Supporting information

On-site detection of methicillin-resistant *staphylococcus aureus* (MRSA) utilizing G-quadruplex based isothermal exponential amplification reaction (GQ-EXPAR)

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Table S1. Sequences of G-quadruplex based isothermal exponential amplification reaction (GQ-EXPAR) probes.

	5' – 3'	Modification
Padlock probe	(p) - TAATAGCCATCATCAT***CCCTA ACCCTAACCCTAACCCTTACCCTAACCCTAACC CTAACCC***TGTACATCTTTAACAT	5′ – phosphate modify
Target DNA (synthetic)	ATGATGATGGCTATTAATGTTAA	
PCR primer for *mecA* gene (forward)	ATGATGATGGCTATTAATGTTAAA	
PCR primer for *mecA* gene (reverse)	ATAGCTCATCATACACTTTA	
*mec*A gene	ATGACGTCTATCCATTTATGTATGGCATGAGTAACGAAGAATATAATAAATTAACCGAAGAT AAAAAAGAACCTCTGCTCAACAAGTTCCAGATTACAACTTCACCAGGTTCAACTCAAAA AATATTAACAGCAATGATTGGGTTAAATAACAAAACATTAGACGATAAAACAAGTTATAAA ATCGATGGTAAAGGTTGGCAAAAAGATAAATCTTGGGGTGGTTACAACGTTACAAGATAT GAAGTGGTAAATGGTAATATCGACTTAAAACAAGCAATAGAATCATCAGATAACATTTTC TTTGCTAGAGTAGCACTCGAATTAGGCAGTAAGAAATTTGAAAAAGGCATGAAAAAACT AGGTGTTGGTGAAGATATACCAAGTGATTATCCATTTTATAATGCTCAAATTTCAAACAAAA ATTTAGATAATGAAATATTATTAGCTGATTCAGGTTACGGACAAGGTGAAATACTGATTAAC CCAGTACAGATCCTTTCAATCTATAGCGCATTAGAAAATAATGGCAATATTAACGCACCTC ACTTATTAAAAGACACGAAAAACAAAGTTTGGAAGAAAAATATTATTTCCAAAGAAAATAT CAATCTATTAACTGATGGTATGCAACAAGTCGTAAATAAAACACATAAAGAAGATATTTATA GATCTTATGCAAACTTAATTGGCAAATCCGGTACTGCAGAACTCAAAATGAAACAAGGA GAAACTGGCAGACAAATTGGGTGGTTTATATCATATGATAAAGATAATCCAAACATGAT GATGGCTATTAATGTTAAAGATGTACAAGATAAAGGAATGGCTAGCTACAATGCCAAAATC TCAGGTAAAGTGTATGATGAGCTATATGAGAACGGTAATAAAAAATACGATATAGATGAATAA	

Notes: P indicates 5′ phosphate modification; underlined text indicates a sequence complementary to the target; bold/italic font indicates sequences that formed a G-quadruplex structure; Red characters represent target region of *mec*A gene.

Table S2. Comparison of Methicillin-Resistant *Staphylococcus aureus* (MRSA) detection methods.

System name	Target	Mechanism	LOD	Advantages(+) & Disadvantages (-)	Ref
C-MRS biosensor	Genomic DNA dsDNA (*mec*A)	CRISPR/Cas12a based magnetic relaxation switching	41 copies/μl	+ High sensitivity - Multi-step reaction - Laboratory level.	[1]
MSP system	Genomic DNA dsDNA (*mec*A)	Multi-signal probes	10 fM	- Multi-step reaction - Request of temperature control. - Laboratory level.	[2]
RPA-CRISPR Cas12a/Cas13a detection system	Genomic DNA dsDNA (*mec*A)	RPA-CRISPR dual gene detection system	5 copies/μl	+ Multiple detection. + Suitable for POCT. - Request of extraction process.	[3]
SOCP	Genomic DNA dsDNA (*mec*A)	signal-off Cas14a1-based platform	1.23 ng/mL	- Request of specific equipment - Laboratory level.	[4]
RCA and G4/hemin DNAzyme Proximity Assembly	Genomic DNA dsDNA (*mec*A)	Rolling Circular Amplification Triggering G-Quadruplex/Hemin DNAzyme Proximity Assembly	9.6 pM	+ Visual assay - Request of temperature control. - Multi-step reaction (time require) - Laboratory level.	[5]
IEXPAR	Genomic DNA dsDNA (*mec*A)	CRISPR/Cas9 induced isothermal exponential amplification reaction	81 fM	+ Isothermal amplification - Request of extraction process. - Laboratory level.	[6]
DTDP	Genomic DNA	Dual-Cas Tandem Diagnostic	1 CFU/mL	+ High sensitivity	[7]

	dsDNA (*mec*A)	Platform		- Request of extraction process. - Request of temperature control.	
GQ-EXPAR	mRNA (*mec*A)	G-quadruplex based isothermal exponential amplification reaction	0.1 fmol	+ Isothermal amplification reaction. + Bacterial capture using 3D-nanostructures. + Omit the extraction process. + Suitable for POCT.	Our work

