

## SUPPLEMENTARY INFORMATION

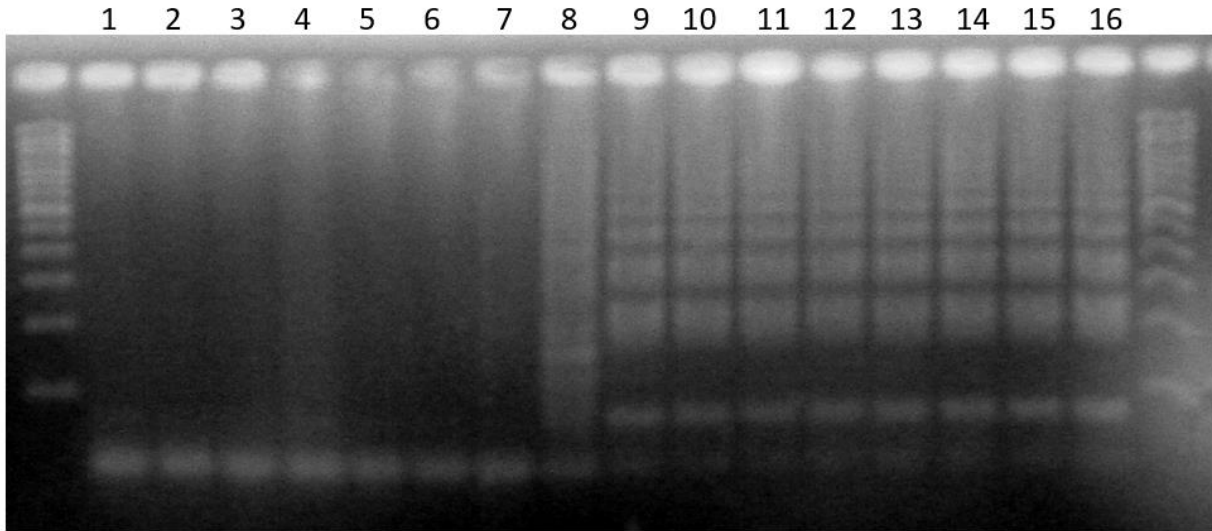
### Emulsion-based Isothermal Nucleic Acid Amplification for Rapid SARS-CoV-2 Detection via Angle-dependent Light Scatter Analysis

Alexander S. Day, Tiffany-Heather Ulep, Babak Safavinia, Tyler Hertenstein, Elizabeth Budiman, Laurel Dieckhaus, and Jeong-Yeol Yoon\*

