1 **Supporting Information**

2

3 **Early warning measurement of SARS-CoV-2 variants of concern in wastewaters**
4 **by mass spectrometry**

5

6 Jiaxi Peng[1,4,5], Jianxian Sun[1], Minqing Ivy Yang[2], Richard M. Gibson[3], Eric J. Arts[3],
7 Abayomi S. Olabode[3], Art F. Y. Poon[3], Xianyao Wang[1], Aaron R. Wheeler[1,4,5],
8 Elizabeth A. Edwards[2], Hui Peng[1,6,*]

9

10 [1] Department of Chemistry, University of Toronto, 80 St George Street, Toronto, ON,
11 M5S 3H6, Canada
12 [2] Department of Chemical Engineering and Applied Chemistry, University of Toronto,
13 200 College Street, Toronto, ON, M5S 3E5, Canada
14 [3] Department of Microbiology and Immunology, Western University, 1151 Richmond
15 Street, London, ON, N6A 5C1, Canada
16 [4] Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, 160
17 College Street, Toronto, ON, M5S 3E1, Canada
18 [5] Institute of Biomedical Engineering, University of Toronto, 164 College Street,
19 Toronto, ON, M5S 3G9, Canada
20 [6] School of the Environment, University of Toronto, 80 St George Street, Toronto, ON,
21 M5S 3H6, Canada

22

23 ***Corresponding authors:** Dr. Hui Peng, Email: hui.peng@utoronto.ca, Department of
24 Chemistry, University of Toronto, Toronto, Ontario, M5S 3H6, Canada.

25

26 The supporting information provides text and figures addressing: (1) RT-qPCR
27 quantification of the SARS-CoV-2 viral signal; (2) AS-qPCR assays; (3) NGS detection
28 of Omicron variant; (4) sequences of primers and amplicons; (5) nPCR-LC-MS method
29 for detection of the Alpha variant; (6) spectra of standard oligomers; (7) method
30 comparison for VOCs detection.
31

32 **Wastewater sample collection and RNA extraction.** Twenty-four-hour flow-
33 dependent composite post-grit wastewater influent samples were collected from three
34 wastewater treatment plants in the City of Toronto, including Ashbridges Bay (TAB),
35 Highland Creek (THC) and North Toronto (TNT), and six wastewater treatment plants
36 in Halton region including Mid Halton (MH), Georgetown (GT), Acton (ACT),
37 Oakville Southeast (OSE), Oakville Southwest (OSW), and Burlington Skyway (SKY),
38 as described in our previous study.[1] Samples from each site were delivered with
39 icepacks. Samples were mixed well before being split into centrifuge bottles. Sample
40 volume used for centrifuge and RNA extraction for each site were determined based on
41 their solid content. In this study, sample volume for each site were: 80 mL for TAB, 80
42 mL for THC, 120 mL for TNT, 40 mL for ACT, 60 mL for GT, 120 mL for MH, 120
43 mL for OSE, 200 mL for OSW, and 120 mL for SKY. After being centrifuged at 10,000
44 x g for 45 minutes at 4°C, supernatant was removed and the remaining wet pellet was
45 transferred to a 2 mL screw-cap microcentrifuge tube and further centrifuged at 13,000
46 × g for 1 minute. The final pellet wet weights were recorded. RNA was extracted from
47 pellet using Qiagen's RNeasy PowerMicrobiome Kit (Qiagen, Germantown, MD), with
48 minor modification: 10 µL beta-mercaptoethanol and 100 µL phenol: chloroform:
49 isoamyl alcohol (25: 24: 1, v/v) mixtures (Invitrogen, CAT# 15593031, USA) were
50 added to the bead beating tube along with the lysis buffer in the kit. When pellet weight
51 exceeded 250 mg, the pellet will be split into two portions for extraction. A whole
52 process control was included in each batch of extraction, which is a blank control with

53 no sample but following the same treatment as samples.

54 **RT-qPCR method.** RNA concentration was quantified by NanoDrop for each sample,

55 and RNA extracts with A260/A280 at about 2.1, and A260/A230 at about 2.4 were

56 applied in RT-qPCR. Bio-Rad CFX Opus 384 Real-Time PCR System was applied for

57 RT-qPCR assays. For inhibition check, RNA was diluted 10-fold, and results from RT-

58 qPCR were compared with those of original RNA. Singleplex RT-qPCR quantification

59 of the SARS-CoV-2 viral signal was performed by targeting the N1 and N2 gene

60 regions. Quantification was performed via 6 point standard curves using a custom,

61 synthetic linearized plasmid (ConcatP) containing the complete N1/N2 genes.