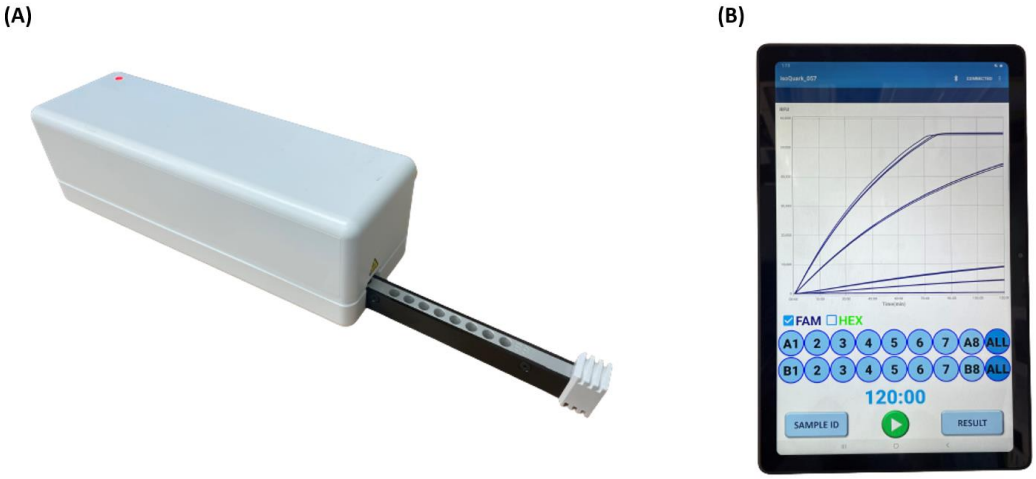

Figure. S1. Portable isothermal polymerase chain reaction (PCR) in the analysis equipment for the G-quadruplex based Isothermal Exponential Amplification reaction (GQ-EXPAR). (A) Portable isothermal PCR, and (B) Galaxy Tab connected to portable isothermal PCR.