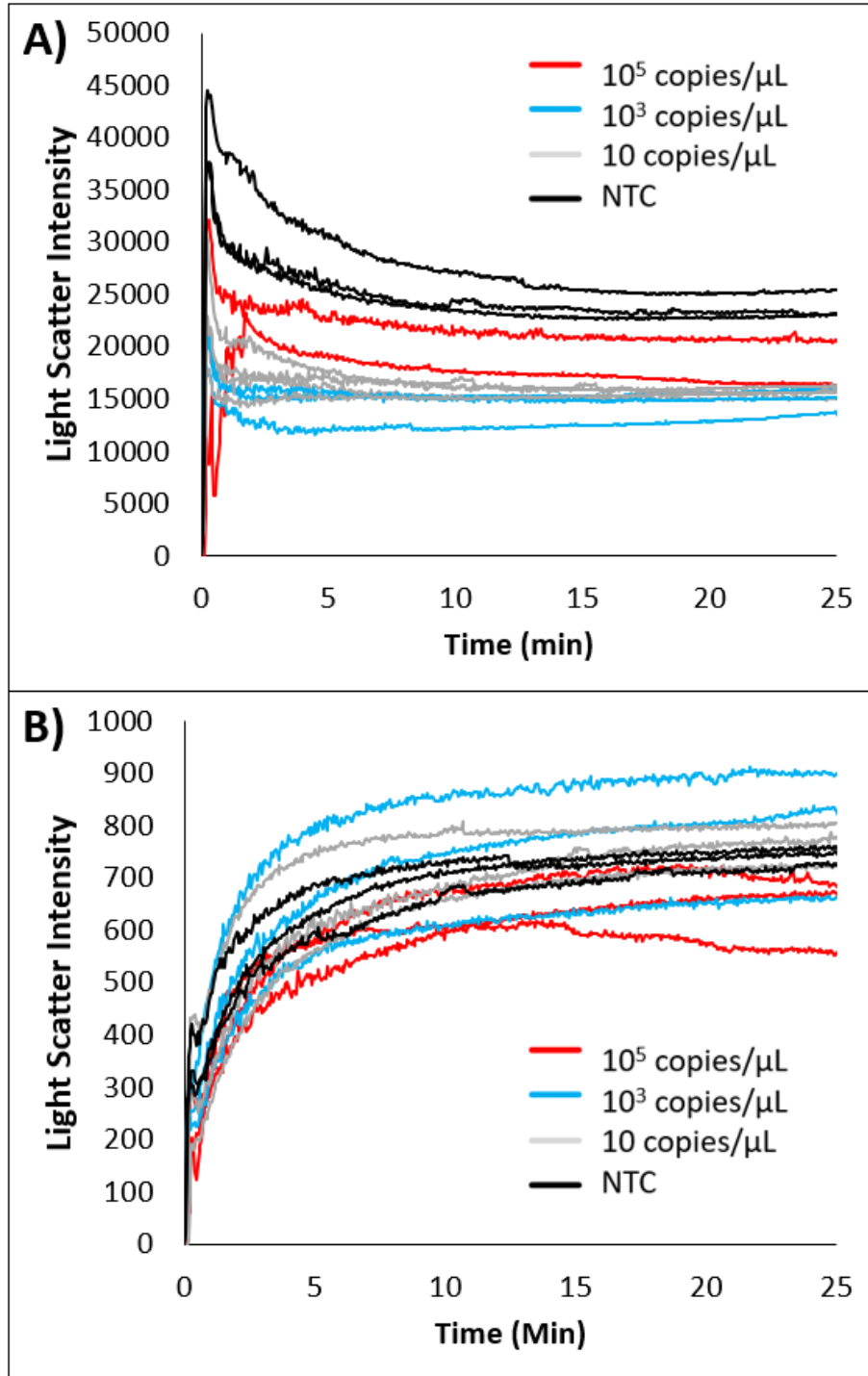
Target	Primer	Sequence (5' – 3')	Size (bp)
E. coli O157:H7 <u>rfbe</u> gene	F3	AACAGTCTTGTACAAGTCCA	20
	B3	GGTGCTTTTGATATTTTCCG	21
	FIP	CTCTCTTTCCTCTGCGGTCCGATG TTTTTCACACTTATTGGAT	43
	BIP	TAAGGAATCACCTTGCAGATAAAC TAGTACATTGGCATCGTGT	43
	Loop F	CCAGAGTTAAGATTGAT	17
	Loop B	CGAAACAAGGCCAGTTTTTTTACC	23
SARS-CoV-2 N gene	F3	TGGCTACTACCGAAGAGCT	19
	B3	TGCAGCATTGTTAGCAGGAT	20
	FIP	TCTGGCCCAGTTCCTAGGTAGTCC AGACGAATTCGTGGTGG	41
	Fluorescent FIP	TCTGGCCCAGTTCCTAGGTAGTCC AGACGAATTCGTGGTG/ <u>iFluor</u> T/G	41
	BIP	AGACGGCATCATATGGGTTGCACG GGTGCCAATGTGATCT	40
	Loop F	GGACTGAGATCTTTCATTTTACCG T	25
	Loop B	ACTGAGGGAGCCTTGAATACA	21

Supplementary Table S1: Primer sequences for both E. coli and SARS-CoV-2 targets. Note that “/iFluorT/” indicates that a Fluorescein molecule was added to the primer sequence to allow for target-specific detection, as described in Gadkar et al. in 2018.

- |                                |                                     |
|--------------------------------|-------------------------------------|
| 1. NTC – 15 min                | 9. $10^3$ copies/ $\mu$ L – 15 min  |
| 2. NTC – 20 min                | 10. $10^3$ copies/ $\mu$ L – 20 min |
| 3. NTC – 25 min                | 11. $10^3$ copies/ $\mu$ L – 25 min |
| 4. NTC – 30 min                | 12. $10^3$ copies/ $\mu$ L – 30 min |
| 5. 10 copies/ $\mu$ L – 15 min | 13. $10^5$ copies/ $\mu$ L – 15 min |
| 6. 10 copies/ $\mu$ L – 20 min | 14. $10^5$ copies/ $\mu$ L – 20 min |
| 7. 10 copies/ $\mu$ L – 25 min | 15. $10^5$ copies/ $\mu$ L – 25 min |
| 8. 10 copies/ $\mu$ L – 30 min | 16. $10^5$ copies/ $\mu$ L – 30 min |

