

Pushbutton-activated microfluidic cell-free biosensor for multiplexed pathogen detection

Dong Hyun Han,^{†a} Yurim Kim,^{†a} Yu Jin Park,^b Dong-Yeon Song,^b Jongho Park,^a Jeonghwan Oh,^a Dong-Myung Kim^{*b} and Je-Kyun Park^{*a}

^a Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong-gu, Daejeon 34141, Republic of Korea. E-mail: jekyun@kaist.ac.kr; Tel: +82 42 350 4315.

^b Department of Chemical Engineering and Applied Chemistry, Chungnam National University, 99 Daehak-ro, Daejeon 34134, Republic of Korea. E-mail: dmkim@cnu.ac.kr; Tel: +82 42 821 5899.

[†] These authors contributed equally.

Supplementary Information includes:

Table S1 Sequence information encoding the 16S rRNAs of the target pathogens under the T7 promoter.

Table S2 Sequence information targeting the 3'-end of each 16S rDNA sequence.

Table S3 Sequence information of pK7/dT7P-sfGFP constructs.

Table S4 Sequence information of IA and TASER probes.

Table S5 Sequence information of IA and TASER probes for *E. coli* before optimization.

Fig. S1 Schematic of the interconnection between the upper and lower fluidic channels via through-hole.

Fig. S2 Reproducibility test based on multiple users. Each sample containing 1 μ M of *E. coli* 16S rRNA was aliquoted and mixed with IA-TASER reagents via the pushbutton operation ($n = 3$).

Fig. S3 Performance comparison using non-optimized and optimized invasive and flap probes in the IA-TASER assay.

Fig. S4 Enlarged images of the detection zone showing sfGFP fluorescence signals from various concentrations of 16S rRNA captured with the hand-held transilluminator.

Fig. S5 Representative image showing the analysis of sfGFP fluorescence intensity in the mPAMD using a smartphone app.

Table S1. Sequence information encoding the 16S rRNAs of the target pathogens under the T7 promoter.