62 Standards were diluted in poly-A carrier solution and the poly-A solution was set as the

63 carrier control. A 4-fold serial dilution of standards with concentrations from 3.9 to 1.6

64 $\times 10^4$ copies/reaction was applied for N1 and N2. The TaqMan Fast Virus 1-Step Master

65 Mix (Applied Biosystems, CAT# 4444436, USA) was utilized for all RT-qPCR

66 reactions in this study. Samples that met the following quality control criteria were

67 included for data analysis: 1) standard curves were linear ($R^2 \geqslant 0.99$); 2) the primer

68 efficiency was between 95%–105%; 3) y-intercept was between 36.7–37.9; 4) slop was

69 between -3.38–-3.22. 4 μL of RNA template/standard was loaded into each qPCR

70 reaction, and primers and probes were present at concentrations of 500 nM and 125 nM,

71 respectively, for a final reaction volume of 10 μL. The PCR conditions were: RT at

72 50°C for 5 min, 95°C for 20 sec, 45 cycles of 95°C for 3 sec followed by 60°C for 30

73 sec. Standard curves for each target, three negative controls (no template control, whole

74 process control, and carrier control), and two positive controls (with concentrations
75 similar to the tested samples) were run along with samples on each 384 PCR clear plate.
76 All quantifications for each target were made using standard curves that were run on
77 the same plate. N1 and N2 were run with triplicates. The relative standard deviation
78 (RSD) was below 20%. The limit of detection (LOD) was set at 3.94 copies/reaction
79 with a detection frequency of 92% which is close to the detection rate of 95%
80 recommended by the Minimum Information for Publication of Quantitative Real-Time
81 PCR Experiments (MIQE) guidelines.[2] If there was any amplification in negative
82 controls greater than 3.94 copy/reaction, the LOD was estimated to be the average of
83 all the negative controls. Samples with no amplification or quantification lower than
84 1.8 copies/reaction (half of the theoretical LOD) were replaced with 1.8 copies/reaction
85 and flagged as below LOD. The LOD for qPCR in wastewater samples was 0.8
86 copies/mL, which was similar to nPCR-LC-MS method. All probes and primers utilized
87 in this study are presented in Table S1. The reporting table from the MIQE guidelines[2]
88 is presented in Table S3.
89
90 **Allele-specific quantitative PCR assays.** An allele-specific qPCR assay targeting at
91 D3 area of N gene was used for the detection of Alpha variant as a previously published
92 work.[3] Bio-Rad CFX maestro software was used to analyze qPCR results. Threshold
93 was set at a point that was significantly higher than baseline and was set at 100 for all
94 target in this study. qPCR reactions were run in triplicate using 2 μl of RNA in a final

95 reaction volume of 10 µl. Reaction contains D3L forward primer (500 nM) combined
96 with the CDC-2019-nCoV_N1 probe (125 nM) and CDC-2019-nCoV_N1 reverse
97 primers (500 nM). Cycling protocol was as follows: RT at 50°C for 5 min, followed by
98 95°C for 20 sec, and 45 cycles of 95°C for 3 sec, 55°C for 45 sec. Quantification was
99 performed via 6 point standard curves using concatP. Standard dilutions and
100 concentrations were the same as N1/N2. Detection of universal, Omicron, and Delta
101 variants were run according to a N200 triplex qPCR assay.[4] The proportion of each
102 VOC detected by AS-qPCR is calculating by the equation: VOC%=Quantity of target
103 VOC/Quantity of universal. qPCR reactions were run in triplicate using 4 µL
104 RNA/DNA template and a final reaction volume of 10 µL. Concentrations of primers
105 were 500 nM, and concentrations of probes were 100 nM for universal, 400 nM for
106 delta and Omicron/Alpha. Cycling protocol was: RT at 50°C for 15 min, 95°C for 2
107 min, 45 cycles of 95°C for 3 sec followed by 57°C for 30 sec. Quantification was
108 performed via 6 point standard curves using concatP for universal/Omicron and a
109 purified PCR product amplified from TWIST Bioscience control 23 for delta/universal.
110 Standard dilutions and concentrations were the same as N1/N2. All qPCR plates were
111 run with negative controls (no template control and carrier control) and positive
112 controls (Resident RNA sample with known Omicron and delta outbreak, respectively).
113
114 **Next-generation sequencing.** For each sample used to compare the Omicron%
115 quantified by LC-MS, a second subsample was taken from the same sample bottle and