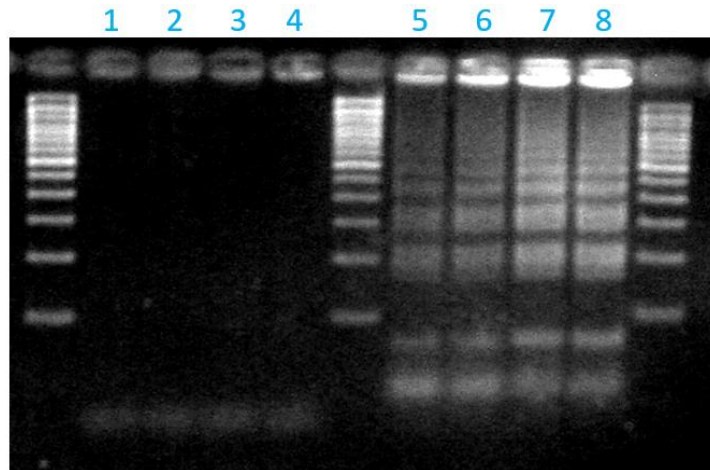


Supplementary Figure S1: Gel electrophoresis image showing time-series results for different SARS-CoV-2 concentrations across different amplification times. As the legend indicates, lanes 1-4 contain amplified NTC samples, lanes 5-8 10 copies/ $\mu$ L samples, 9-12  $10^3$  copies/ $\mu$ L samples, and 13-16  $10^5$  copies/ $\mu$ L samples.

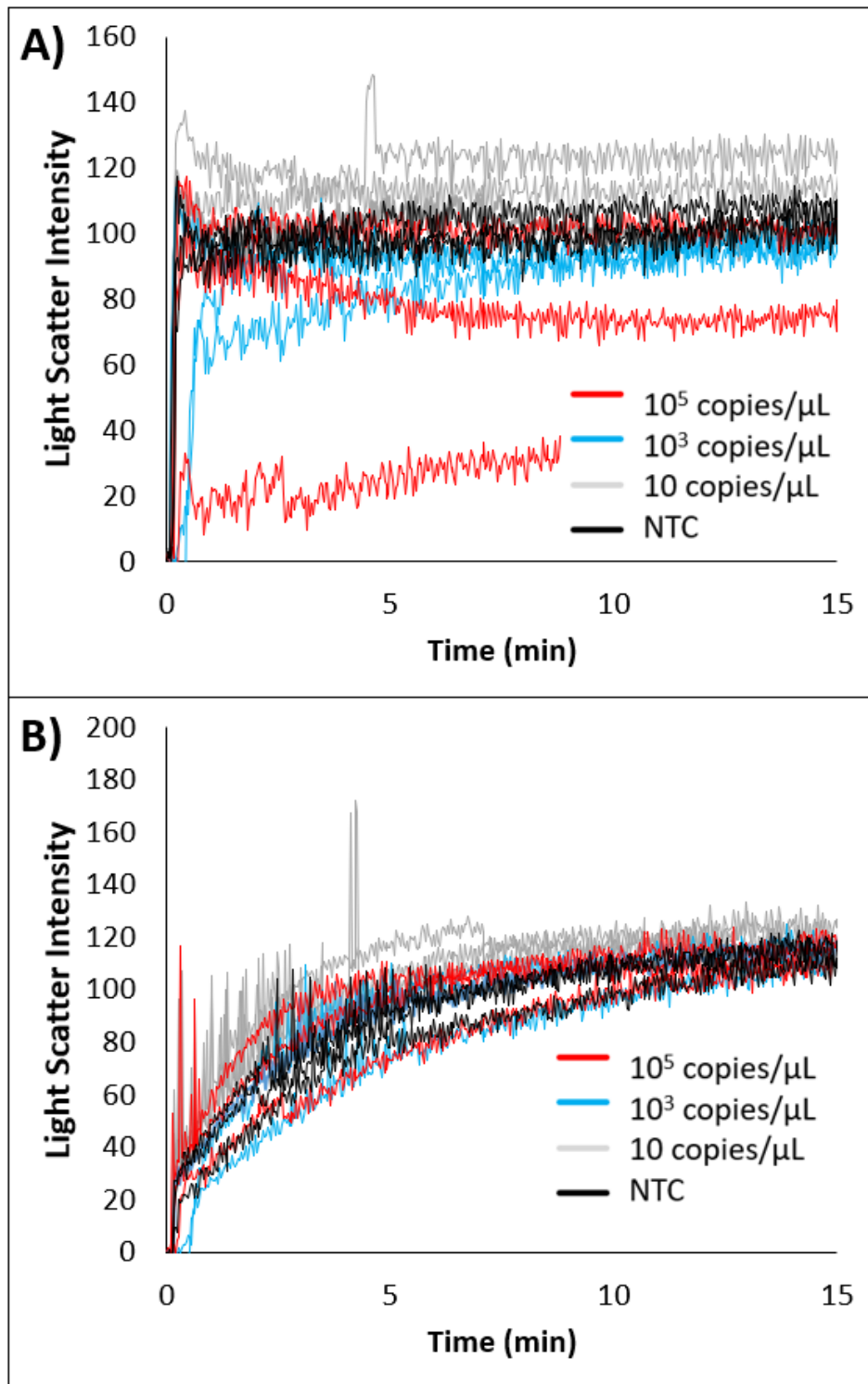


Supplementary Figure S2. *In situ* light scatter intensity changes for emulsion LAMP reaction of SARS-CoV-2 via spectrophotometer. Changes over time are shown at A) 30° and B) 60° angle with respect to 650 nm incident wavelength with varying initial SARS-CoV-2 positive control concentration of 10<sup>5</sup>, 10<sup>3</sup>, 10, and 0 copies per μL

