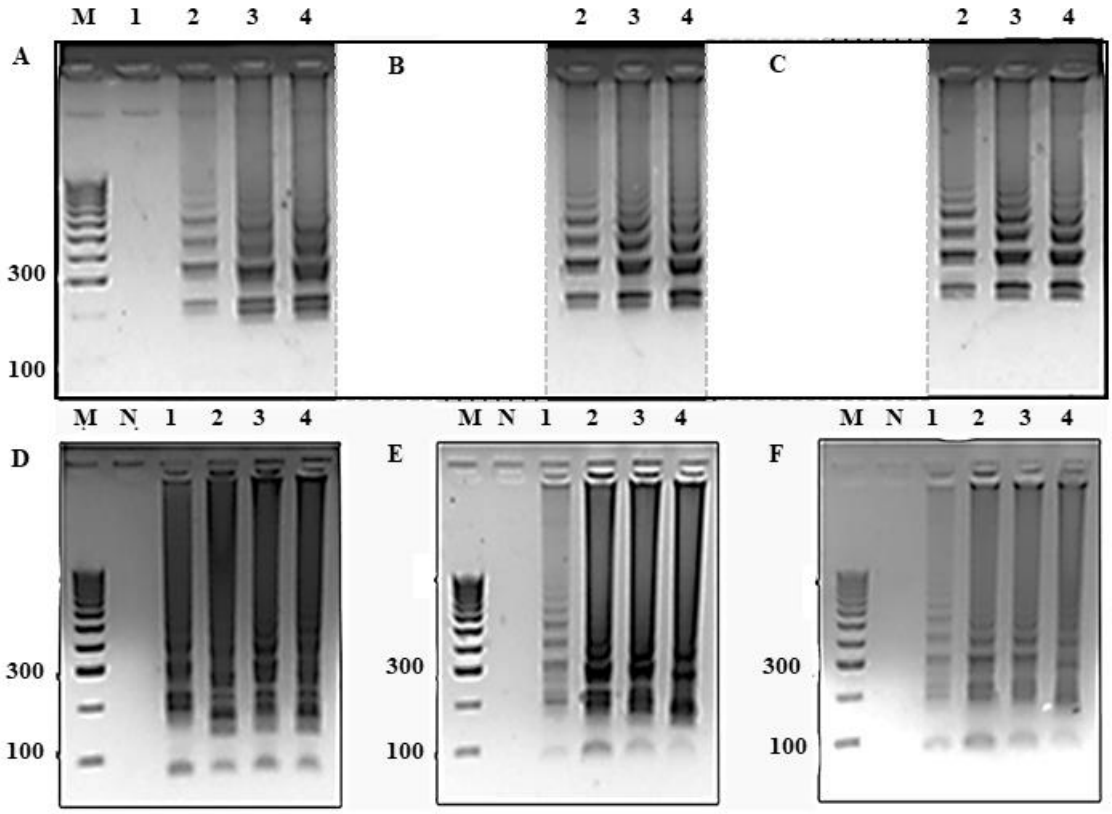
Rapid colorimetric loop-mediated isothermal amplification for hypersensitive point-of-care *Staphylococcus aureus* enterotoxin A gene detection in milk and pork products

Grittaya Srimongkol[a], Boonsong Ditmangklo[b], Ilada Choopara[a], Jiraporn Thaniyavarn[c], Deborah Dean[d,e,f], Sirirat Kokpol[b], Tirayut Vilaivan[b], and Naraporn Somboonna[c*]

