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Supplemental Information

Temporal Detection and Phylogenetic Assessment of SARS-CoV-2 in Municipal Wastewater

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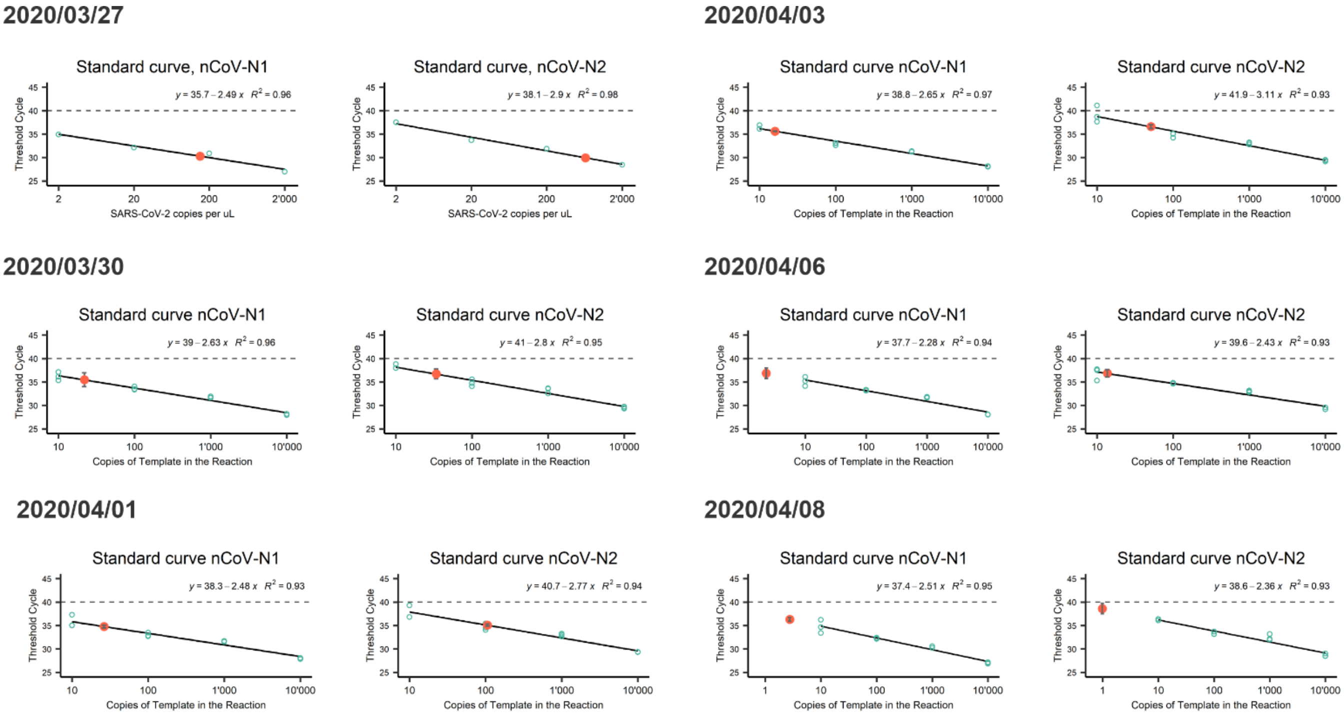

Supplemental Figure S1, related to Figure 1. Standard curves for absolute quantification of SARS-CoV-2 titers. Standard curves were generated for each RT-qPCR run. A 10-fold dilution series of positive control plasmid (IDT#10006625) encoding for SARS-CoV-2 N gene were used, three technical replicates at each dilution (green circles). Data was plotted as Cycle Threshold (Ct) on y-axis versus log10(copy number) on x-axis. Trend lines were fitted to the data by linear regression analysis in RStudio v1.2.1335, Linear equations and R2 values are shown for each standard curve. Red dots show mean Ct values for wastewater samples. Error bars represent standard deviation of Ct values.