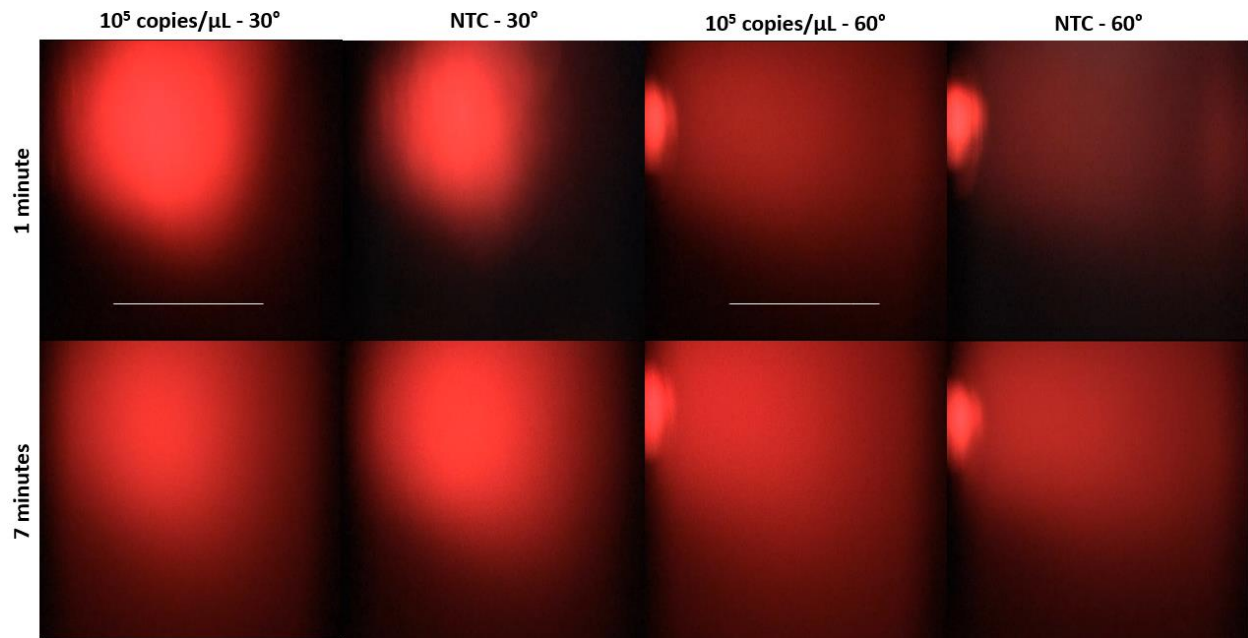
- 1: NTC – No prior eLAMP
  - 2:  $10^1$  copies/ $\mu\text{L}$  – No prior eLAMP
  - 3:  $10^3$  copies/ $\mu\text{L}$  – No prior eLAMP
  - 4:  $10^5$  copies/ $\mu\text{L}$  – No prior eLAMP
- 
- 5: NTC – Prior eLAMP
  - 6:  $10^1$  copies/ $\mu\text{L}$  – Prior eLAMP
  - 7:  $10^3$  copies/ $\mu\text{L}$  – Prior eLAMP
  - 8:  $10^5$  copies/ $\mu\text{L}$  – Prior eLAMP



Supplementary Figure S3: Gel electrophoresis images from the emulsion LAMP reactions (for 30 minutes) with varying initial concentrations of SARS-CoV-2, broken and extracted, and followed by additional 15-minute conventional LAMP reaction (lanes 5-8). Lanes 1-4 shows the same results without the emulsion LAMP reaction.



Supplementary Figure S4. *In situ* light scatter intensity changes for emulsion LAMP reaction of SARS-CoV-2 via smartphone. Changes over time are shown at A)  $30^\circ$  and B)  $60^\circ$  angle with respect to 650 nm incident wavelength with varying initial SARS-CoV-2 positive control concentration of  $10^5$ ,  $10^3$ , 10, and 0 copies per  $\mu\text{L}$



Supplementary Figure S5. Representative images of ongoing emulsion LAMP reactions containing either no SARS-CoV-2 (columns 1 and 3) or  $10^5$  copies/ $\mu\text{L}$  (columns 2 and 4) at one minute and seven minutes into the reaction process. Column headers indicate which images have the incident light source at an angle of either  $30^\circ$  or  $60^\circ$  relative to the smartphone camera. Scale bars indicate 5 mm.