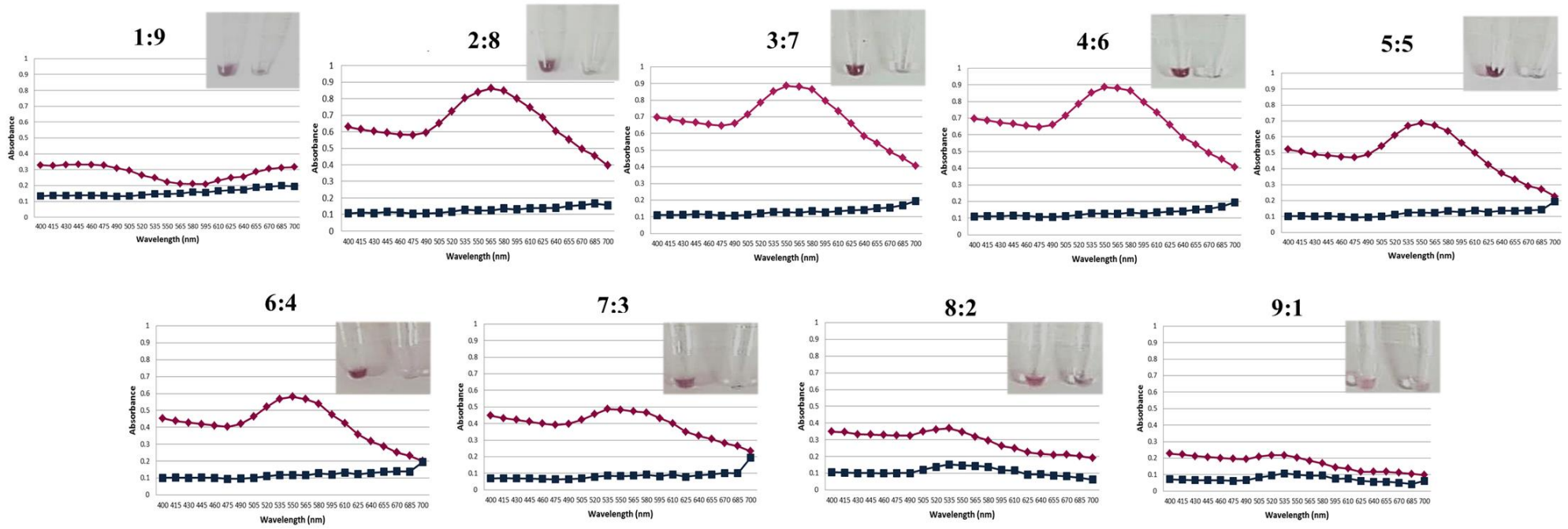
Supplementary Figures and Tables

Supplemental Figure 1. Incubation periods and temperatures of (A-C) Goto's and (D-F) our LAMPs.

In (A and D) incubation temperature was at 60 ˚C, (B and E) 63 ˚C, and (C and F) 65 ˚C. Lane M represents 100 bp DNA ladder (GeneDireX Inc., Miaoli County, Taiwan); N, negative control; 1-4, incubation period at 15, 30, 45 and 60 min, respectively. (Original photographs of A-C is in Supplementary Information).


M 1 2 3 4
A
300
100
2 3 4
B
2 3 4
C
M N 1 2 3 4
D
300
100
M N 1 2 3 4
E
300
100
M N 1 2 3 4
F
300
100

Supplemental Figure 2. Optimized ratios of LAMP-to-AuNP volume for optimal color contrast between positive (red) and negative (purple/blue/gray) test results, determined by visualization and spectrophotometer at 400-700 nm absorbance. An optimal ratio was 5:5 LAMP:AuNP.


1:9
2:8
3:7
4:6
5:5
6:4
7:3
8:2
9:1
Absorbance
Wavelength (nm)
400 415 430 445 460 475 490 505 520 535 550 565 580 595 610 625 640 655 670 685 700

Supplemental Table 1. Bacterial strains.

Bacteria species	***Strain numbers***	***Sources***
Staphylococcus aureus (sea)	*ATCC13565*	*Department of Microbiology, Faculty of Science, Chulalongkorn University*
Staphylococcus aureus	*ATCC25928*	
Staphylococcus aureus	*ATCC144925*	
Staphylococcus aureus (sea)	*ATCC25923*	*Department of Medical Sciences, Ministry of Public Health*
Staphylococcus epidermidis	*ATCC12228*	
Staphylococcus saprophyticus	*ATCC15305*	
Vibrio funissii	*N/A**	*Bamrasnaradura Infectious Diseases Institute, Ministry of Public Health*
Shigella flexneri	*N/A*	
Escherichia coli	*ATCC25922*	
Escherichia coli	*ATCC35218*	
Salmonella typhimurium	*ATCC14026*	
Enterobacter cloacae	*N/A*	
Aeromonas sobria	*N/A*	
Enterococcus faecalis	*N/A*	