116 shipped to the University of Western for NGS analysis. A 30 mL aliquot of the
117 composite influent wastewater was centrifuged at 4200 × g at 4°C for 20 minutes. The
118 supernatant was discarded, except for approximately 500 µL used to resuspend the
119 solids pellet. The resuspended solids pellet was added to 700 µL of RLT lysis buffer
120 (QIAgen; 79216) containing 2-Mercaptoethanol (1:100) and bead beated using 0.7 mm
121 ceramic garnet beads (PowerBead Tubes, QIAgen; Cat # 13123-50) on a MP Bio
122 Fastprep-24 tissue homogenizer for 4 cycles of 20 seconds at 4.0 M/s. The sample was
123 centrifuged at 14,000 x g for three minutes to pellet any solids, and the supernatant was
124 retained. The supernatant was added to an equal volume of 70% ethanol and mixed
125 thoroughly prior to extraction using the RNeasy Mini Kit (QIAgen; 74106), as per
126 manufacturer's specifications, beginning with the addition of the sample to the spin-
127 column. Nucleic acids were eluted in 50 µL of nuclease-free water. The complement
128 DNA (cDNA) was synthesized using the SuperScript™ IV First-Strand Synthesis
129 System (Invitrogen; 18091050) as described in others' work.[5] After obtaining the
130 cDNA, a series of 400-bp SARS-CoV-2 amplicons were generated using the ArticV3
131 primer set, as previously described.[6] PCR was carried out with the following steps:
132 initial denaturation at at 98°C for 30 seconds, followed by 35 cycles of 95°C for 15
133 seconds and 63°C for 5 minutes, with a final elongation at 4°C using a Life
134 Technologies thermal cycler. Artic primer set 1 and set 2 samples were pooled and
135 purified using the AMPure XP Beads (0.8X) (Beckman Coulter; A63881) and
136 quantified using Qubit 4 fluorometer (Invitrogen) using the dsDNA High Sensitivity

137 Kit (Invitrogen; Q32854). The pooled PCR products (5µL) was used to generate
138 barcoded sequencing libraries using the Nextera XT kit (Illumina; FC-131-1024) and
139 index primer sets according to the manufacturer's specifications. The libraries were
140 purified and concentrated using the AMPure XP Beads (1.8×) (Beckman Coulter;
141 A63881), then were resuspended in 10 mM Tris-HCl with 50 mM NaCl, pH 8.0. The
142 final concentrated pooled libraries were sequenced using the Illumina's MiSeq platform.
143 Raw reads from the Illumina system were demultiplexed using the software provided
144 by the manufacturer. The resulting FASTQ files were trimmed and cleaned using
145 cutadapt (version 1.18)[7] and then mapped to the SARS-CoV-2 reference sequence
146 (Genbank accession NC_045512) using minimap2 (version 2.17).[8] Mutation
147 frequencies and coverage statistics were extracted from the program outputs using
148 Python (https://github.com/PoonLab/gromstole/). To estimate PANGO lineage
149 frequencies, we fit a quasibinomial regression model in R to the frequencies and
150 coverage of mutations associated with the given lineage "constellation"
151 (https://github.com/cov-lineages/constellations). We used the quasibinomial model
152 instead of the binomial model, which assumes that the variance is equal to $p(1-p)/N$, to
153 accommodate overdispersion in frequencies among mutations that may result from
154 variation in sampling or PCR amplification among amplicons. We used this method to
155 independently estimate the frequencies of Delta and Omicron variants for each sample,
156 which were the predominantly circulating lineages at the study sites. Note that this
157 approach does not constrain these lineage frequencies to sum to 100%. To facilitate

158 comparison to other estimates in this study, these frequencies were normalized to sum

159 to 100%, which assumes that no other lineage was present in the sample population.

