Accurate detection and quantification of SARS-CoV-2 genomic and subgenomic mRNAs by ddPCR and meta-transcriptomics analysis

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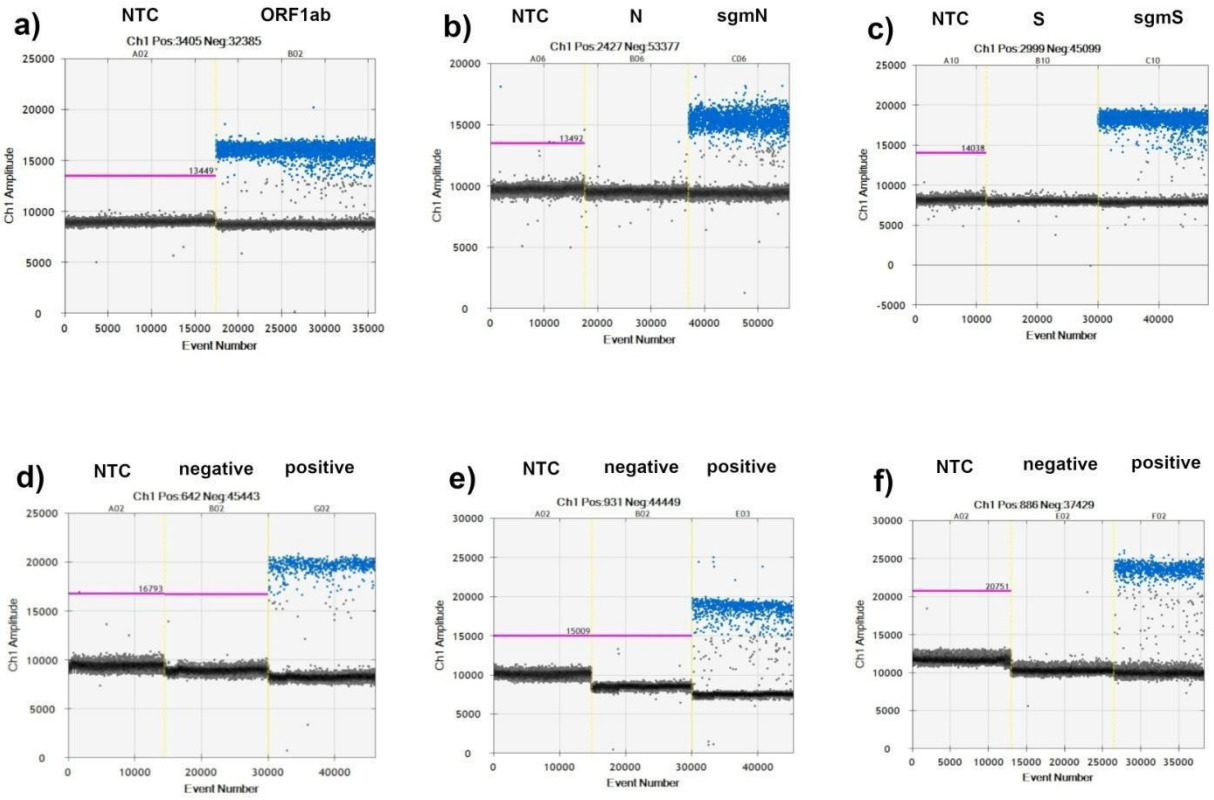
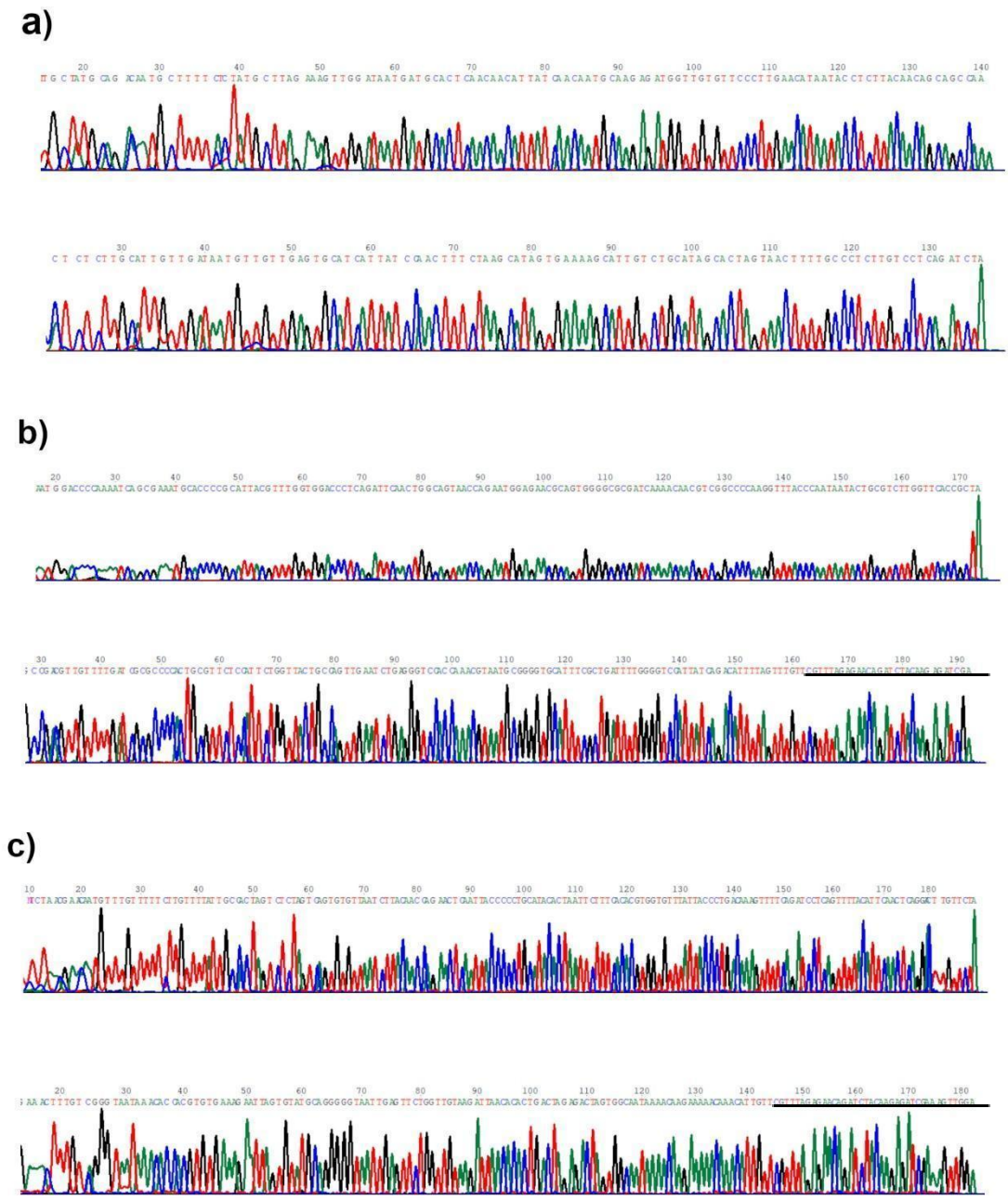
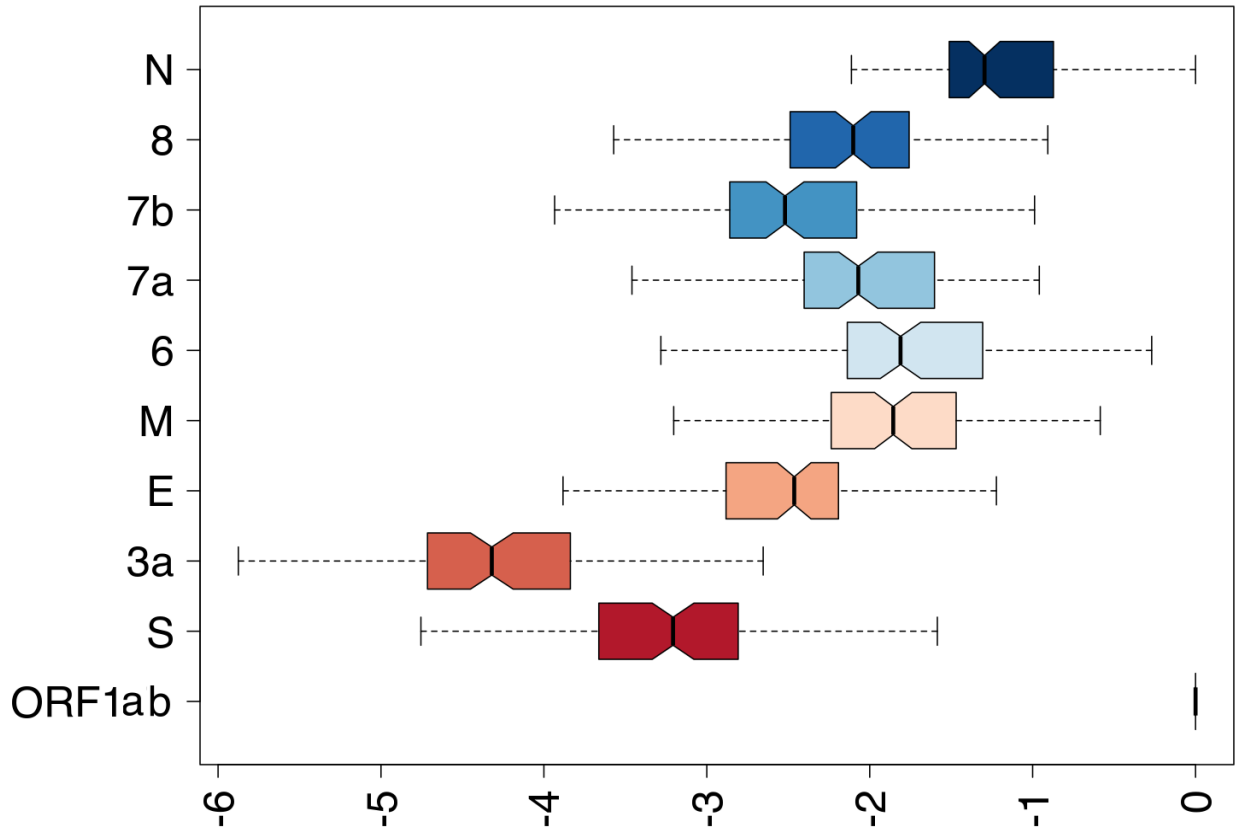