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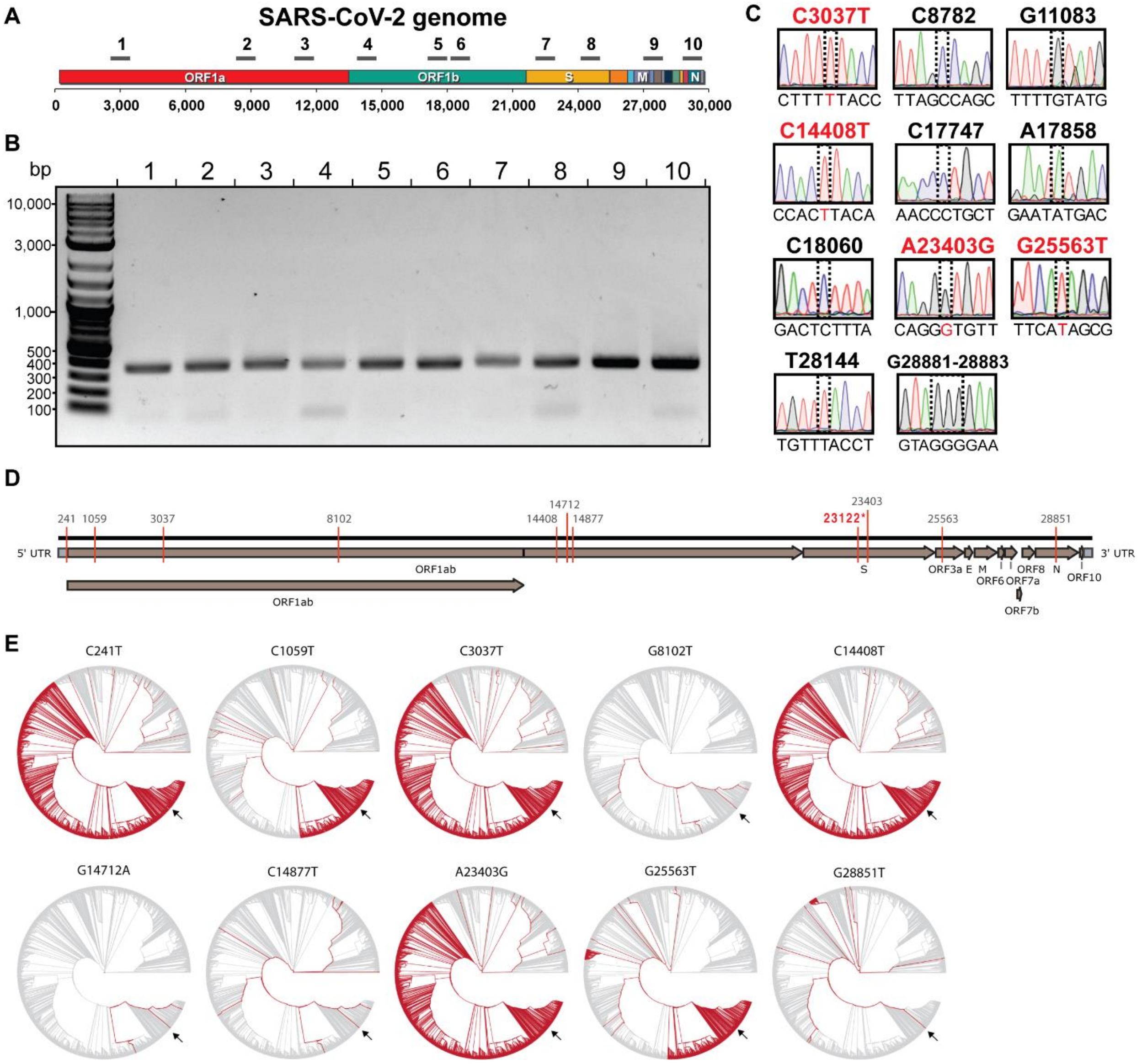

Supplemental Figure S2, related to Figure 2. A) Map of the SARS-CoV-2 genome. The solid black lines indicate the approximate location of 10 amplicons spanning phylogenetically informative regions of the genome. **B)** Agarose gel of the corresponding PCR products. **C)** Ten PCR products shown in **B** were gel-purified and used to sequence 13 polymorphic sites in SARS-CoV-2 genome [44]. Sanger traces (9-bp windows) are shown for each polymorphic site (dotted boxes). Nucleotide numbering is based on the reference genome Wuhan-Hu-1/2019 [25]. Highlighted in red are nucleotide substitutions relative to the reference. **D)** Genetic map of SARS-CoV-2 with mutations present in Bozeman wastewater sequence highlighted. *****Variant A23122T was further filtered out as an artifact of sequencing library preparation (see explanation in the main text). **E)** Phylogenetic tree with each mutation present in Bozeman wastewater mapped in red. The SARS-CoV-2 sequence from Bozeman wastewater is indicated by black arrow.