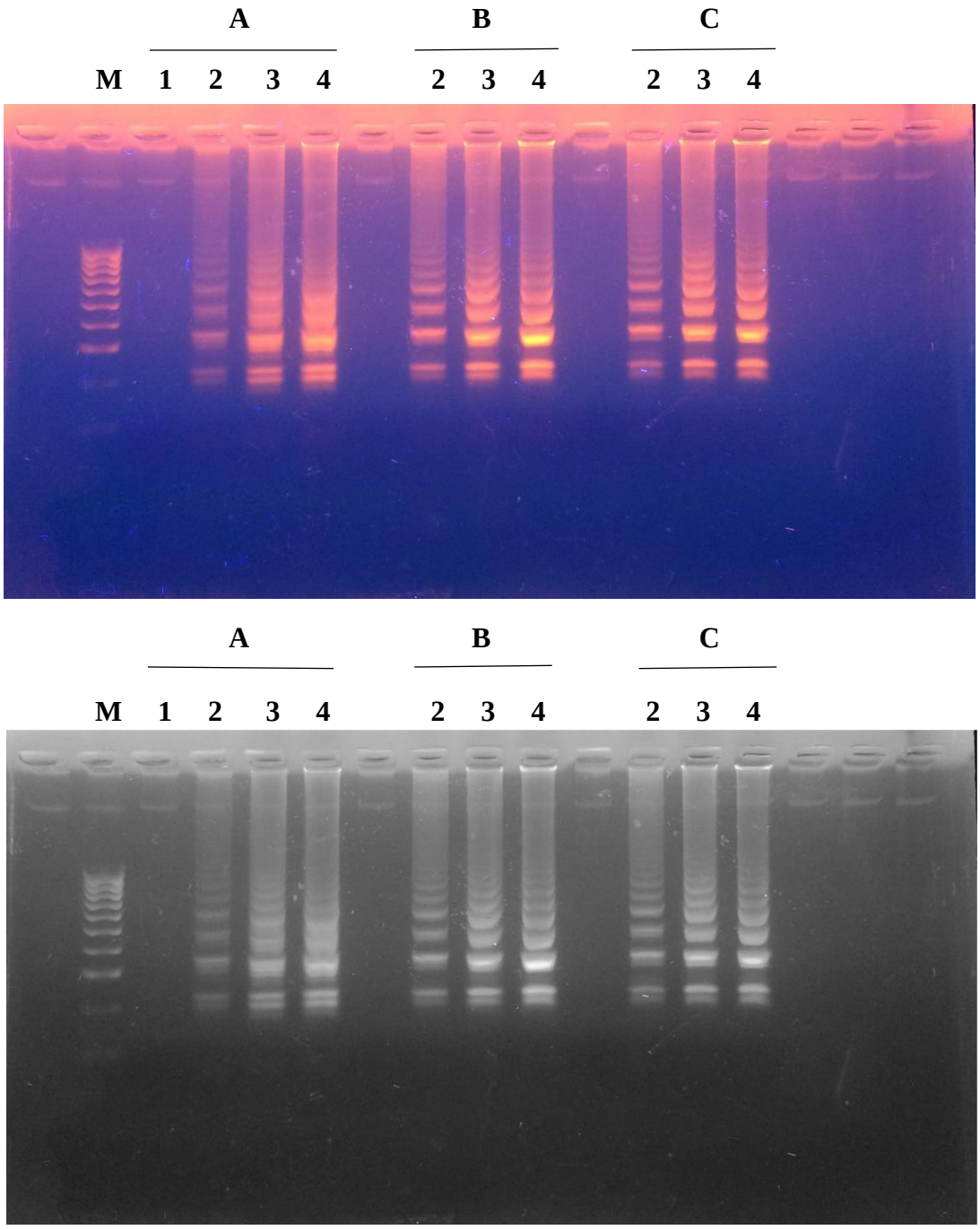
* N/A represents information on ATCC number that is not available, because strain was isolated and sequenced to identify species at local source.

Supplemental Table 2. Oligonucleotide primer and probe sequences.

