections of multiple respiratory viruses by Rapid field-deployable sequencing

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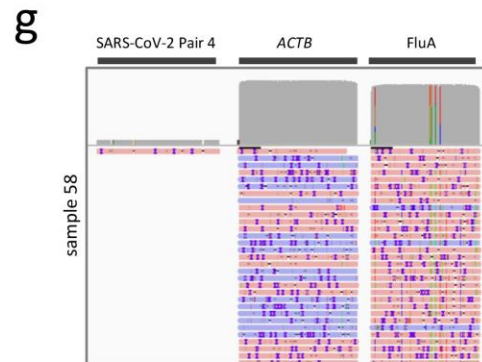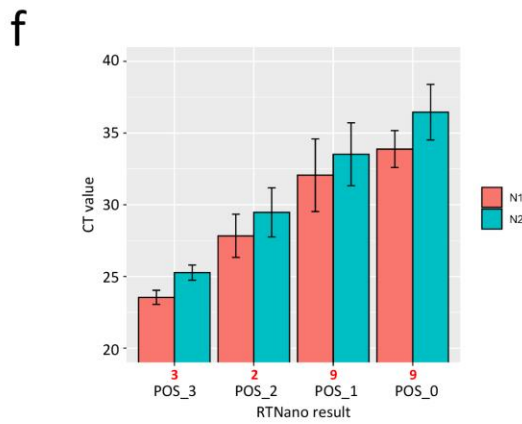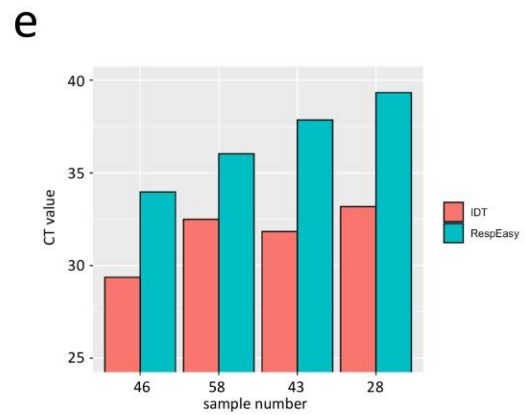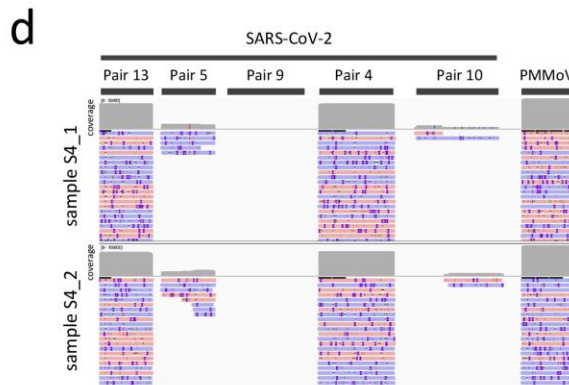
This PDF file includes:

Figs. S1, S2
Tables S1, S2

a

b

	position					
sample		Pair 13	Pair 5	Pair 9	Pair 4	Pair 10
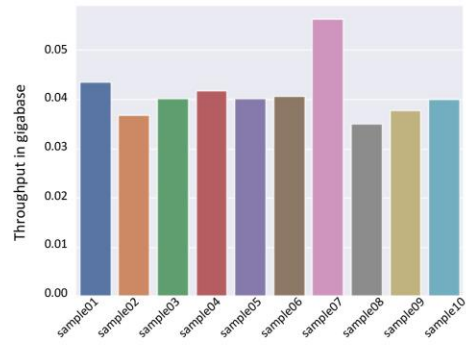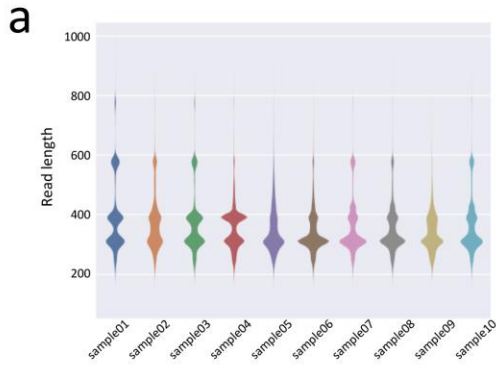
Read number per amplicon

