1 *Supplementary data*

2 **Fluorescence nucleobase analogue-based strategy with high signal-to-**
3 **noise ratio for ultrasensitive detection of food poisoning bacteria**

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**Table S1.** Oligonucleotide sequences used in this study.

Name	Sequence (5' -> 3')
S1 [a]	AAG CTG CAA TCC GGG *2AP*GG GTG GG
S2 [a]	TGG GCT TTT AAG ATA ACC
a (blocker-14mer)	CCG GAT TGC AGC TT
b (blocker-15mer)	CCC GGA TTG CAG CTT
c (blocker-16mer)	TCC CGG ATT GCA GCT T
d (blocker-17mer)	CTC CCG GAT TGC AGC TT
e (blocker-18mer)	CCT CCC GGA TTG CAG CTT
f (blocker-19mer)	CCC TCC CGG ATT GCA GCT T
g (blocker-20mer)	ACC CTC CGG ATT GCA GC TT
h (blocker-21mer)	CAC CCT CCC GGA TTG CAG CTT
i (blocker-22mer)	CCA CCC TCC CGG ATT GCA GCT T
j (blocker-23mer)	CCC ACC CTC CGG ATT GCA GC TT
e-1 (blocker-16mer)	CCT CCC GGA TTG CAG C
e-2 (blocker-14mer)	CCT CCC GGA TTG CA
e-3 (blocker-12mer)	CCT CCC GGA TTG
e-4 (blocker-10mer)	CCT CCC GGA T
e-4-phos (blocker-10mer)	CCT CCC GGA T-phosphate
e-5 (blocker-8mer)	CCT CCC GG
CT (26mer)	GGT TAT CTT AAA AGG GAT TGC AGC TT
SA (26mer)	TCA GAC TAT TAT TGG TTG ATA CAC CT
NG (26mer)	CTG CCG CCG ATA TAC CTA GCA AGC TC
KP (26mer)	GCC GCC ATT ACC ATG AGC GAT AAC AG
MG (26mer)	CTC AAG TAT CTC AAT GCT GTT GAG AA
Helper-linked forward primer (*UidA* gene; *E. coli*) [b]	GGT TAT CTT AAA AGG GAT TGC AGC TTG CGA GGT ACG GTA GGA GTT G
Helper-linked reverse primer (*UidA* gene; *E. coli*) [b]	GGT TAT CTT AAA AGG GAT TGC AGC TTG AAG GGC GAA CAG TTC CTG A
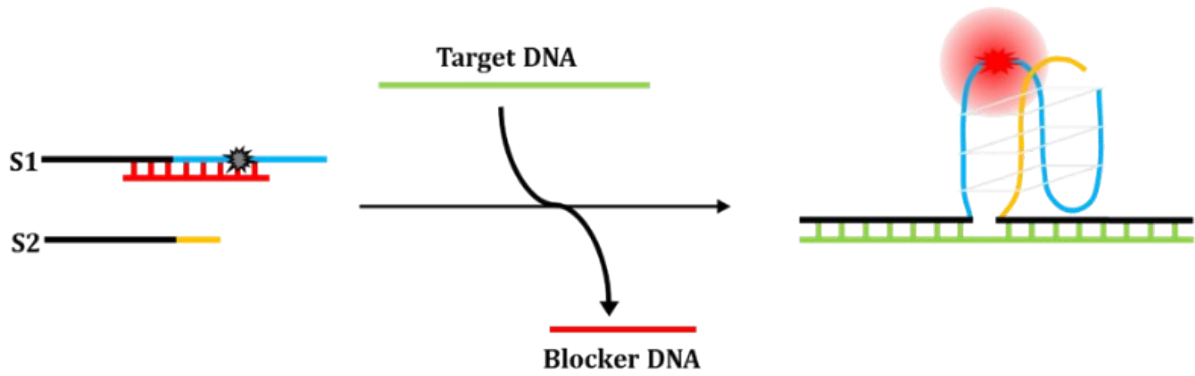
29 **Scheme S1.** Schematic illustration (not drawn to scale) of the fluorescence-based target
30 DNA detection strategy using a 2-AP-containing split G-quadruplex in combination with the
31 blocker DNA. The S1 and S2 probes contain two split G-rich segments (sky blue and orange,
32 respectively), which are divided at a 3:1 spilt ratio based on the number of guanine bases, in
33 addition to a target-specific overhang sequence (black). The S1 probe only contains 2-AP.

35 **Table S2.** Comparison of our method with other fluorescence methods for target DNA
36 detection.