Supplementary Fig. 1. ddPCR 1D plot of SARS-CoV-2 ddPCR assays on different templates.
ddPCR 1D amplitude plots of ORF1ab (panels a and d), N sgmRNA (panels b and e), S sgmRNA (panels c and f), using as template:

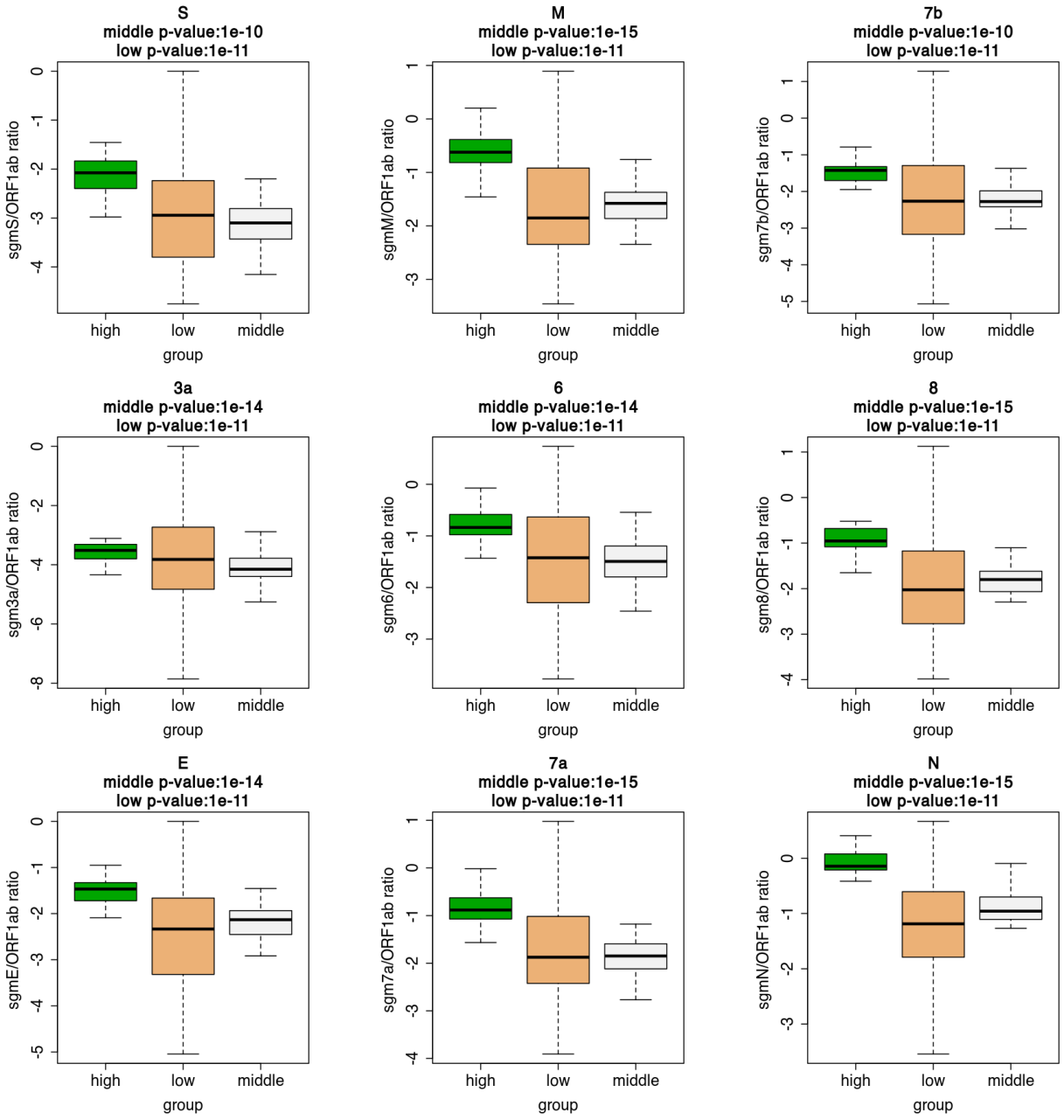
a) from the left: NTC sample; custom genomic ORF1ab DNA sequence;
b) from the left: NTC sample, custom DNA sequence corresponding to N gene, custom DNA sequence corresponding to N sub-genomic mRNA;
c) from the left: NTC sample, custom DNA sequence corresponding to S gene, custom DNA sequence corresponding to S sub-genomic mRNA;
d) from the left: NTC sample; SARS-CoV-2 negative RNA sample; SARS-CoV-2 positive RNA sample;
e) from the left: NTC sample; SARS-CoV-2 negative RNA sample; SARS-CoV-2 positive RNA sample;
f) from the left: NTC sample; SARS-CoV-2 negative RNA sample; SARS-CoV-2 positive RNA sample.