Sequence name	Sequence
T-blunt vector	<p>AGCGCCAATACGCAAACCGCCTCTCCCGCGCGTGGCCGATTCAATATGCAGCTGGCACGACAGGTTTCCCGACT GGAAAGCGGGCAGTGTAGCGCAACGCAATTAATGTGAGTTAGCTCACTCATTAGGCACCCCAGGCTTACACTTTATGC TTCCGGCTCGTATGTTGTGTGGAATTGTGAGCGGATAACAATTTACACAGGAAACAGCTATGACCATGATTACGCCA AGCTTGGTACCGAGCTCGGATCCACTAGTAACGGCCGCGAGTGTGCTGGAATTCGCCCTNNNNNNNNNNNNNNNN NNNNNNNNNNNNNNNNNNNNNAGGGCGAATTCTGCAGATATCCATCACACTGGCGGCCGCTCGAGCATGCATCTA GAGGGCCAATTCGCCCTATAGTGAATCGTATTACAATTCACCTGGCCGCTCGTTTACAACGTCGTGACTGGGAAAACC CTGGCGTTACCAAACCTTAATCGCCTTGCAGCACATCCCCCTTTCCCGAGCTGGCGTAATAGCGAAGAGGCCCGACC ATCGCCTTCCCAACAGTTGCGCATACGTACCGAGTTAAGGTTTACACCTATAAAAAGAGAGAGCCGTTATC GTCTGTTTGTGGATGTACAGAGTGATATTATTGACACGCCGGGGCGACGGATGGTGATCCCCCTGGCCAGTGCACGTC TGCTGTAGATAAAGTCTCCCGTGAACCTTACCCTGGTGGTGCATATCGGGGATGAAAGCTGGCGCATGATGACCACCG ATATGGCCAGTGTGCCGGTCTCCGTTATCGGGGAAGAAGTGGCTGATCTCAGCCACCGGAAAAATGACATCAAAAAC GCCATTAACCTGATGTTCTGGGGAATATAAATGTACAGCATGAGATTATCAAAAAGGATCTTACCTAGACTCCTTTTC ACGTAGAAAAGCTCCGACGAAACGGTGCACCCCGATGAATGTAGCTACTGGCTATCTGGACAAGGAAAA CGCAAGCGCAAAGAGAAAGCAGGTAGCTTGCAGTGGGCTTACATGGCGATAGCTAGACTGGGCGGTTTTATGGACAG CAAGCGAACCGGAATTGCCAGCTGGGGCGCCCTCTGGTAAGGTTGGGAAGCCCTGCAAAGTAAACTGGATGGCTTC TTGCCGCAAGGATCTGATGGCGCAGGGGATCAAGCTGATCAAGAGACAGGATGAGGATCGTTTCGCATGATTGA ACAAGATGGATTGCACGCAAGTTCTCCGGCCGTTGGTGTGAGAGGCTATTTCGGCTATGACTGGCAACAACAGACAA TCGGCTGCTCTGATGCCGCCGTTTCCGGCTGTACGCGCAGGGGCGCCCGTTCTTTTTGTCAAGACCGACCTGTCCGG TGCCCTGAATGAACTGCAAGACGAGGCAGCGCGCTATCTGGCTGGCCACGACGGGCGTTCTTGCAGCTGTGCT CGACGTTGTCACTGAAGCGGGAAGGGACTGGCTGTATTTGGCGAAGTCCGGGGCAGGATCTCTGTCACTCACT TGCTCTCGCGAAGAAGTATCCATCATGGCTGATGCAATCGCGCGGCTGCATACGCTTGAATCCGGCTACCTGCCAT CGACCACCAAGCGAAACATCGCATCGAGCGAGCACGTAAGGATGGAAGCCGGTCTTGTGATCAGGATGATCTGG ACGAAGAGCATCAGGGGCTCGCGCCAGCCGAACTGTTCCGCAAGGCTCAAGGCGAGCATGCCCGACGGCAGGATCTC GTCGTGACCCATGGCGATGCCTGCTTGCAGAAATCATGTTGGAAAAATGGCCGCTTTTGTGATTCACTGACTGTGGC CGGCTGGGTGGCGGACCCGCTACGAGCATAGCGTTGGCTACCCGATATTGCTGAAGATATTGCTGCGCCGCAATG GGCTGACCGCTTCTCTGTGCTTACCGTATCGCGCTCCCGATTCGACGCGCATCGCTTATCGCCTTCTTACGAG TTCTTCTGAATTGAAAAAGGAAGAGTATGAGTATTCAACATTTCCGTGTGCGCCCTATTCCCTTTTTGCGGCATTTTGC CTTCTGTTTTTGTCTACCCAGAAACGCTGGTGAAGTAAAAGATGCTGAAGATCAGTTGGGTGCACGAGTGGGTTAC ATCGAAGTGGATCAACAGCGGTAAGATCCTTGTAGAGTTTTCGCCCCGAAGAAGCTTTTCAATGATGAGCACTTTT AAAGTCTGCTATGTGGCGCGGATTATCCCGTATTGACGCCGGCAAGAGCAACTCGGTCGCCGCATCACTATTCT CAGAATGACTTGGTTGAGTACTCACCAGTCACAGAAAAGCATCTTACGGATGGCATGACAGTAAGAGAATTATGCAG TGCTGCCATAACCATGAGTGATAAACAATGCGGCCAACTTACTTCTGACAACGATCGGAGGACCGAAGGAGCTAACCG CTTTTTTGCACAACATGGGGGATCATGTAATCGCCTTGTGCTGGGAACCGGAGCTGAATGAAGCCATAACAAACG ACGAGCGTGACACCACGATGCCTGTAGCAATGGCAACAACGTTGCGCAAACTATTAACCTGGCGAATCACTACTAG CTTCCCGGCAACAATTAATAGACTGGATGGAGGCGGATAAAGTTGCAGGACCACTTCTGCGCTCGGCCCTTCCGGCTG GCTGGTTTTATTGCTGATAAATCTGGAGCCGGTGAAGGTTGGTCTCGCGGTATCATTGCAGCACTGGGGCCAGATGGTA AGCCTTCCCGTATCGTAGTTATCTACACGACGGGGAGCCAGGCAACTATGGATGAACGAAATAGACAGATCGTGTAG ATTTGCTCCTCACTGATTAAGCATTGGTAAGTGTACAGCAACGTTTACTCATATATACTGATTGATTTAAAACCTC ATTTTTAATTTAAAAGGATCTAGGTGAAGATCCTTTTTGATAATCTCATGACCAAAAATCCCTAACGTTAGTTTTCGTT CCACTGAGCGTACAGCCCGTAGAAAAGATCAAAGGATCTTCTTGTAGATCCTTTTTTCTGCGGTAATCTGTGCTGTG CAAAACAAAAAACACCGCTACCAGCGGTGGTTTGTGGCCGATCAAGAGCTACCAACTCTTTTTCCGAAGGTAAC GGCTTCCAGCAGCGCAGATACCAAACTGTTCTTCTAGTGTAGCCGATGTTAGGCCACCCTCAAGAAGCTGTGA GCACCGCTACATACTCGCTCTGCTAATCCTGTTACTAGTGGCTGCTGCCAGTGGCGATAAGTGTGCTTACCAGG TGGATTCAAGACGATAGTTACCGGATAAAGGCGCAGCGGTGGGCTGAACGGGGGTTCTGTGCACACAGCCAGCTTG GAGCGAACGACCTACACGAACTGAGATACCTACAGCTGAGCTATGAGAAAAGCGCCACGCTTCCGAAAGGGAGAAA GGCGGACAGGTATCCGGTAAGCGCGAGGTCGGAACAGGAGCGCACGAGGGAGCTTCCAGGGGAAACGCGCTGG TATCTTTATAGCTGTGCGGTTTCCGCCACTCTGACTTACGCTGATTTTTTGTGATGCTCAGGGGGCGGAGCC TATGAAAAACGCCAGCAACGCGCCTTTTTACGGTCTCTGGCCTTTTGTGCTGCTTGTGCTCATGTTCTTTCTCTGC GTTATCCCCTGATTCTGTGGATAACCGTATTACCCTTTGAGTGAGCTGATACCGCTCGCCGACGCCGAACGACCGA CGCGACGCAATCAGTGAGCGAGGAAGCGGAAG</p>
<i>F. tularensis</i>	<p>TAATACGACTACTATAGGGGTAACAGGTTTAGGATGCTGACGAGTGGCGACGGGTGAGTAACCGGTAGGAATCT GTCCATTTGAGGGGGATAACCAGTTGGAACGACTGTTAATACCGCATAATATCTGTGGATTAAGGTTGGCTTTCGGGC TGTCGAGATGGATGAGCCTGCGTTGGATTAGCTAGTTGGTGGGGTAAGGGCCACCAAGGCTACGATCCATAGCTGA TTTGTAGAGGATGATCAGCCACATTGGGACTGAGACCGGCCAAAACCTCTACGGGAGGCGAGCAGTGGGGAATATTGG ACAATGGGGGCAACCCTGATCCAGCAATGCCATGTGTGTAAGAGGCCCTAGGGTTGTAAGCACTTTAGTTGGG AGGAAAGCCTCAAGGTTAATAGCCTTGGGGGAGGACGTTTACCAAAAAGAAATAAGCACCAGCTTAACTCGTGCCAGCAG CCGCGGTAATACGGGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGGTTCTGTAGGTGGTTTGTAAAGTCAG ATGTGAAAAGCCCAGGGTCAACCTTGGAACTGCATTTGATACTGGCAAACCTAGAGTACGGTAGAGGAATGGGGAATT TCTGGTGTAGCGGTGAAATGCGTAGAGATCAGAAGGAACACCAATGGCGAAGGCAACATTCTGGACCGATACTGACA CTGAGGGACGAAAAGCGTGGGGATCAAAACAGGATTAGATAACCCTGGTAGTCCACGCTGTAAACGATGAGTACTG TTGGAGTCCGTTGTAAGGCTCTAGTGGCGCAGCTAACCGGATAAGTACTCCGCTGGGGACTACGGCCGCAAGGCTA AACTCAAAGGAATTGACGGGGACCCGCACAAGCGGTGGAGCATGTGGTTTAAATTCGATGCAACGCGAAGAACCTTA CCTGGTCTTGACATCCTGCGAACTTTCTAGAGATAGATTGGTGCCTTCCGGAAACGCGAGTACAGGTTGCACGGCTG TGTGAGCTGTTGTGAAAATGTTGGTTAAGTGGTCCCGCAACGACGCGCAACCCTATTGATAGTTACATCATTAAAGTT GGGTACTCTATTGAGACTGCCGCTGACAAGGCGGAGGAAGTGGGGACGACGTCAGTCAATCATGGCCCTTACGACC AGGGTACACACGTGCTACAATGGGTATTACAGAGGGCTGCGAAGGTGCGAGCTGGAGCGAAACTCAAAAAGTACT CTTAGTCCGGATTGCACTGCAACTCGATGAAAGTCCGAAATCGCTAGTAATCGCAGGTCAGAATACTGCGGTG AATACGTTCCCGGCTTTGTACACACCGCCCTCACACCATGGGAGTGGGTTGCTCCAGAAGTAGATAGCTTAAACGAA TGGCGCTTACACCGGATGATTTCATGACTGGGTTG</p>