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| Key detection component/principle	Dynamic range	Detection limit	Assay time	Reference
Graphene oxide and SYBR Green I dye	0–50 nM	0.5 nM	70 min	1
DNA oligonucleotide-stabilized silver nanoclusters	25–1000 nM	14 nM	50 min	2
ssDNA-adsorbed graphene oxide	0–50 nM	1 nM	35 min	3
Bioluminescence resonance energy transfer	20–130 nM	20 nM	30 min	4
2-AP-containing split G-quadruplex	10–2000 nM	7.24 nM	45 min	5
2-AP-containing split G-quadruplex in addition to blocker DNA	1–2000 nM	0.17 nM	45 min	This work

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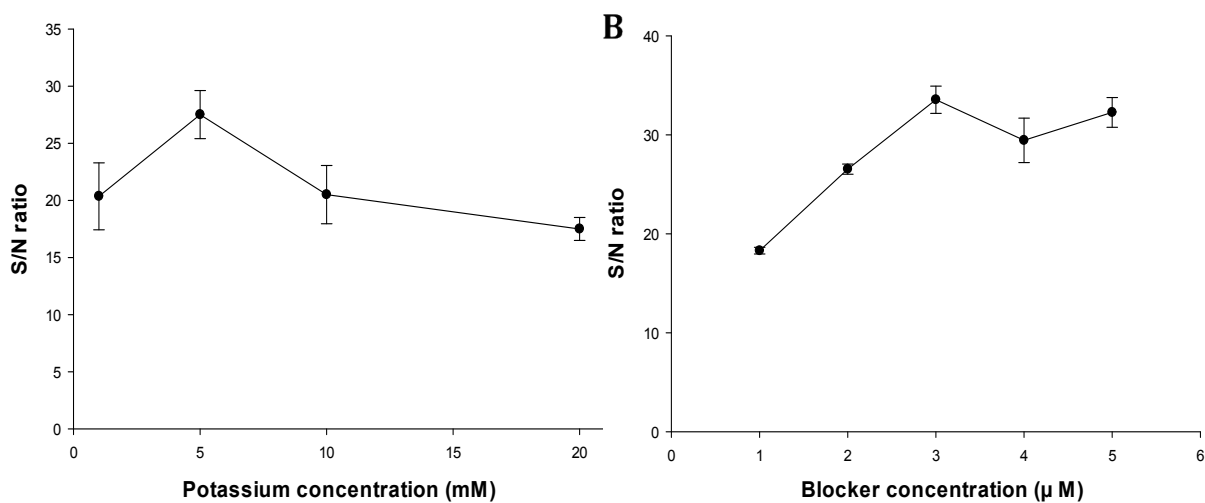
Figure S1. Optimization of experimental conditions for successful operation of the developed system. Concentrations of (A) potassium ions and (B) blocker DNA. The S/N ratio was calculated as $(F - F_0)/F_0$, where F_0 and F are fluorescence signals in the absence and presence of target DNA, respectively. The final concentration of S1, S2, and target DNA was 2 μM. The error bars show the standard deviation among three replicates.