160 **Table S1: Oligonucleotide sequences**

Primer/probe	Sequence (5'→3')	Amplicon size	Experiment information	Reference
2019-nCoV_N1 forward primer	GACCCCAAAATCAGC GAAAT	73bp	N1 RT-qPCR, Figure 3	U. S. CDC
2019-nCoV_N1 reverse primer	TCTGGTTACTGCCAGT TGAATCTG	73 bp		U. S. CDC
2019-nCoV_N1 probe (IDT)	**6-FAM-** ACCCCGCAT/**ZEN**/TAC GTT TGGTGGACC-**IBFQ**	/		U. S. CDC
2019-nCoV_N2 forward primer	TTACAA ACATTGGCCGCAAA	68 bp	N2 RT-qPCR, Figure 3	U. S. CDC
2019-nCoV_N2 reverse primer	GCGCGACATTCCGAAG AA	68 bp		U. S. CDC
2019-nCoV_N2 probe (IDT)	**6-FAM-** ACAATTTGC/**ZEN**/CCC CAGCGCTTCAG-**IBFQ**	/		U. S. CDC
N200 forward primer	TAGTCGCAACAGTTCA AGAAAT	742 bp	N200 triplex qPCR, Figure 3	Meghan Fuzzen[4]
N200 reverse primer	CTGGTTCAATCTGTCA AGCAG	742 bp		
N200 probe universal (IDT)	FAM- TCCTGCTAGAATGGC- BHQ-1 plus	/		
N200 probe delta (IDT)	Cal Fluor Gold 540- CAGCAGTATGGGAACT -BHQ-1 plus	/		
N200 probe Omicron/Alpha (IDT)	Quasar670- CAGCAGTAAACGAAC- BHQ-2 plus	/		
D3L forward primer	CATCTAAACGAACAA ACTAAATGTCTCTA	106 bp	D3L AS-qPCR, Figure 1d	Tyson Graber[3]
2019-nCoV_N1 reverse primer	TCTGGTTACTGCCAGT TGAATCTG	106 bp		U. S. CDC
2019-nCoV_N1 probe (IDT)	**6-FAM-** ACCCCGCAT/**ZEN**/TAC	/		U. S. CDC

	GTT TGGTGGACC-**IBFQ**			
2019-nCoV_N1 reverse primer Wide N1 forward primer	TCTGGTTACTGCCAGTTGAATCTG GAGTATCATGACGTTCGTGTTGT	139 bp	Alpha variant detection, nPCR-LC-MS, first round PCR, Figure 1	U. S. CDC This study
N200 forward primer N200 reverse primer	TAGTCGCAACAGTTCAAGAAAT CTGGTTCAATCTGTCAAGCAG	742 bp	Multiple variant detection, nPCR-LC-MS, first round PCR, Figures 2 and 3	Meghan Fuzzen[4]
D3 nest forward primer D3 nest reverse primer	AACGAACAAACTAAA TTGGGGTCCATT	37 bp	Alpha variant detection, nPCR-LC-MS, Nested PCR, Figure 1	This study
P13 nest forward primer P13 nest reverse primer	TCAGCGAAATGCA CCAAACGTAATGCG	31 bp	Omicron variant detection, nPCR-LC-MS, Nested PCR, Figures 2b and 3	This study
D63 nest forward primer D63 nest reverse primer	AACATGGCAAGGAAG TCGAGGGAATTTAAGG	33 bp	Delta variant detection, nPCR-LC-MS, Nested PCR, Figure 2c	This study
R203/G204 nest forward primer R203/G204 nest reverse primer	CTCCAGGCAGCAG TTCTAGCAGGAGAAG	37 bp	Delta variant detection, nPCR-LC-MS, Nested PCR, Figure 2d	This study