Supplemental Table S1, related to Fig 1. Total wastewater flow volumes and SARS-CoV-2 RNA concentration in composite samples

Sampling date	SARS-CoV-2 RNA concentration estimated with N1, copies / L*	SARS-CoV-2 RNA concentration estimated with N2, copies / L*	Total daily flow volume, L
March 30, 2020	528.9 ± 249	665.6 ± 261.5	22,871,458
April 1, 2020	432.7 ± 66.3	1710.8 ± 179.2	24,097,932
April 3, 2020	254.7 ± 5.4	836.3 ± 144.6	23,287,853
April 6, 2020	57.6 ± 38	257.4 ± 93.8	23,882,163
April 8, 2020	46 ± 9.2	23.5 ± 14.6	23,628,540
April 15, 2020	76 ± 24	46.7 ± 4.2	22,761,681
April 22, 2020	44.6 ± 5.6	22.2 **	22,492,917
April 29, 2020	43.5 ± 34.4	24.6 ± 1.5	22,273,363
May 6, 2020	0 ***	0	22,720,042
May 13, 2020	0	0	22,473,990
May 20, 2020	0	0	22,750,325
May 27, 2020	0	0	22,807,106
June 3, 2020	5400.5 ± 1046.1	6116.2 ± 1034.8	22,121,947
June 5, 2020	4174.6 ± 749.1	5624.6 ± 2509.2	21,754,762
June 8, 2020	2464.2 ± 502.4	1429 ± 322.4	21,016,606
June 10, 2020	971.9 ± 21.5	1947.9 ± 62.4	23,102,368
June 12, 2020	1762.7 ± 733.1	2291.4 ± 680.9	22,591,338

*Data shown as mean ± sem, n = 3

** Only one of three replicates showed amplification

*** Samples tested negative and thus were called 0 copies/L

Supplemental Table S2, related to Methods. Primer design used for RT-PCR (Supplemental Fig S2A,B)

PCR product	Primer	Sequence (5'-3')
1	nCoV-2019_10_LEFT	TGAGAAGTGCTCTGCCTATACAGT
	nCoV-2019_10_RIGHT	TCATCTAACCAATCTTCTTCTTGCTCT
2	nCoV-2019_29_LEFT	ACTTGTGTTCCTTTTTGTTGCTGC
	nCoV-2019_29_RIGHT	AGTGTACTCTATAAGTTTTGATGGTGTGT
3	nCoV-2019_37_LEFT	ACACACCACTGGTTGTTACTCAC
	nCoV-2019_37_RIGHT	GTCCACACTCTCCTAGCACCAT
4	nCoV-2019_48_LEFT	TGTTGACACTGACTTAACAAAGCCT
	nCoV-2019_48_RIGHT	TAGATTACCAGAAGCAGCGTGC
5	nCoV-2019_59_LEFT	TCACGCATGATGTTTCATCTGCA
	nCoV-2019_59_RIGHT	AAGAGTCCTGTTACATTTTCAGCTTG
6	nCoV-2019_60_LEFT	TGATAGAGACCTTTATGACAAGTTGCA
	nCoV-2019_60_RIGHT	GGTACCAACAGCTTCTCTAGTAGC
7	nCoV-2019_77_LEFT	CCAGCAACTGTTTGTGGACCTA
	nCoV-2019_77_RIGHT	CAGCCCCTATTAAACAGCCTGC
8	nCoV-2019_84_LEFT	TGCTGTAGTTGTCTCAAGGGCT
	nCoV-2019_84_RIGHT	AGGTGTGAGTAAACTGTTACAAACAAC
9	nCoV-2019_93_LEFT	TGAGGCTGGTTCTAAATCACCCA
	nCoV-2019_93_RIGHT	AGGTCTTCCTTGCCATGTTGAG
10	nCoV-2019_95_LEFT	TGAGGGAGCCTTGAATACACCA
	nCoV-2019_95_RIGHT	CAGTACGTTTTTGCCGAGGCTT