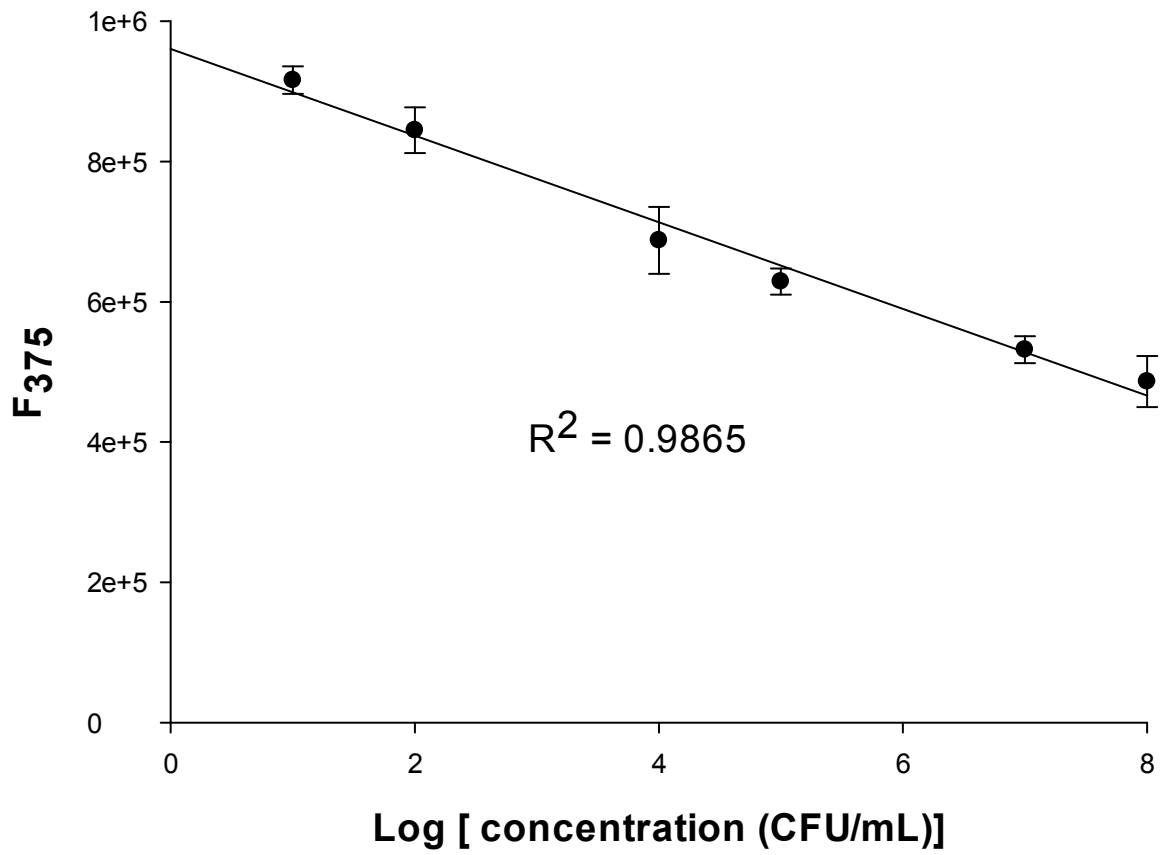
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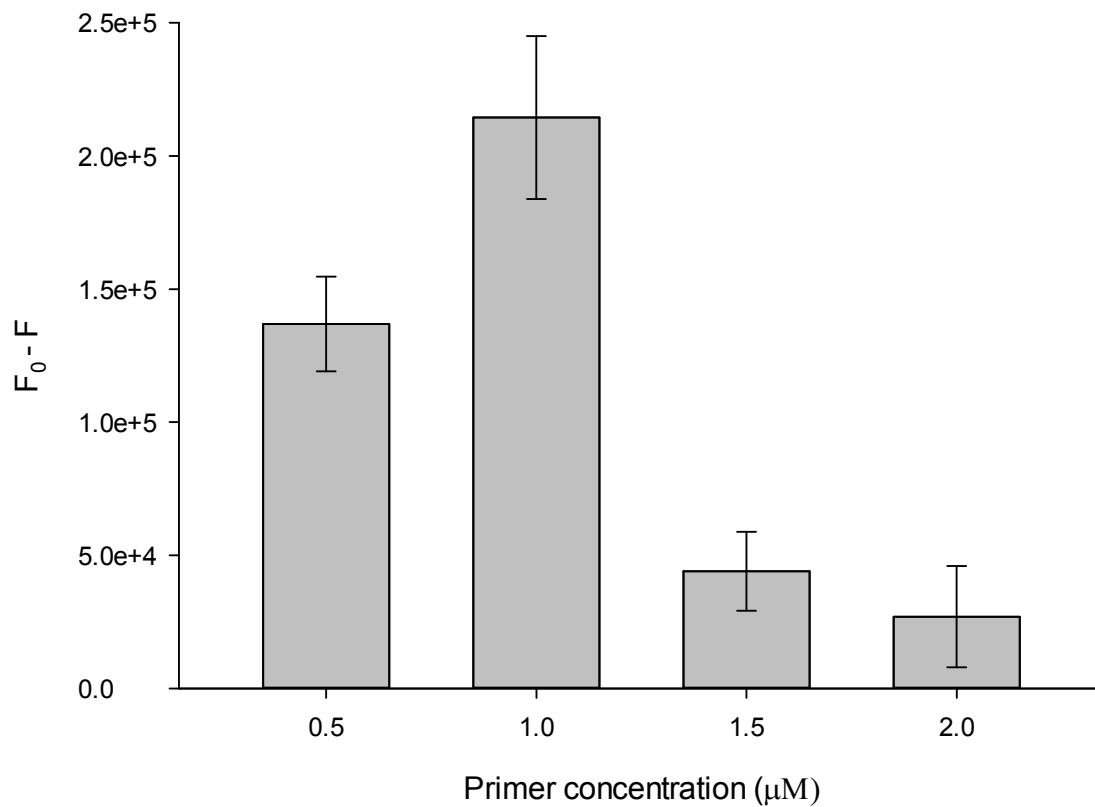
Figure S2. Optimization of primer concentration. F_0 and F are fluorescence signals in the absence and presence of *E. coli* gDNA, respectively. The copy number of E. coli gDNA is 10^4. The error bars show the standard deviation among three replicates.
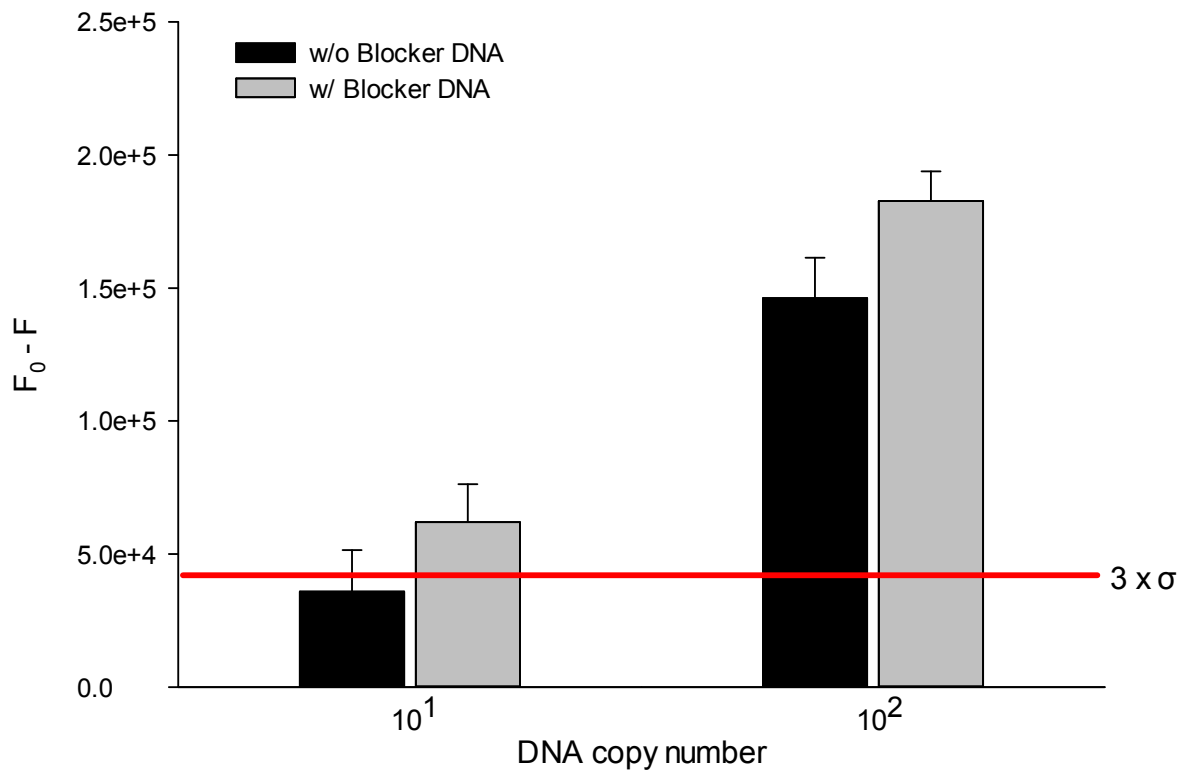
Figure S3. Detection of *E. coli* gDNA (10 and 100 copies) with two strategies: one is without (w/o) Blocker DNA originally used in our previous work, while the other is with (w/) blocker DNA proposed in this work. F_0 and F are fluorescence signals in the absence and presence of *E. coli* gDNA, respectively. The horizontal red line represents the threshold line defined by 3 x σ where σ is the standard deviation.