sample	Pair 13	Pair 5	Pair 9	Pair 4	Pair 10
sample01	34401	24945	2287	66325	41788
sample02	20938	1043	29	20735	11830
sample03	32322	9850	332	51450	22589
sample04	37847	6151	3379	81953	3586
sample05	27862	1503	668	6248	438
sample06	59938	10427	1920	17949	3992
sample07	63907	21127	22480	38411	26992
sample08	32394	14967	8632	23044	8291
sample09	34126	1569	651	10686	481
sample10	27783	17773	13889	27925	18077

c

Read number per amplicon

sample	Pair 13	Pair 5	Pair 9	Pair 4	Pair 10	*ACTB*
sample01_1	8065	404	21	12235	13	8357
sample01_2	5430	155	15	4918	37	5
sample02	1222	35	6	1052	4	10569

d

e

f

g
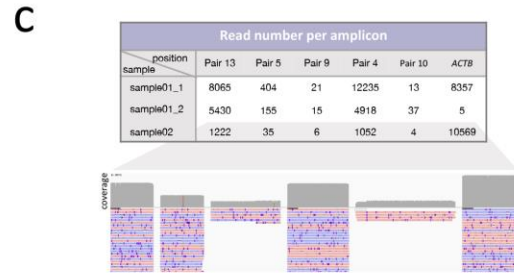
Figure S1. Sequencing analysis of multiplex RPA, Related to Figure 1.

a, The read length distribution and throughput of ten SAR-CoV-2^{+} sample sequencing.

b, The read number for each amplicon in the sequencing of ten SAR-CoV-2^{+} samples. All amplicons were covered by reads.

c, The read number for each amplicon in the trial sequencing of multiplex RPA of SARS-CoV-2 and *ACTB*. Sample 01 is used as RPA template to determine the primer concentration in two trials (sample01_1 and sample01_2). Sample 02 is used in a repeat trial using the same primer mix as sample01_2.

d, The IGV alignment plot showing robust amplification of PMMoV with SARS-CoV-2. A SARS-CoV-2^{+} sample (S4) was used as input sample in two trials with different primer concentration.

e, The CT values of FluA[+] samples in Resp'Easy[TM] and IDT FluA assays.

f, The average rRT-PCR CT values of SARS-CoV-2 RTNano[+] samples (PCR[+] of both N1 and N2 primers) of different confidence level using 7-amplicon NIRVANA.

g, IGV plots showing the read alignment to SARS-CoV-2, *ACTB* and FluA amplicon in sample 58 using 7-amplicon NIRVANA.