161
162

163 **Table S2. Sequences of amplicons expected from nested PCR**

Primer/probe	Sequence (5’→3’)	m/z
Wild-type D3	AACGAACAAACTAAAATGTCTGATAATGGACCCCAAa*	2270.79
D3L	AACGAACAAACTAAAATGTCTCTAAATGGACCCCAAa	2262.78
D3L_del	AACGAACAAACTAAATGTCTCTAAATGGACCCCAAa	2200.37
Wild-type P13	TCAGCGAAATGCACCCCGCATTACGTTTGGa	2368.90
P13L	TCAGCGAAATGCACTCCGCATTACGTTTGGa	2372.65
Wild-type D63	AACATGGCAAGGAAGACCTTAAATTCCCTCGAa	2023.54
D63G	AACATGGCAAGGAAGGCCTTAAATTCCCTCGAa	2026.74
R203K/G204R	CTCCAGGCAGCAGTAAACGAACTTCTCCTGCTAGAAa	2261.18
R203M	CTCCAGGCAGCAGTATGGGAACTTCTCCTGCTAGAAa	2270.58
Wild-type D3 anti-sense	TTGGGGTCCATTATCAGACATTTTAGTTTGTTCGTTa	2275.17
D3L anti-sense	TTGGGGTCCATTTAGAGACATTTTAGTTTGTTCGTTa	2282.96
D3L_del anti-sense	TTGGGGTCCATTTAGAGACATTTAGTTTGTTCGTTa	2222.36
Wild-type P13 anti-sense	CCAAACGTAATGCGGGGTGCATTTCGCTGAa	2388.89
P13L anti-sense	CCAAACGTAATGCGGAGTGCATTTCGCTGAa	2384.90
Wild-type D63 anti-sense	TCGAGGGAATTTAAGGTCTTCCTTGCCATGTTa	2028.94
D63G anti-sense	TCGAGGGAATTTAAGGCCTTCCTTGCCATGTTa	2025.94
R203K/G204R anti-sense	TTCTAGCAGGAGAAGTTCGTTTACTGCTGCCTGGAGa	2285.78
R203M anti-sense	TTCTAGCAGGAGAAGTTCCCATACTGCTGCCTGGAGa	2276.78

164 * Taq polymerase has a nontemplate-dependent terminal transferase activity that adds a single deoxyadenosine (a) to the 3´-ends
165 of PCR products.
166

167

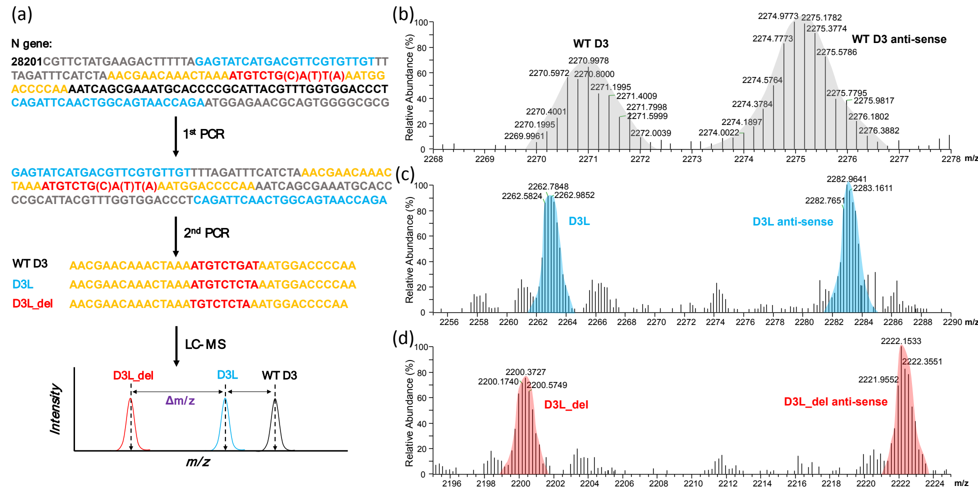
Table S3. Check-list of experimental details as requested by MIQE guidelines[2]