The plot shows F_{375} versus Log [concentration (CFU/mL)] with a linear fit. The y-axis is labeled F_{375} with values 0, 2e+5, 4e+5, 6e+5, 8e+5, 1e+6. The x-axis ranges from 0 to 8. Annotation: $R^2 = 0.9865$.

Figure S4. Linear relationship between the fluorescent intensity at 375 nm (F_{375}) and concentration of *E. coli* (colony forming units, CFU) spiked in the lettuce sample. The final concentration of S1 and S2 was 2 µM, and that of the blocker DNA was 3 µM. The error bars show the standard deviation among three replicates.

References

71 **References**

72 1. H. Qiu, N. Wu, Y. Zheng, M. Chen, S. Weng, Y. Chen, and X. Lin, *Int. J. Nanomedicine*, 2015,
73   **10**, 147.

74 2. G. Y. Lan, W. Y. Chen, and H. T. Chang, *Biosens. Bioelectron.*, 2011, **26**(5), 2431-2435.

75 3. M. Zhang, B. C. Yin, W. Tan, and B. C. Ye, *Biosens. Bioelectron.*, 2011, **26**(7), 3260-3265.

76 4. K. A. Cissell, S. Campbell, and S. K. Deo, *Anal. Bioanal. Chem.*, 2008, **391**(7), 2577.

77 5. S. H. Hwang, W. Y. Kwon, H. Eun, S. Jeong, J. S. Park, K. J. Kim, K. S. Park, *Artif. Cell.*
78   *Nanomed. B.*, 2018, **46**(sup3), S950-S955.