<i>Y. pestis</i>	TAATACGACTCACTATAGGGGCAGCGGGAAGTAGTTACTACTTTGCCGGCAGCGGGCGACGGGTGAGTAATGTCT GGGGATCTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATGACCTCGCAAGAGCAAAGTGGG GGACCTTAGGGCCTCACGCCATCGGATGAACCCAGATGGGATTAGCTAGTAGGTGGGGTAATGGCTCACCTAGGCGA CGATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAAGTACGACACGGTCCAGACTCTACGGGAGGCAGCA GTGGGGAATATTGCACAATGGGGCGAAGCCTGATGCAGCCATGCCGCGTGTGTGAAGAAGGCCTTCGGGTTGTAAG CACTTTCAGCGAGGAGGAAGGGGTTGAGTTAATACGCTCAATCATTGACGTTACTCGCAGAAGAAGCACCGGCTAA CTCCGTGCCAGCAGCCGCGTAATACGGGAGGTTGAGCGTGAACCGGTTAATCGGAATTACTGGGCGTAAAGCGCACGAGGCG GTTTGTTAAGTCAGATGTGAAATCCCGCGCTAACGTGGGAAGTGCATTTGAAACTGGCAAGCTAGAGTCTTGTA GGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTGG ACAAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCTGTAAACGA TGTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGTACCGGTTAAGTCGACCCGCTGGGAGTACGGCC GCAAGGTTAAACTCAAATGAATTGACGGGGGCCGCAAGCGGTGGAGCATGTGGTTAATTCGATGCAACGCGA AGAACCTTACCTACTCTTGACATCCACAGAATTGGCAGAGATGCTAAAGTGCCTTCGGGAAGTGTGAGACAGGTGCT GCATGGCTGTCTCAGCTCGTGTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATCCTTTGTTGCCAG CACCAATGGGAACTCAAGGGAGACTGCGCGAAGGAGGAAAGGTTGGGATGAGTGCCTTACGTTGAGTGCCTTACG GCCCTTACGAGTAGGGCTACACACGTGCTACAATGGCAGATACAAAGTGAAGCGAACTCGCGAGAGCCAGCGGACCA CATAAAGTCTGTCTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTAGATCAG AATGCTACGGTGAATACGTTCCCGGGCCTGTACACACCCCGCTCACACCATGGGAGTGGGTTGCAAAAAGAAGTAG GTAGCTTAACCTTCGGGAGGGCGCTTACCACTTTGTGATTCTACTGACTGGGGTG
<i>V. cholerae</i>	TAATACGACTCACTATAGGGGCAGCAGAGGAATTGTTCTTGGGTGGCGAGCGGGCGACGGGTGAGTAATGCCT GGGAAATTGCCCGGTAGAGGGGGATAACCATTGGAACGATGGCTAATACCGCATAACCTCGCAAGAGCAAAGCAG GGGACCTTCGGGCCTTGCCTACCGGATATGCCAGGTGGGATTAGCTAGTTGGTGAGGTAAGGGCTCACCAAGGCG ACGATCCCTAGCTGGTCTGAGAGGATGATCAGCCACACTGGAAGTACGACACGGTCCAGACTCCTACGGGAGGCAGC AGTGGGGAATATTGCACAATGGGGCGCAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTA GTACTTTCAGTAGGGAGGAAGGTGGTTAAGTTAATACCTTAATCATTGACGTTACTACAGAAGAAGCACCGGCTAA CTCCGTGCCAGCAGCCGCGTAATACGGAGGGTGCAAGCGTTAATCGGAATTACTGGGCGTAAAGCGCATGCAGGTG GTTTGTTAAGTCAGATGTGAAAGCCTGGGCTAACCTAGGAATCGCATTTGAAACTGACAAGCTAGAGTACTGTAGA GGGGGTGAGAAATTCAGGTGTAGCGGTGAAATGGCTAGAGATCTGAAGGAATACCGGTGGCGAAGGCGGCCCTGG ACAGATACTGACACTCAGATGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACCGA TGTCTACTTGGAGTTGTGCCCTAGAGGGCGTGGCTTCCGAGCTAACCGGTTAAGTAGACCGCCTGGGAGTACGGTC GCAAGATTAATACTCAAATGAATTGACGGGGGCCGCAAGCGGTGGAGCATGTGGTTAATTCGATGCAACGCGA AGAACCTTACCTACTCTTGACATCCAGAGAATCTAGCGGAGACGCTGGAGTGCCTTCGGGAGCTCTGAGACAGGTGCT GCATGGCTGTCTCAGCTCGTGTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATCCTTTGTTGCCAG CACGTAATGGTGGGAACTCCAGGGAGACTGCCGGTATAAACCGGAGGAAGGTGGGACGACGTCAAGTCATCATGG CCCTACGAGTAGGGCTACACACGTGCTACAATGGCTATACAGAGGGCAGCGAAACCGCGAAGGTGAGCGAATCTC ACAAAGTACGTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGCAAATCAGA ATGTTGCGGTGAATACGTTCCCGGGCCTTGTACACACCCCGCTCACACCATGGGAGTGGGCTGCAAAAAGAAGCAGG TAGTTTAACTTCGGGAGGACGCTTGCCACTTTGTGGTTCATGACTGGGGTG
<i>E. coli</i>	TAATACGACTCACTATAGGGGTAACAGGAAGAAGCTTGCTTCTTGCTGACGAGTGGCGGACGGGTGAGTAATGTCTG GGAAACTGCCTGATGGAGGGGGATAACTACTGGAAACGGTAGCTAATACCGCATAACGTCGCAAGACCAAAGAGGG GGACCTTCGGGCCTTGGCATCGGATGTGCCAGATGGGATTAGCTAGTAGGTGGGGTAACGGCTCACCTAGGCGAC GATCCCTAGCTGGTCTGAGAGGATGACCAGCCACACTGGAATTGAGACACGGTCCAGACTCCTACGGGAGGCAGCAG TGGGGAATATTGCACAATGGGGCGAAGCCTGATGCAGCCATGCCGCGTGTATGAAGAAGGCCTTCGGGTTGTAAGT ACTTTCAGCGGGGAGGAAGGGAGTAAAGTTAATACCTTTGCTCATTGACGTTACCCGCGAAGAAGCACCGGCTAAC TCCGTGCCAGCAGCCGCGTAATACGGAGGGTGAACGCTTAATCGGAATTACTGGGCGTAAAGCGCACGACGGCGG TTTGTTAAGTCAGATGTGAAATCCCGGGCTAACCTGGGAAGTGCATCTGATACTGGCAAGCTTGAAGTCTCGTAGAG GGGGGTAGAATTCCAGGTGTAGCGGTGAAATGCGTAGAGATCTGGAGGAATACCGGTGGCGAAGGCGGCCCTTGG CGAAGACTGACGCTCAGGTGCGAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAACGAT GTCGACTTGGAGGTTGTGCCCTTGAGGCGTGGCTTCCGGAGCTAACCGGTTAAGTCGACCGCCTGGGGAGTACGGCCG CAAGGTTAAACTCAAATGAATTGACGGGGGCCGCAAGCGGTGGAGCATGTGGTTAATTCGATGCAACGCGAA GAACCTTACCTGGTCTTGACATCCACAGAATTTCCAGAGATGGATTGGTGCCTTCGGGAAGTGTGAGACAGGTGCTG CATGGCTGTCTCAGCTCGTGTGTGAAATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTATCTTTGTTGCCAGC GGTCCGGCCGGGAATCAAAGGAGACTGCCAGTGATAAACTGGAGGAAGGTGGGGATGACGTCAAGTCATCATGGCC CTTAGCACCAGGGCTACACACGTGCTACAATGGCGCATACAAAGAGAAGCGACCTCGCGAGAGCAAAGCGACCTCAT AAAGTGCCTCGTAGTCCGGATTGGAGTCTGCAACTCGACTCCATGAAGTCGGAATCGCTAGTAATCGTGGATCAGAAT GCCACGGTGAATACGTTCCCGGGCCTGTACACACCCCGCTCACACCATGGGAGTGGGTTGCAAAAAGAAGTAGGTA GCTTAACTTCGGGAGGGCGCTTACCACTTTGTGATTCTACTGACTGGGGTG