ITEM TO CHECK	Provided (Y/N)	CHECKLIST
EXPERIMENTAL DESIGN		
Definition of experimental and control groups	Y	Provided in method section, SI
Number within each group	Y	Provided in method section, SI
SAMPLE		
Description	Y	Provided in method section, SI
Volume/mass of sample processed	Y	Provided in method section, SI
Microdissection or macrodissection	N	N/A
Processing procedure	Y	Provided in method section, SI
If frozen - how and how quickly?	N	N/A
If fixed - with what, how quickly?	N	N/A
Sample storage conditions and duration (especially for FFPE samples)	Y	Provided in method section, SI
NUCLEIC ACID EXTRACTION		
Procedure and/or instrumentation	Y	Provided in method section, SI
Name of kit and details of any modifications	Y	Provided in method section, SI
Source of additional reagents used	Y	Provided in method section, SI
Details of DNase or RNAse treatment	N	N/A
Contamination assessment (DNA or RNA)	N	Not done
Nucleic acid quantification	Y	Provided in method section, SI
Instrument and method	Y	Provided in method section, SI
Purity (A260/A280)	Y	Provided in method section, SI
RNA integrity method/instrument	N	Not done
RIN/RQI or Cq of 3' and 5' transcripts	N	N/A
Inhibition testing (Cq dilutions, spike or other)	Y	Provided in method section, SI
REVERSE TRANSCRIPTION		
Complete reaction conditions	Y	Provided in method section, SI
Amount of RNA and reaction volume	Y	Provided in method section, SI
Priming oligonucleotide (if using GSP) and concentration	Y	Provided in method section and table S1, SI
Reverse transcriptase and concentration	Y	Provided in method section, SI
Temperature and time	Y	Provided in method section, SI
Manufacturer of reagents and catalogue numbers	Y	Provided in method section, SI
Cqs with and without RT	N	N/A
Storage conditions of cDNA	N	N/A
qPCR TARGET INFORMATION		
If multiplex, efficiency and LOD of each assay.	Y	Provided in method section, SI
Sequence accession number	Y	Published assays or provided in results section
Amplicon length	Y	Provided in Table S1
In silico specificity screen (BLAST, etc)	N	Not done
Location of each primer by exon or intron (if applicable)	N	N/A
What splice variants are targeted?	N	N/A
qPCR OLIGONUCLEOTIDES		
Primer sequences	Y	Provided in Table S1
Probe sequences	Y	Provided in Table S1
Location and identity of any modifications	N	N/A
Manufacturer of oligonucleotides	Y	Provided in method section, SI
qPCR PROTOCOL		
Complete reaction conditions	Y	Provided in method section, SI
Reaction volume and amount of cDNA/DNA	Y	Provided in method section, SI
Primer, (probe), Mg++ and dNTP concentrations	Y	Provided in method section, SI
Polymerase identity and concentration	Y	According to kit instructions
Buffer/kit identity and manufacturer	Y	Provided in method section, SI
Additives (SYBR Green I, DMSO, etc.)	N	N/A
Complete thermocycling parameters	Y	Provided in method section, SI
Manufacturer of qPCR instrument	Y	Provided in method section, SI
qPCR VALIDATION		
Specificity (gel, sequence, melt, or digest)	N	N/A
For SYBR Green I, Cq of the NTC	N	N/A
Standard curves with slope and y-intercept	Y	Provided in method section, SI
PCR efficiency calculated from slope	Y	Provided in method section, SI
r2 of standard curve	Y	Provided in method section, SI
Linear dynamic range	N	Not accessed
Cq variation at lower limit	N	Not determined
Evidence for limit of detection	Y	Provided in method section, SI
If multiplex, efficiency and LOD of each assay.	Y	Provided in method section, SI
DATA ANALYSIS		
qPCR analysis program (source, version)	Y	Provided in method section, SI
Cq method determination	Y	Provided in method section, SI
Outlier identification and disposition	N	N/A
Results of NTCs	Y	Provided in method section, SI
Justification of number and choice of reference genes	N	N/A
Description of normalisation method	N	N/A
Number and stage (RT or qPCR) of technical replicates	Y	Provided in method section, SI
Repeatability (intra-assay variation)	Y	Provided in method section, SI
Statistical methods for result significance	Y	Provided in method section, SI
Software (source, version)	Y	Provided in method section, SI