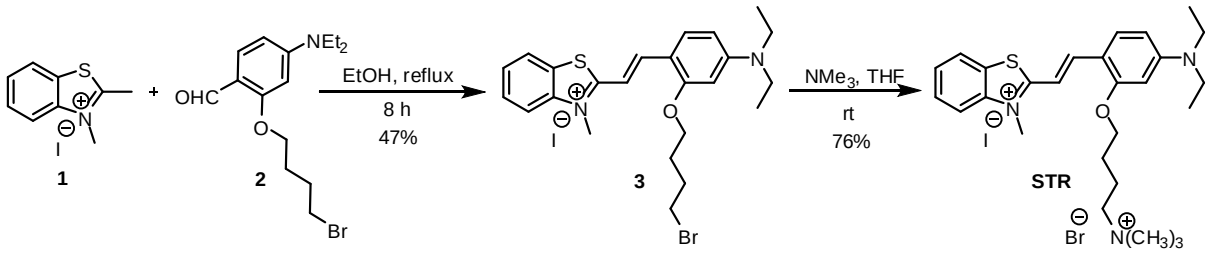
Reaction	***Primer and probe names***	***Sequences (5′- 3′)***
PCR[30]	*SEA-F*	*TTGGAAACGGTTAAAACGAA*
	SEA-R	*GAACCTTCCCATCAAAAACA*
Goto et al.'s LAMP[2]	*SEA_FIP*	*GATCCAACTCCTGAACAGTTACAATACAGTACCTTTGGAA ACG*
	SEA_BIP	*CTGATGTTTTTGATGGGAAGGTTCCCGAAGGTTCTGTAGA AGT*
	SEA_F3	*TCAATTTATGGCTAGACGGT*
	SEA_B3	*CTTGAGCACCAAATAAATCG*
	SEA_LB	*AGAGGGGATTAATCGTGTTTCA*
Our LAMP	*Sa_SEA FIP*	*CTGTAAAAAAGCCTTTAAACAATATTTTGCTAAAACTGAAA ATAAAGAGAGTC*
	Sa_SEA BIP	*GGTATAACGATTTATTAGTAGATTTTTATACAAGTCTACTTT TTTCCCTT*
	Sa_SEA F3	*TCTATTATTACAATGAAAA*
	Sa_SEA B3	*ATTGATAACCATAATAAGCA*
	Sa_SEA LF	*TGCTAGTTAAAAATGTCGTATGAT*
	Sa_SEA LB	*GATTCAAAGGATATTGTTGAT*
Our LAMP-AuNP	*Probe-Sa_SEA*	*HS-AGGCTTTTTTACA(G/A)ATCATTC*

Supplementary Information

Supplementary Information 1. Original photographs of Supplemental Figure 1A, 1B and 1C



Supplementary Information 2. Synthesis of STR dye



A sample of 2,3-dimethyl-1,3-benzothiazol-3-ium iodide (**1**) (80 mg, 0.3 mmol) and *2-(4-*bromobutoxy)-*4-(*diethylamino)-benzaldehyde (**2**) (200 mg, 0.6 mmol)[39] was refluxed in absolute ethanol for 8 h. The precipitate formed was recrystallized from ethanol to give the intermediate **3** as a gray solid (78 mg, 47%): ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.20 (d, J = 7.4 Hz, 1H), 8.08 (d, J = 14.5 Hz, 1H), 8.00 (d, J = 8.0 Hz, 1H), 7.90 (d, J = 9.2 Hz, 1H), 7.73 (t, J = 7.7 Hz, 1H), 7.62 (t, J = 7.25 Hz, 1H), 7.48 (d, J = 15.1 Hz, 1H), 6.51 (d, J = 8.74 Hz, 1H), 6.20 (s, 1H), 4.20 (t, J = 6.0 Hz, 2H), 4.11 (s, 3H), 3.65 (t, J = 6.3 Hz, 4H), 3.54 (m, 4H), 3.40 (t, J = 6.6 Hz, 1H), 2.00 (m, 4H), 1.15 (t, J = 6.9 Hz, 6H)

Trimethylamine (1 mL, 5 mmol) was added to a solution of **3** (30 mg, 0.05 mmol) in tetrahydrofuran (2 mL) and the reaction was stirred for 30 h at room temperature. The reddish-purple precipitate was filtered to give the dye **STR (**30 mg, 76%): ^{1}H NMR (400 MHz, DMSO-d_6): δ = 8.22 (d, J = 7.4 Hz, 1H), 8.10 (d, J = 14.5 Hz, 1H), 8.02 (d, J = 8.00 Hz, 1H), 7.90 (d, J = 9.2 Hz, 1H), 7.73 (t, J = 7.7 Hz, 1H), 7.62 (t, J = 7.2 Hz, 1H), 7.48 (d, J = 15.1 Hz, 1H), 6.51 (d, J = 8.7 Hz, 1H), 6.20 (s, 1H), 4.20 (t, J = 6.0 Hz, 2H), 4.11 (s, 3H), 3.51 (m, 4H), 3.44 (m, 2H), 3.09 (s, 9H), 1.89 (m, 4H), 1.15 (t, J = 6.9 Hz, 6H)

The dye **STR** exhibited maximum absorption at 525 nm and emission at 591 nm and the fluorescence emission increased by more than 18-fold in the presence of DNA.