<i>B. pseudomallei</i>	<p>TAATACGACTCACTATAGGGGACGACACGGGCTTCGGCCCTGGTGGCGAGTGGCGAACCGGGTGAAGTAATACATCGGAAC ATGTCCTGTAGTGGGGATAGCCCGGCGAAAGCCGGATTAATACCGCATAACGATCTAAGGATGAAAGCGGGGACCT TCGGGCTCGCGCTATAGGGTTGGCCGATGGCTGATTAGCTAGTTGGTGGGGTAAAGGCCTACCAAGGCGACGATCA GTAGCTGGTCTGAGAGGACGACCAGCCACTGGGACTGAGACACGGCCAGACTCTACGGGAGGCAGCAGTGGGG AATTTTGGACAATGGGCGCAAGCCTGATCCAGCAATGCCGCTGTGTGAAGAAGGCCTTCGGGTTGTAAAGCACTTTT GTCCGAAAGAAATCATTCTGGCTAATACCCGGAGTGGATGACGGTACCGGAAGAATAAGCACCCGGCTAACTACTGTG CCAGCAGCCCGGTAATACGTAGGGTGGAGCGTAACTCGAAGTACTGGGCGTAAAGCGTGGCGGGGTTTGGCT AAGACCGATGTGAAATCCCGGGCTCAACTGGGAAGTGCATTGGTACTGGCAGGCTAGAGTATGGCAGAGGGGGG TAGAATTCACGTGTAGCAGTAAATGCGTAGAGATGTGGAGGAATACCGATGGCGAAGGCAGCCCCCTGGGCCAAT ACTGACGCTCATGCACGAAAGCGTGGGGAGCAAAACAGGATTAGTACCCTGGTAGTCCACGCCCTAAACGATGTCAA CTAGTTGTTGGGGATTCAATTCCTTAGTAACGTAGCTAACCGGTGAAGTTGACCGCTGGGGAGTACGGTCGCAAGAT TAAACTCAAAGGAATTGACGGGGACCCGCAACGCGGTGGATGATGTGGATTAATTCGATGCAACGCGAAAAACCT TACCTACCCTTGACATGGTCGGAAGCCCCGATGAGAGTTGGGCGTGTCTGAAAGAGAACCAGCGCGCACAGGTGCTGCAT GGCTGTGCTCAGCTCGTGTGATGAGTGTGGGTTAAGTCCCGCAACGAGCGCAACCCCTGTCTTAGTTGCTACGCA GAGCAACTCTAAGGAGACTGCCGGTACAAACCGGAGGAAGTGGGGATGACGTCAAGTCTGGCGGAGCAATTCAGG TAGGGCTTACACGTCATACAATGGTCGGAACAGAGGGTCCGCAACCCCGGAGGGGGAGCCAATCCAGAAAAACCGA TCGTAGTCCGGATTGCACTCTGCAACTCGAGTGCATGAAGCTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGGT GAATACGTTCCCGGGTCTTGTACACACCCCGCTCACACCGATGGGAGTGGGTTTTACCAGAAGTGGCTAGTCTAACC CAAGGAGCAGGTCACCACGGTAGGATTCATGACTGGGTG</p>
<i>R. conorii</i>	<p>TAATACGACTCACTATAGGGGACTAATTGGGGCTTGTCTCAGTTAGTTAGTGGCAGACGGGTGAGTAACACGTGGGA ATCTACCCATTAGTACGGAATAACTTTTAAAAATAAAAGCTAATACCGTATATTTCTGCGGAGGAAAGATTTATCGC TGATGGATGAGCCCGCTCAGATTAGGTAGTTGGTGAAGTAATGGCTACCAAGCCGACGATCTGTAGCTGGTCTGAG AGGATGATCAGCCACTGGGACTGAGACACGGCCAGACTCTACGGGAGGCAGCAGTGGGGAATATTGGACAATG GGCAAAGCTGATCCAGCAATACCGAGTGAAGTGAAGCTTACGGGTTGTAAGGTTGTAAGGCTTTTAGCAAGGAAGATA ATGACGTTACTTGCAGAAAAAGCCCCGCTAACTCCGTGCCAGCAGCCGCGTAAGACGGAGGGGGCTAGCGTTGTT CGGAATTAAGTGGCGTAAAGAGTGCAGTGGGTTTGTAGTAAAGTGAAGTGAAGCCCGGGGCTAACCTCGGAATT GCTTTAAAACTACTAATCTAGAGTGTAGTGGGGATGATGGAATTCCTAGTGTAGAGGTGAAATTCCTAGATATTG GAGAAACACCGGTGGCGAAGGCGGTCTATCTGGGACTGACAGCTGATGCACGAAACCGGAGCAAAACAGG ATTAGATACCCTGGTAGTCCACGCGTAAACGATGAGTGTAGATATCGGAAGATTCTTTTCGGTTTCGACGCTAAC GCATTAAGCACTCCGCTGGGGAGTACGGTCCGAAGATTAACACTCAAAGGAATTGACGGGGGCTCGCACAAAGCGGT GGAGCATGCGGTTAATTCGATGTTACGCGAAAAACCTTACCAACCCTTGACATGGTGGTCCGCGGATCGCAGAGATGC TTTTCTAGCTCGGCTGGACCACACAGGTGTGATGGTGTGCTCAGCTCGTGTGAGTGTGGTGTAAAGTCT CCGCAACGAGCGCAACCCCTATTCTTATTTGCCAGCGGGTATGCCGGAACTATAAGAAAACTGCCGGTATAAGCC GGAGGAAGGTGGGGACGACGTCAGTCAATCATGCGCCTTACGGGTTGGGCTACACGCGTGTACAATGGTGTTTACA GAGGGAAGCAAGACGGCGACGTGGAGCAAAATCCCTAAAAAGACATCTCAGTTCGGATTGTTCTCTGCAACTCGAGAGC ATGAAGTTGGAATCGCTAGTAATCGCGGATCAGCATGCCGCGTGAATACGTTCTCGGGCCTGTACACACTGCCCGT CACGCCATGGGAGTTAGTTTTACCTGAAGGTGGTGAAGTCAACCGCAAGAGGCAGCCAACCCAGGTAATAATTAGCGACT GGGGTG</p>