168

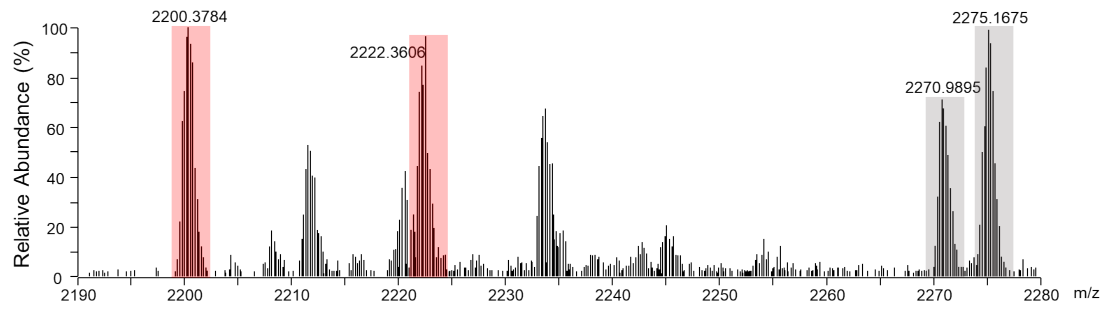
169

170



171 **Figure S1.** (a) The pipeline of the nPCR-LC-MS method for detection of the Alpha
172 variant. The signature mutation at position 28280 in B.1.1.7 was selected to target the
173 Alpha variant. Sequence in blue is the primer for the first PCR step. Sequence in yellow
174 is the primer for the second PCR (nested PCR) step. Sequence in red is the target
175 mutation region. Spectra of amplicons containing wild-type D3 (b), D3L (c), and
176 D3L_del (d) using corresponding synthesized oligomer as templates: both sense strand
177 (left peak) and anti-sense strand (right peak) were detected in each sample.