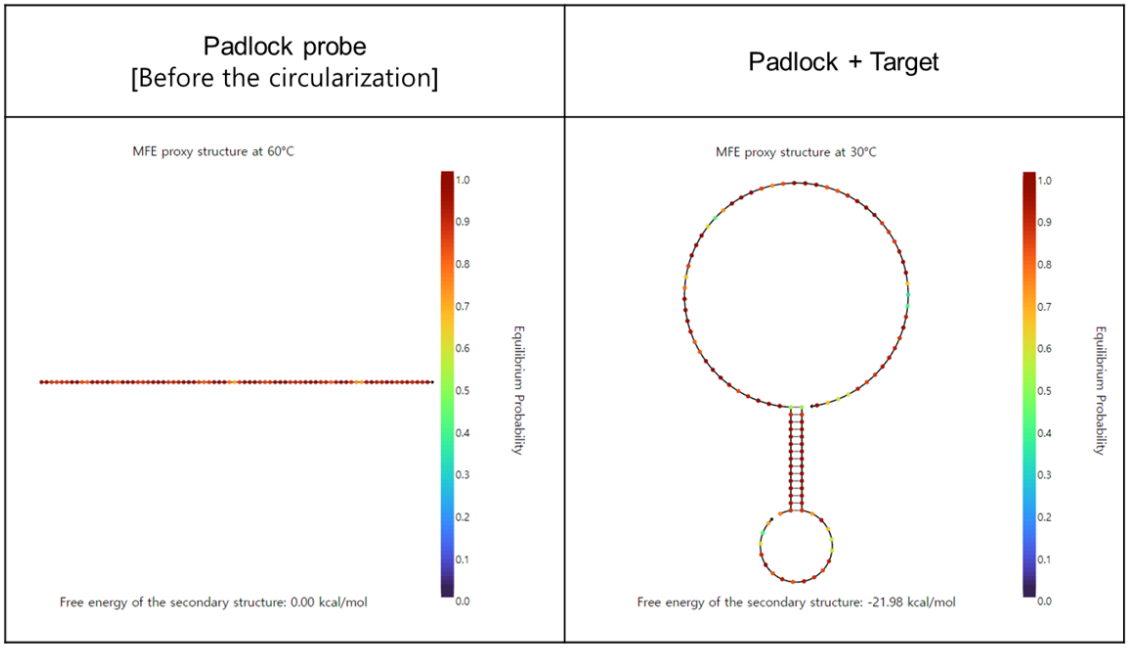

Figure. S2. Nucleic Acid Package (NUPACK software) analysis of the alignment and thermodynamic properties of the DNA probes in the diagnostic system. Confirmation of nonspecific binding using a single-stranded padlock probe. Assessment of binding affinity between the padlock probe and target at system reaction temperature.

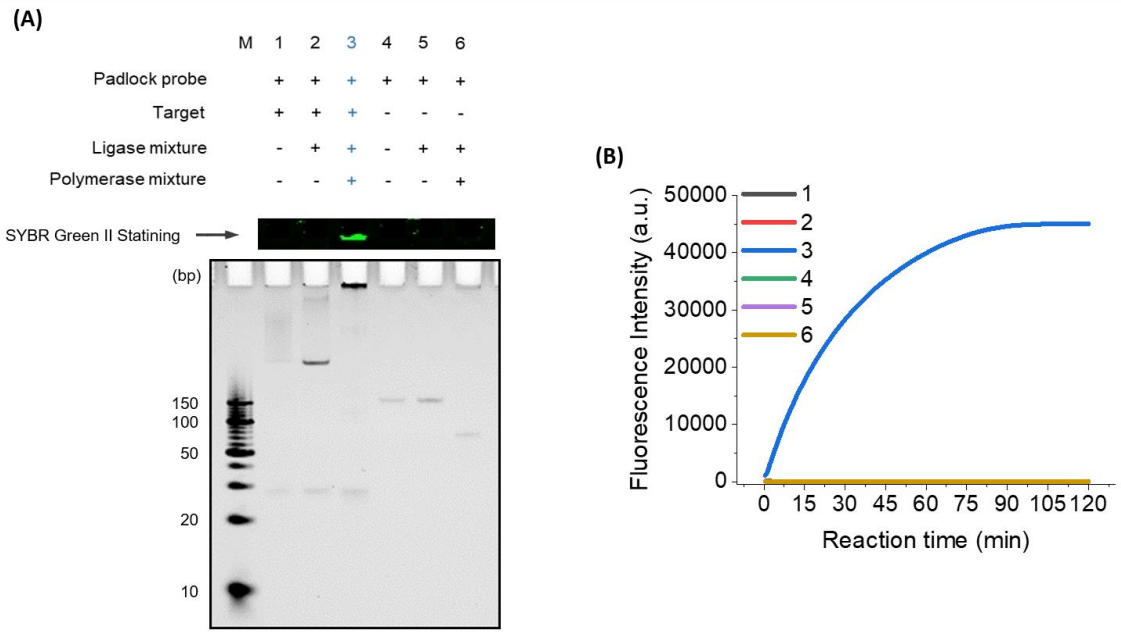

Figure. S3. Evaluation of G-quadruplex based Isothermal Exponential Amplification reaction (GQ-EXPAR). (a) 15% native polyacrylamide gel analysis and (b) real-time fluorescence intensity responses under various conditions. [Padlock probe] = (a) 50 nM, (b) 500 nM, [Target] = (a) 10 nM, (b) 100 nM, [T4 DNA Ligase] = 200 unit, [Phi29 DNA Polymerase] = 10 unit, [dNTP] = 200 μM, [Thioflavin T] = (a) 25 μM.

Figure. S4. Diagram of the fluorescence image analysis equipment for G-quadruplex-based Isothermal Exponential Amplification reaction (GQ-EXPAR).