<i>B. abortus</i>	<p>TAATACGACTCACTATAGGGCCCCGCAAGGGGAGCGGCAGACGGGTGAGTAACGCGTGGGAACGTACCATTGCTAC GGAATAACTCAGGGAACCTTGTGCTAATACCGTATGTGCCCTTCGGGGAAAGATTTATCGGCAATGATCGGCCCGC GTTGGATTAGTGTGGTGGGGTAAAGGCTACCAAGCGCAGATCCATAGCTGGTCTAGAGAGGATGATCAGCCAC ACTGGGACTGAGACACGGCCAGACTCTACGGGAGGCAGTGGGGAATATTGGACAATGGGCGCAAGCCTGATC CAGCATGCCCGTGAAGTGAAGGCCCTAGGGTTGTAAAGCTCTTTCACCGGTGAAGATAATGACGGTAACCGGA GAAGAAGCCCCGGCTAACTTCGTGCCAGCAGCCGCGTAAATACGAAGGGGGCTAGCGTTGTTTCGGATTACTGGGCG TAAAGCGCACGTAGGCGGACTTTTAAAGTCAAGGGTGAATCCCGGGGCTCAACCCCGGAACCTTTGATACTGGA AGTCTTGAGTATGGTAGAGGTGAGTGGAAATCCGAGTGTAGAGGTGAAATTCGTAGATATTTCGGAGGAACACAGTG GCGAAGGCGGCTCACTGGACCATTACTGACGCTGAGGTGCGAAAGCGTGGGGAGCAAAACAGGATTAGATACCCTGGT AGTCCACGCCGTAACGATGAATGTTAGCCGTCGGGGTGTTTACACTTCGGTGGCGAGCTAACGCATTAACACTCC GCCTGGGAGTACGGTCCGAAGATTAACACTCAAAGGAATTGACGGGGGCCCCGCAAGCGGTGGAGCATGTGGTTT AATTCGAAGTCAACCGCAGAACCTTACCAGCCCTGACATCCCGTCCGCGTTAGTGGAGACTACTCTTCAGTTAG GCTGGACCGGAGACAGGTGCTGCATGGTGTGCTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGC AACCTTCGCCCTTAGTTGCCAGATTAGTTGGGCACTTAAGGGGACTGCCGGTATAAGCCGAGAGGAAGGTGGG GATGACGTCAGTCTCATGCCCCCTACGGGCTGGGCTACACACGCTGCTACAATGGTGTGACAGTGGGCGAGCGAGC ACGGAGTGTGAGCTAATCTCCAAAAGCCATCTCAGTTCGAGTGTGCACTGCAACTCGAGTCAATGGAATGGAATC GCTAGTAATCGCGGATCAGCATGCCGCGGTGAATACGTTCCCGGGCCTGTACACACCCGCCGTCACACCATGGGAGT TGGTTTTACCGAAGGCGCTGTGCTAACCGCAAGGAGGCAGGCGACCACGGTAGGGTACGCGACTGGGGTG</p>
<i>S. aureus</i>	<p>TAATACGACTCACTATAGGGAACGGACGAGAAGCTTGTCTCTGATGTTAGCGGCGGACGGGTGAGTAACACGTGG ATAACCTACCTATAAGACTGGGATAACTTCGGGAAACCGGAGCTAATACCGGATAATATTTTGAACCGCATGGTTCAA AAGTGAAGACGGTCTTGTCTCACTTATAGATGGATCCCGCTGCATTAGCTAGTTGGTAAAGTAAACCGCTTACCA GGCAACGATACGTAGCCGACCTGAGAGGGTGTATCGGCCACTGGAAGTGAACACCGTCCAGACTCTACGGGAGG CAGCAGTAGGGAATCTCCGCAATGGGCGAAAGCCTGACGGGACACCGCCGCTGAGTGTGAAGGTCTTCGGATCG TAAACTCTGTTATTAGGGAAGAACATATGTGTAAAGTAACTGTGCACATCTTGACGGTACTTAATCAGAAAAGCCACGG CTAACTAGTCCAGCAGCCGCGTAAATACGTAGGTGAAGTGAAGCGTTATCCGGAATATTGGGCGTAAAGCGCGCTA GGCGGTTTTTTAAGTCTGATGTGAAAGCCCACGGCTCAACCGTGGAGGGTCAATTGAAAACCTGGAAAACCTGAGTGCAG AAGAGGAAAGTGAATTCATGTGTAGCGGTGAAATGCGCAGAGATATGGAGGAACACAGTGGCGAAGGCGACTTT CTGGTCTGTAAGTGAAGTGTGGGGTTTCCGCCCTTAGTGTGACGCTAAGCATTAAAGCCTAGCTGCTGGGAGTA CGACCGCAAGGTTGAAAACCTCAAAGGAATTGACGGGGACCCGCAACGCGGTGGAGCATGTGGTTAATTCGAAGCAA CGCAAGAACCTTACCAATCTTGACATCTTTGACAACCTTAGAGATAGAGCCTTCCCCTTCGGGGGACAAAAGTGAC AGGTGGTGCATGGTTGCTCAGCTCGTGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCCTAAGCTTA GTTGCCATTAAGTTGGGCACTTAAGTTGACTCCGGTGACAAACCGGAGGAAGTGGGGATGACGTCATAATCA TCAATGCCCTTATGATTTGGGCTACACACGCTGCTACAATGGCAATAACAAAGGGCAGCGAAACCCGAGGTCAGCA AATCCCATAAAGTTGTTCTCAGTTCGGATTGATGCTGCAACTGACTACATGAAGCTGGAATCGCTAGTAATCGTAG ATCAGCATGCTACGGTGAATACGTTCCCGGGTATTGTACACACCCCGCTCACACCAGGAGGTTTGAATACCCGAA GCCGGTGGAGTAACCTTTTAGGAGCTAGCCGTCGAAGGTGGGACAAATGATTGGGGTG</p>