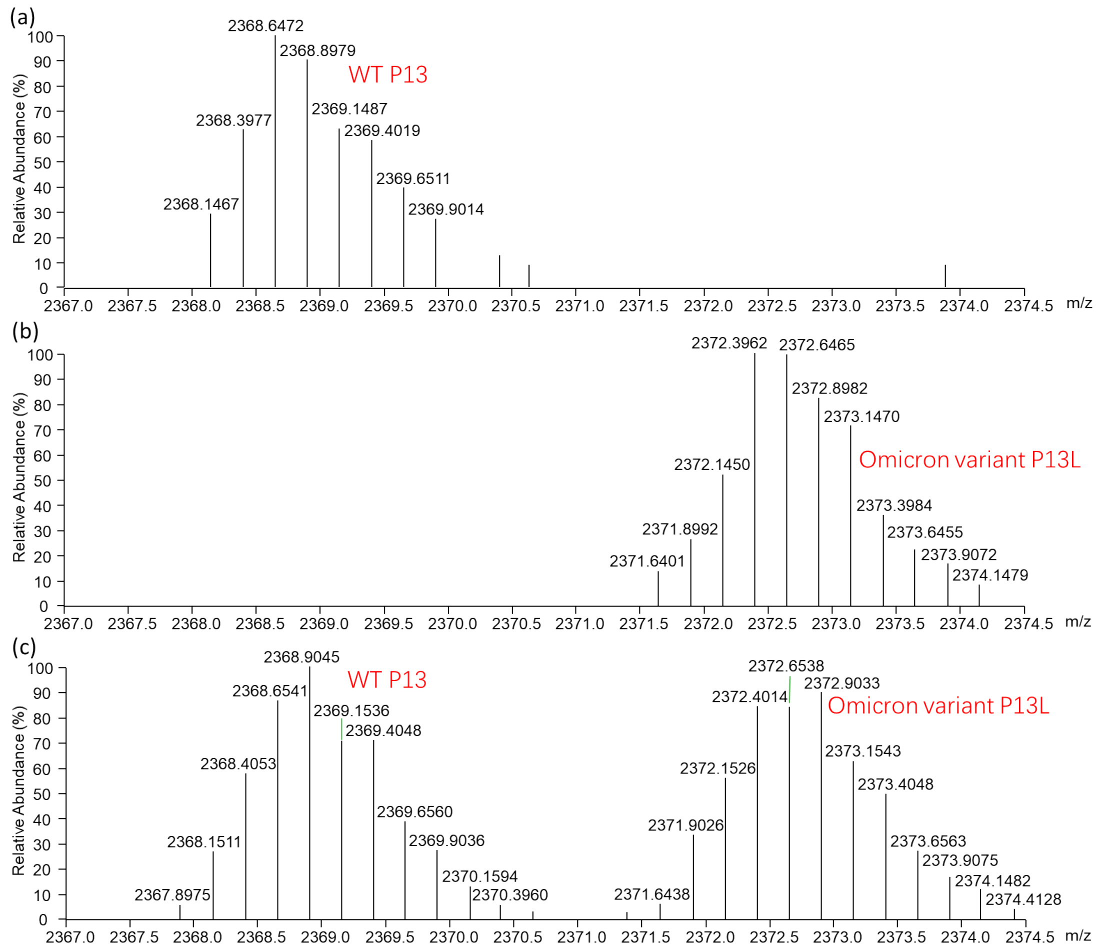
178



179

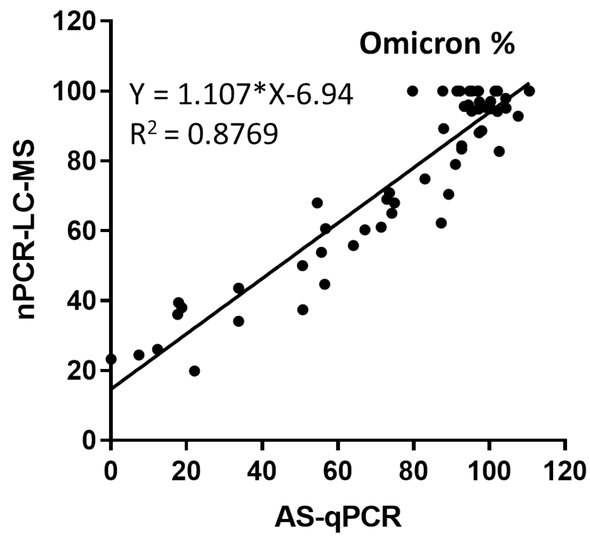
180 **Figure S2.** Identification of standard oligomers of wild-type D3 (grey) and D3L_del
181 (red).
182

183



184 **Figure S3.** Identification of wild-type P13 and Omicron variant P13L by LC-MS. (a)
185 Standard oligomer of wild-type P13 identified by LC-MS. (b) Standard oligomer of
186 Omicron variant P13L identified by LC-MS. (c) Mixture of standard oligomers of wild-
187 type P13 and Omicron variant P13L with molar ratio 1:1.

188



189
190 **Figure S4.** Comparison of identification results for Omicron proportion from
191 wastewater by nPCR-LC-MS and qPCR.
192
193
194

References

1. Pileggi, V.; Shurgold, J.; Sun, J. S.; Yang, M. I.; Edwards, E. A.; Peng, H.; Tehrani, A.; Gilbride, K. A.; Oswald, C. J.; Wijayasri, S.; Al-Bargash, D.; Stuart, R.; Khansari, Z.; Raby, M.; Thomas, J.; Fletcher, T.; Simhon, A. Quantitative Trend Analysis of SARS-CoV-2 RNA in Municipal Wastewater Exemplified with Sewershed-Specific COVID-19 Clinical Case Counts. *ACS ES&T Water* **2022**.
2. Bustin, S. A.; Benes, V.; Garson, J. A.; Hellemans, J.; Huggett, J.; Kubista, M.; Mueller, R.; Nolan, T.; Pfaffl, M. W.; Shipley, G. L.; Vandesompele, J.; Wittwer, C. T. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. *Clinical chemistry* **2009,** *55*, (4), 611-22.
3. Graber, T. E.; Mercier, E.; Bhatnagar, K.; Fuzzen, M.; D'Aoust, P. M.; Hoang, H. D.; Tian, X.; Towhid, S. T.; Plaza-Diaz, J.; Eid, W.; Alain, T.; Butler, A.; Goodridge, L.; Servos, M.; Delatolla, R. Near real-time determination of B.1.1.7 in proportion to total SARS-CoV-2 viral load in wastewater using an allele-specific primer extension PCR strategy. *Water Res* **2021,** *205*, 117681.
4. Fuzzen, M.; Harper, N. B. J.; Dhiyebi, H. A.; Srikanthan, N.; Hayat, S.; Peterson, S. W.; Yang, I.; Sun, J. X.; Edwards, E. A.; Giesy, J. P.; Mangat, C. S.; Graber, T. E.; Delatolla, R.; Servos, M. R. *medRxiv* **2022**.
5. Landgraff, C.; Wang, L. Y. R.; Buchanan, C.; Wells, M.; Schonfeld, J.; Bessonov, K.; Ali, J.; Robert, E.; Nadon, C. Metagenomic sequencing of municipal wastewater provides a near-complete SARS-CoV2 genome sequence identified as the B.1.1.7 variant of concern from a Canadian municipality concurrent with an outbreak. *medRxiv* **2021**.
6. Freed, N. E.; Vlkova, M.; Faisal, M. B.; Silander, O. K. Rapid and inexpensive whole-genome sequencing of SARS-CoV-2 using 1200 bp tiled amplicons and Oxford Nanopore Rapid Barcoding. *Biol Methods Protoc* **2020,** *5*, (1), bpaa014.
7. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet.journal* **2011,** *17*, (1), 10.
8. Li, H. Minimap2: pairwise alignment for nucleotide sequences. *Bioinformatics* **2018,** *34*, (18), 3094-3100.