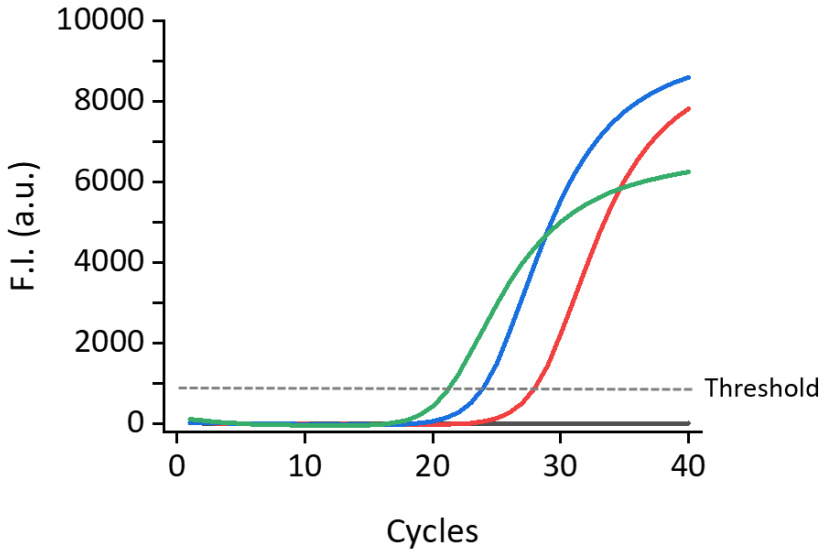

Figure. S5. Quantitative reverse transcription polymerase chain reaction (PCR) analysis of total methicillin-resistant *Staphylococcus aureus* (MRSA) RNA expression at varying concentrations (n = 3). Real-time fluorescence intensity indicates concentration of MRSA total RNA (green, 100 ng; blue, 10 ng; red, 1 ng).

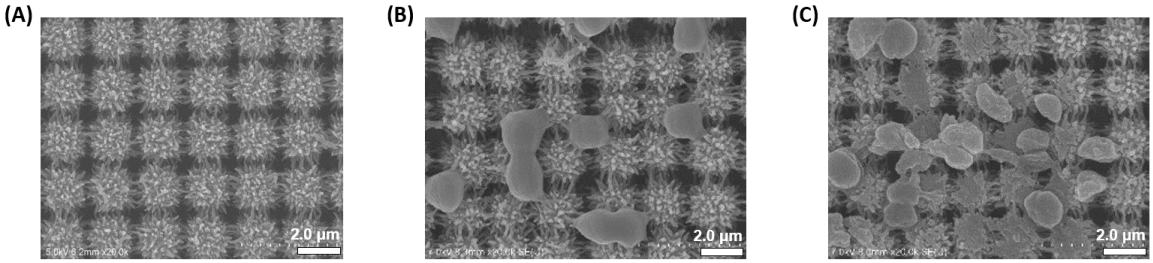

Figure. S6. Morphological images obtained using scanning electron microscopy of bacteria captured on 3D-nanostructures: (a) bare 3D-nanostructure, (b) captured bacteria in 3D-nanostructures, and (c) after thermal lysis of the bacteria captured in 3D-nanostructures (scale bar: 2.0 µm)

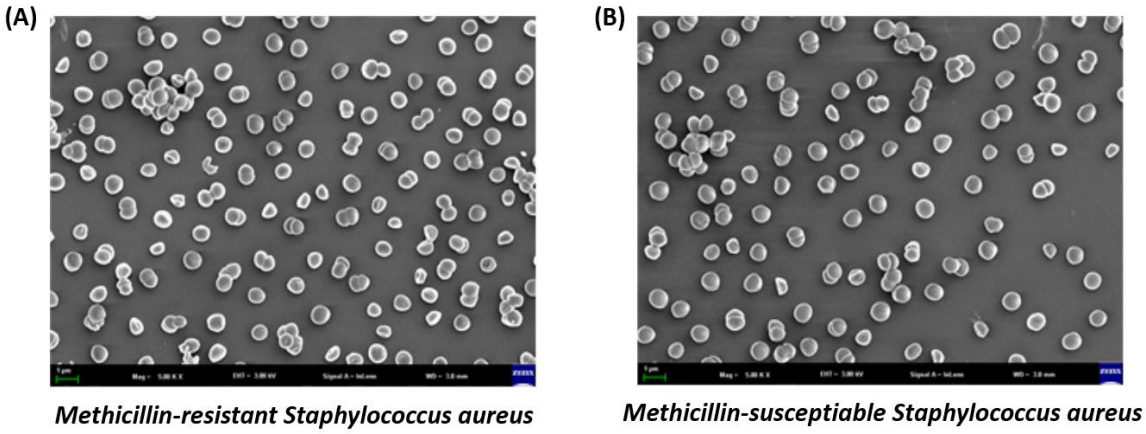

Figure. S7. Morphological images of (a) methicillin-resistant *Staphylococcus aureus* (MRSA) and (b) methicillin-susceptible *Staphylococcus aureus* (MSSA) obtained using scanning electron microscopy (SEM).

References

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