Table S2 Sequence information targeting the 3'-end of each 16S rDNA sequence.

Name	Sequence (5' to 3') ^[a]
T716UP8U-F	TCGAUCCUGCGAAAUG <u>UAAUACGACU</u> CTAUAGGGUGACCACAACGGTTTCCCTCTAG
T7P	TAATACGACTCACTATAGG
T7T-R	CAAAAAACCCCTCAAGACCCGTTTA
<i>F. tularensis</i> -R	CACCCCAGTCATGAATCACT
<i>Y. pestis</i> -R	CACCCCAGTCATGAATCACAAAGT
<i>V. cholerae</i> -R	CACCCCAGTCATGAACCACA
<i>E. coli</i> -R	CACCCCAGTCATGAATCACAAAGT
<i>B. pseudomallei</i> -R	CACCCCAGTCATGAATCCTACC
<i>R. conorii</i> -R	CACCCCAGTCGCTAATTTTACCG
<i>B. abortus</i> -R	CACCCCAGTCGCTGA
<i>S. aureus</i> -R	CACCCAATCATTGTCCACC

[a] Underlined letters indicate T7 promoter sequence

Table S3 Sequence information of pK7/dT7P-sfGFP constructs.

pK7/dT7P-sfGFP	<p>AGTGAGCGAGGAAGCGGAAGAAGCTCGCACATTCAGCAGCGTTTTTCAGCGCGTTTTTCGATCAGCGTTTTCAATGTTGG TATCAACACCAGGTTAACTTTGAACTTATCGGCACTGACGGTACTGATTTTGAACCTTTGCTTTGCCACGGAACGGT CTGCGTTGTCCGGGAAGATGCGTGATCTGATCCTTCAACTCAGCAAAAAGTTTCGCCAATACGCAAACCGCCTCTCCCGC GCGTTGGCCGATTCAATTAATGCAGCTGGCACGACAGGTTTCCCGACTGGAAAGCGGGCAGTGAGCGCAACGCAATTA ATGTGAGTTAGCTCACTCATTAGGCACCCAGGCTTACACGACCACAACGGTTTTCCCTCTAGAAATAATTTTGTTTAA CTTTAAGAAGGAGATATACATATGAGCAAAGGTGAAGAACTGTTTACCGGCGTTGTGCCGATTCTGGTGGAAC GGATGGCGATGTGAACGGTCAAAAATTAGCGTGCCTGGTGAAGGTGAAGGCGATGCCACGATTGGCAAACCTG ACGCTGAAATTTATCTGCACCACCGGCAAACTGCCGGTGCCGTGGCCGACGCTGGTGACCACCTGACCTATG GCGTTCAAGTGTTTTAGTCGTATCCGGATCACATGAAACGTACAGATTTCTTTAAATCTGCAATGCCGGAAGGC TATGTGACAGGAACGTACGATTAGCTTTAAAGATGATGGCAAATATAAAACGCGCGCCGTTGTGAAATTTGAAG GCGATACCCTGGTGAACCGCATTGAACTGAAAGGCACGGATTTTAAAGAAGATGGCAATATCCTGGGCCATAA ACTGGAATACAACCTTTAATAGCCATAATGTTTATATTACGGCGGATAAAACAGAAAAATGGCATCAAAGCGAATT TTACCGTTCGCCATAACGTTGAAGATGGCAGTGTGACGCTGGCAGATCATTATCAGCAGAATACCCCGATTGGT GATGGTCCGGTGTCTGCTGCCGATAATCATTATCTGAGCACGCAGACCGTTCTGTCTAAAGATCCGAAACGAAA AACGGGACCACATGGTCTGCACGAATATGTGAATGCGGCAGGTATTACGTGGAGCCATCCGCAGTTCGAAAA AGGTGGTTCTCATCACCATCACCATCACTAATAAGTCGACCGGCTGCTAACAAAGCCCGAAGGAAGCTGAGTTGG CTGCTGCCACCGCTGAGCAATAACTAGCATAACCCCTTGGGGCCTTAAACCGGCTTTGAGGGGTTTTTGTCTGAAAG GAGGAATTTATTCATATCAGGATTATCAATACCATATTTTTGAAAAAGCCGTTTCTGTAATGAAGGAGAAAACTAC CGAGGCAGTTCATAGGATGGCAAGATCCTGGTATCGGTCTGCGATTCCGACTCGTCCAACATCAATACAACCTATTA ATTTCCCTCGTCAAAAATAAGGTTATCAAGTGAGAAATCACCATGAGTGACGACTGAATCCGGTGAGAATGGCAAA AGCTTATGCATTTCTTTCCAGACTTGTTCACAGGCCAGCCATTACGCTCGTCATCAAAATCACTCGCATCAACCAAAAC CGTTATTCATTCGTGATTGCGCTGAGCGAGACGAAATACGCGATCGCTGTTAAAAGGACAATTACAAACAGGAATCG AATGCAACCGGCGCAGGAACACTGCCAGCGCATCAACAATATTTTACCTGAATCAGGATATTCTTCTAATACCTGGA ATGCTGTTTTCCCGGGGATCGCAGTGGTGAGTAACCATGCATCATCAGGAGTACGGATAAAATGCTTGATGGTCGGAA GAGGCATAAATCCGTCAGCCAGTTAGTCTGACCATCTCATCTGTAACATCATTGGCAACGCTACCTTTGCCATGTTT CAGAAACAACCTGGCGCATCGGGCTCCCATACAATCAGATAGATTGTCGCACCTGATTGCCGACATTATCGCGAGC CCATTTATACCCATATAAATCAGCATCCATGTTGGAATTTAATCGCGGCTTCGAGCAAGACGTTTCCCGTTGAATATGG CTCATAACACCCCTTGTATTACTGTTTATGTAAGCAGACAGTTTTATTGTTTCATGATGATATATTTTTATCTGTGCAAT GTAACATCAGAGATTTTGAGACACAACGTGGCTTTGTTGAATAAATCGAACTTTTGTGAGTTGAAGGATCAGATCAC GCATCTTCCCGACAACGCAGACCGTTCGGTGGCAAAGCAAAAAGTTCAAAATCACCAACTGGTCCACCTACAACAAG CTCTCATCAACCGTGGCTCCCTCACTTTCTGGCTGGATGATGGGGCGATTACGGCTGGTATGAGTCAGCAACACCTTC TTCACGAGGCAGACCTCTCGACGGATCGTTCCACTGAGCGTCAGACCCCGTAGAAAAGATCAAAGGATCTTCTTGAGA TCCTTTTTTCTGCGCGTAATCTGCTGCTTGCAAACAAAAAACCACCGCTACCAGCGGTGGTTTGTGTTGCCGGATCAA GAGTACCAACTCTTTTCCGAAGGTAACCTGGCTTCAGCAGAGCGCAGATACCAAACTACTGTCCTTCTAGTGTAGCCG TAGTTAGGCCACCCTTCAAGAACTCTGTAGCACCGCTACATACCTCGCTCTGCTAATCCTGTTACCAGTGGCTGCTG CCAGTGGCGATAAGTCGTGTCTTACCGGTTGGACTCAAGACGATAGTTACCGGATAAAGGCGCAGCGGTCCGGCTGA ACGGGGGGTTCGTGCACACAGCCAGCTTGAGAGCAACGACCTACACCGAAGTACCTACAGCGTGAGCATTG AGAAAGCGCCACGCTTCCCGAAGGGAGAAAGGCGGACAGGTATCCGGTAAAGCGGCAGGGTCCGAAACAGGAGAGCGC ACGAGGGAGCTTCCAGGGGAAACGCTGGTATCTTTATAGTCTGTGCGGTTTCGCCACCTCTGACTTGAGCGTCA TTTTTGTGATGCTCGTCAGGGGGCGGAGCCTATGAAAAACGCCAGCAACGCGGCCTTTTACGGTTCCTGGCCTTTT GCTGGCCTTTTGTCTACATGTTCTTCTGCGTTATCCCTGATTCTGTGGATAACCGTATTACCGCCTTTGAGTGAGCT GATACCGCTCGCCGAGCCGAACGACCGAGCGCAGCGAGTC</p>
----------------	---