a
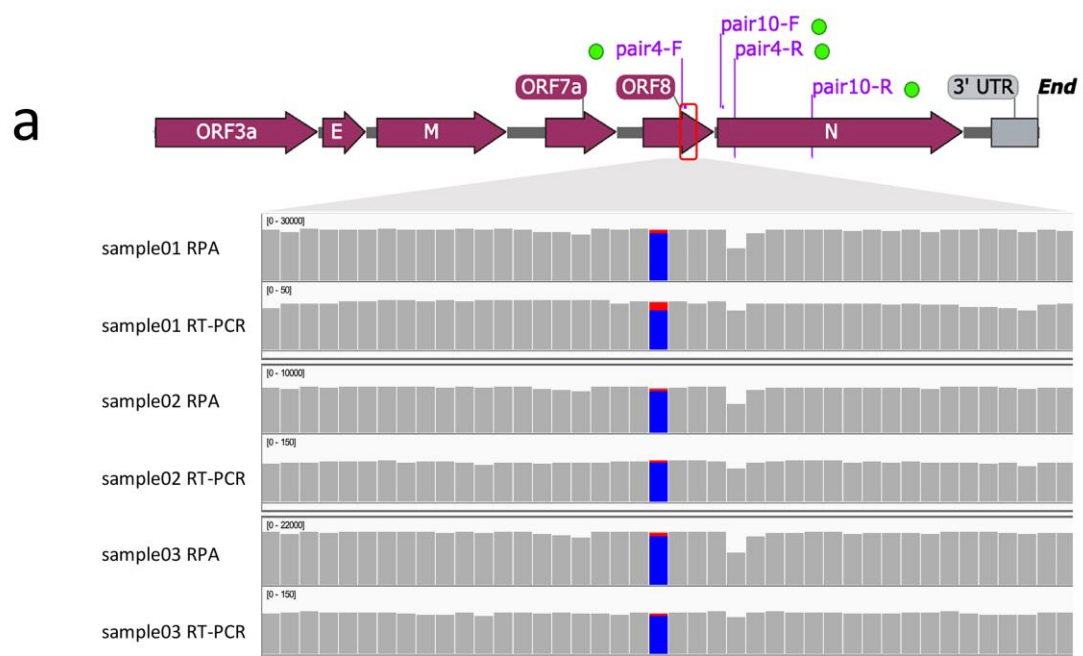
b

Figure S2. Validation of SNVs detected by NIRVANA, Related to Figure 3.

a, IGV plots showing the nt28144 T/C SNV in samples 01-03 from RPA and RT-PCR Nanopore sequencing. The blue bar represents the C base while the red bar represents the T base. All of the 3 SNVs detected in RPA sequencing were confirmed by RT-PCR amplicon sequencing.

b, Equipment used in NIRVANA. The whole workflow can be done with one laptop, one Nanopore MinION sequencer, two pipettes, two boxes of pipette tips, and a heating block (using a miniPCR™ mini16 here). All equipment can be packed into a suitcase.

Table S1. Primers used in this study, Related to Figure 1 and RPA sections in STAR Methods.

Primer	Sequence	Amplicon Size	Primer Amount
pair4-F	GCTGGTTCTAAATCACCCATTCAGT	273 bp	6 µl
pair4-R	TCTGGTTACTGCCAGTTGAATCTG		
pair5-F	TTGGGATCAGACATACCACCCA	194 bp	9 µl
pair5-R	CAACACCTAGCTCTCTGAAGTGG		
pair9-F	CCAGCAACTGTTTGTGGACCT	309 bp	12 µl
pair9-R	AGCAACAGGGACTTCTGTGC		
pair10-F	GACCCCAAAATCAGCGAAAT	394 bp	12 µl
pair10-R	TGTAGCACGATTGCAGCATTG		
pair13-F	CCAGAGTACTCAATGGTCTTTGTTC	195 bp	6 µl
pair13-R	ACCCAACTAGCAGGCATATAGAC		
ACTB-F	CCCAGCCATGTACGTTGCTATCCAGGC	263 bp	4 µl
ACTB-R	ACAGCTTCTCCTTAATGTCACGCACGAT		
influA-F	ATGAGYCTTYTAACCGAGGTCGAAACG	244 bp	12 µl
influA-R	TGGACAAANCGTCTACGCTGCAG		
HAdVs-F	GCCGAGAAGGGCGTGCGCAGGTA	161 bp	9 µl
HAdVs-R	TACGCCAACTCCGCCCACGCGCT		
HCoV-F	ATGGTCAAGGAGTTCCCATTGCTTTCGGAGTA	151 bp	9 µl
HCoV-R	GGGCCGGTACCGAGATAGTAGAAATACCATCTCG		

Table S2. Sample classification rules in RTNano analysis, Related to Figure 3.

Mark	Condition
POS_3	3 regions >= 50 records
POS_2	2 regions >= 50 records OR 1 region >= 50 records and 2 regions >= 5
POS_1	1 region >= 20 records OR 2 regions >= 5 records OR 3 regions >= 1 record
POS_0	only 1 region >= 1 and <20 records or only 2 regions >= 1 and <5 records
NEG	all regions = 0 record AND ACTB >= 1000 records
UNK (unknown)	all regions = 0 record AND ACTB <= 1000 records