Supplementary Fig. 2. Sanger sequencing electropherograms of SARS-CoV-2 ORF1ab, sgmN and sgmS RNAs amplicons. Sanger sequencing was performed on amplicons obtained from SARS-CoV-2 positive samples; forward and reverse sequences electropherograms are shown for ORF1ab (a), sgmN (b) and sgmS (c) RNAs, respectively at the top and the bottom of each panel.
In (b) and (c), the leader sequence (LS) is underlined.



Supplementary Fig. 3. Boxplot of SARS-CoV-2 metatranscriptomic reads ratios.
Log2 ratios distributions between each sgmRNA and the ORF1ab gene are represented. Genes are indicated on the Y axis. Log ratios on the X axis.



Supplementary Fig. 4. Boxplot of SARS-CoV-2 transcripts relative expression from metatranscriptomic sequencing according to viral RNA content groups.

Boxplots represent log2 scaled ratios distributions between each SARS-CoV-2 canonical sgmRNAs and the ORF1ab genomic RNA. Log ratios are represented on the Y axis; viral RNA content groups on the X axis. Significance was calculated by comparing “high” *vs* “middle+low” groups by Mann–Whitney U test / Wilcoxon rank-sum.

SARS-CoV-2 target	Sequences (5'-3')
ORF1ab	GGAAAAGATGGCTGATCAAGCTATGACCCAAATGTATAAACAGGCTAGATCTGAGGACAAGAGGGC AAAAGTTACTAGTGCTATGCAGACAATGCTTTTCACTATGCTTAGAAAGTTGGATAATGATGCACT CAACAACATTATCAACAATGCAAGAGATGGTTGTGTTCCCTTGAACATAATACCTCTTACAACAGC AGCCAAACTAATGGTTGTCATACCAGACTATAACACATATAAAAATACGTGT
Genomic N	AGACTTTTTAGAGTATCATGACGTTCGTGTTGTTTTAGATTTCATCTAAACGAACAAACTAAAATG TCTGATAATGGACCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAA CTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGGTTTAC CCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGA
Genomic S	AATTAGAGAAAACAACAGAGTTGTTATTTCTAGTGATGTTCTTGTTAACAACTAAACGAACAATGT TTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAAT TACCCCCTGCATACACTAATTCTTTCACACGTGGTGTTTATTACCCTGACAAAGTTTTCAGATCCT CAGTTTTACATTCAACTCAGGACTTGTTCTTACCTTTCTTTTCCAATGTTA
N sgmRNA	AGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAACGAACAAACTAAAATGT CTGATAATGGACCCCAAAATCAGCGAAATGCACCCCGCATTACGTTTGGTGGACCCTCAGATTCAA CTGGCAGTAACCAGAATGGAGAACGCAGTGGGGCGCGATCAAAACAACGTCGGCCCCAAGGTTTAC CCAATAATACTGCGTCTTGGTTCACCGCTCTCACTCAACATGGCAAGGAAGA
S sgmRNA	ACCTTCCCAGGTAACAAACCAACCAACTTTCGATCTCTTGTAGATCTGTTCTCTAAACGAACAATG TTTGTTTTTCTTGTTTTATTGCCACTAGTCTCTAGTCAGTGTGTTAATCTTACAACCAGAACTCAA TTACCCCCTGCATACACTAATTCTTTCACACGTGGTGTTTATTACCCTGACAAAGTTTTCAGATCC TCAGTTTTACATTCAACTCAGGACTTGTTCTTACCTTTCTTTTCCAATGTTA

Supplementary Table 1. Custom synthetic DNA sequences (250 bp each) used in this study.
For sgmRNAs, the portion of the Leader Sequence (LS) present in the custom DNA sequence is underlined.