Underlined letters indicate T716UP8U-F binding region

Bold letters indicate sfGFP sequence

Table S4 Sequence information of IA and TASER probes.

Target pathogen	Flap probe ^[b]	Invasive probe
<i>F. tularensis</i>	TCGATCCTGCGAAATGCTCGCACCT* T * C * G * C * A	ACCTTTTGTAGTTTCGCTCCAA
<i>Y. pestis</i>	TCGATCCTGCGAAATGGCTCTCGCG* A * G * T * T * C	GACTTTATGTGGTCCGCTA
<i>V. cholerae</i>	TCGATCCTGCGAAATGATTCTAGGTT* G * A * G * C * C	CTCTAGCTTGTGTCAGTTTCAAATGCA
<i>E. coli</i>	TCGATCCTGCGAAATGTTCTGTGGATGTC* A * A * G * A * C	CACCAATCCATCTCTGGAAAA
<i>B. pseudomallei</i>	TCGATCCTGCGAAATGCGTAGCAACTAAGGA* C * A * A * G * G	GCAGTCTCCTTAGAGTGCTCTTA
<i>R. conorii</i>	TCGATCCTGCGAAATGTCGCCGTCTTG* C * T * T * C * C	GATGTCTTTTAGGGATTGCTCCACA
<i>B. abortus</i>	TCGATCCTGCGAAATGCGCGTTGCTTCGAAT* T * A * A * A * C	TCAAGGGCTGGTAAGGTTCTA
<i>S. aureus</i>	TCGATCCTGCGAAATGCGCATTTAC* C * G * C * T * A	ACTGGTGTTCCTCCATATCTCTA

[b] * indicates the phosphorothioate bond. Bold letters indicate the nucleotides with 2'-O-methoxy-ethyl (2'MOE) modifications.

Table S5 Sequence information of IA and TASER probes for *E. coli* before optimization.

Target pathogen	Flap probe ^[b]	Invasive probe
<i>E. coli</i>	TCGATCCTGCGAAATGAGACTCAAGC* T * T * G * C * C	GAATTCTACCCCCTCTACA

[b] * indicates the phosphorothioate bond. Bold letters indicate the nucleotides with 2'-O-methoxy-ethyl (2'MOE) modifications.

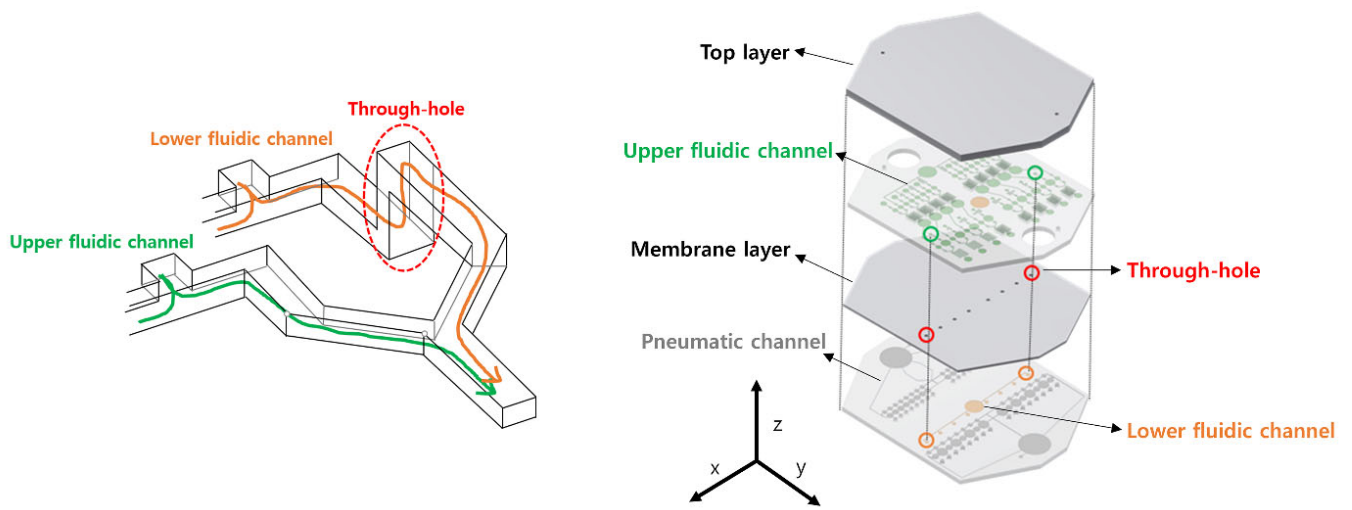


Fig. S1 Schematic of the interconnection between the upper and lower fluidic channels via through-hole. Layer-by-layer schematic of through-hole embedded PAMD. The embedded through-hole structure allows two fluidic paths in a single device.

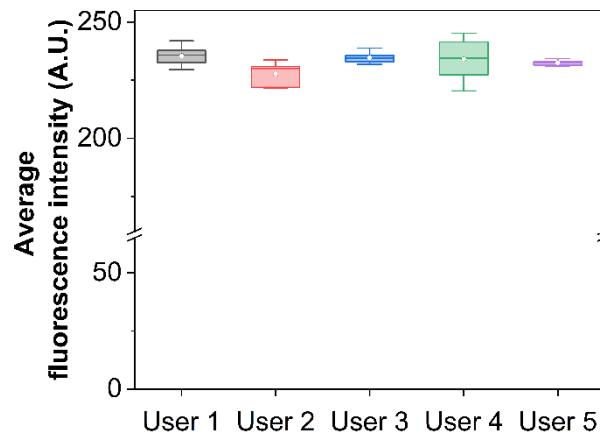


Fig. S2 Reproducibility test based on multiple users. Each sample containing $1 \mu\text{M}$ of *E. coli* 16S rRNA was aliquoted and mixed with IA-TASER reagents via the pushbutton operation ($n = 3$).

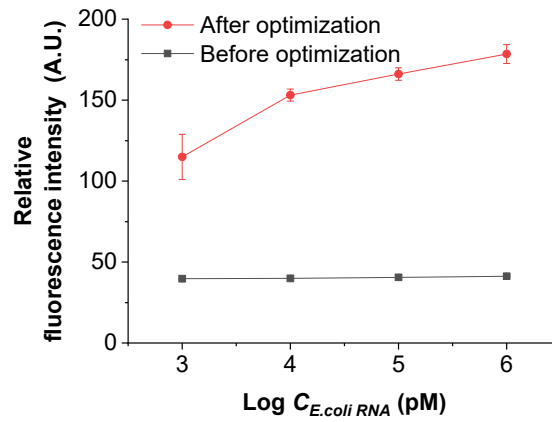


Fig. S3 Performance comparison using non-optimized and optimized invasive and flap probes in the IA-TASER assay.

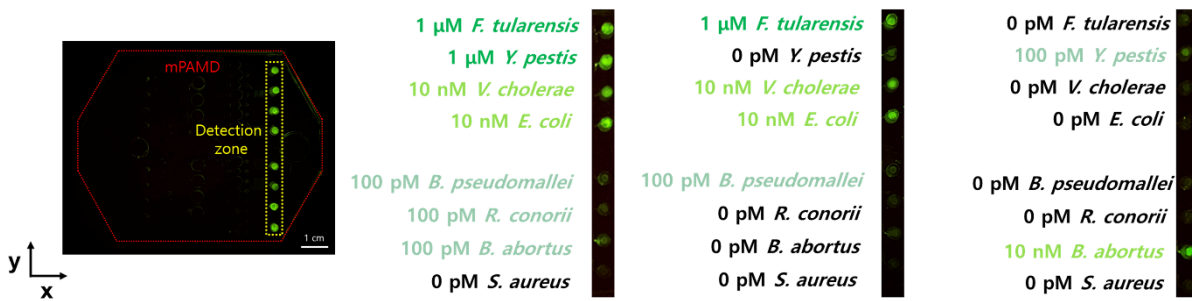


Fig. S4 Enlarged images of the detection zone showing sfGFP fluorescence signals from various concentrations of 16S rRNA captured with the hand-held transilluminator.

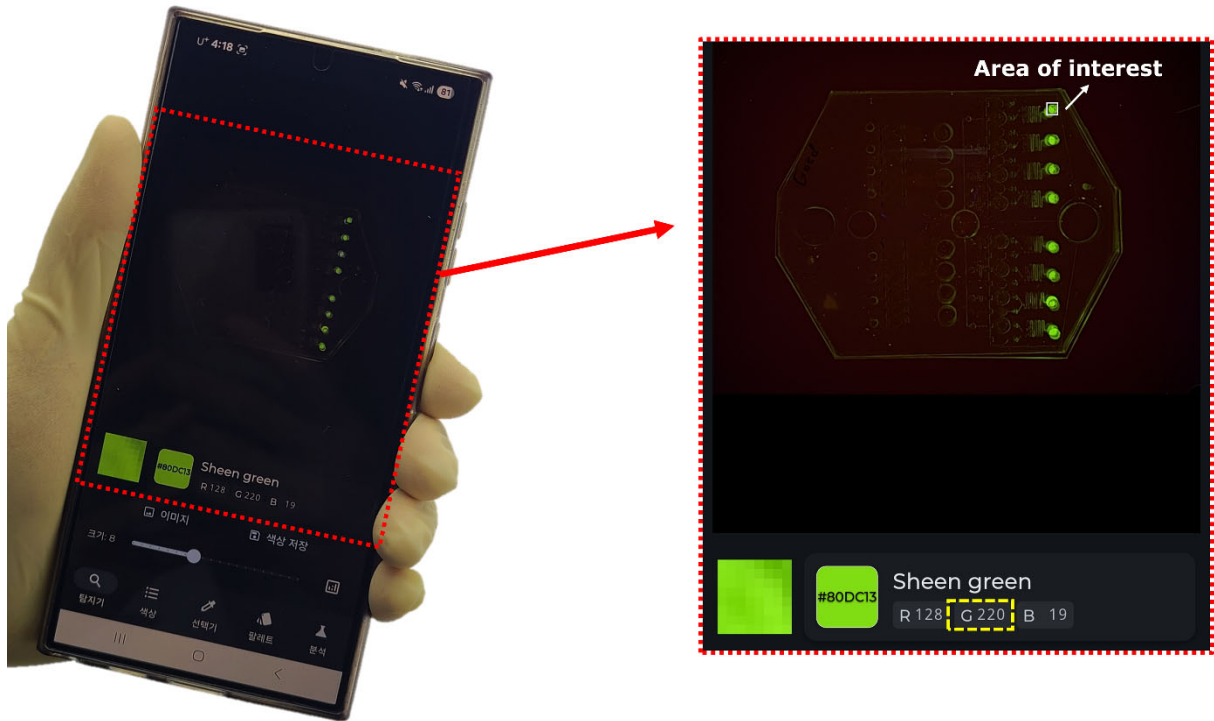


Fig. S5 Representative image showing the analysis of sfGFP fluorescence intensity in the mPAMD using a smartphone app.