# *Electronic Supplementary Information (ESI)*

## Multiplex detection of bacterial pathogens by PCR/SERS assay

Nana Lyu[a], Phani Rekha Potluri[a], Vinoth Kumar Rajendran[a],
Yuling Wang*[a,b] and Anwar Sunna*[a,b]

[a] School of Natural Sciences, Macquarie University, Sydney, NSW 2109, Australia

[b] Biomolecular Discovery Research Centre, Macquarie University, NSW 2109, Australia

* Corresponding authors: yuling.wang@mq.edu.au; anwar.sunna@mq.edu.au;

**Table S1.** Sequences of oligonucleotides and the PCR amplicons used in this study.

| Target | Description | 5'-Sequence-3' |
|---|---|---|
| *P. aeruginosa* | Amplicon (size: 135 bp) | TCCCATAAAAGCCCTCTTCCGCTCCCCGCCAGCCTCCCCGCATCCC GCACCCTAGACGCCCCGCCGCTCTCCGCCGGCTCGCCCGACAAGA AAAACCAACCGCTCGATCAGCCTCATCCTTCACCCATCACAGGA |
| | Forward primer | CAGATCGTCATGTTC/iSpC3/TCCCATAAAAGCCCTCTTCC |
| | Reverse primer | /5Biosg/TCCTGTGATGGGTGAAGGAT |
| | DNA probe | GAACATGACGATCTGTTTTT/3ThioMC6-D |
| *S. aureus* | Amplicon (size: 219 bp) | GGAGAAACTGGCAGACAAATTGGGTGGTTTATATCATATGATAAAG ATAATCCAAACATGATGATGGCTATTAATGTTAAAGATGTACAAGAT AAAGGAATGGCTAGCTACAATGCCAAAATCTCAGGTAAAGTGTAT GATGAGCTATATGAGAACGGTAATAAAAAATACGATATAGATGAAT AACAAAAGCAGTGAAGCATCCGTAACGATGGTTGC |
| | Forward primer | TACACAGCAC/iSpC3/GGAGAAACTGGCAGACAAATTGGGTGGT |
| | Reverse primer | /5Biosg/GCAACCATCGTTACGGATTGCTTCACTG |
| | DNA probe | GTGCTGTGTATTTTT/3ThioMC6-D |
| *S. epidermidis* | Amplicon (size: 275 bp) | TTGTGGGTCAAGAAATCATACCGTATCCTGGTAATAGTGATTTAGC AATCTTAAGAGTGTCACCAAACGAACATAATCAACATATTGGTCAA GTAGTTAAACCTGCAACTATAAGTAGCAATACAGACACTAGAATTA ATGAAAACATCACTGTTACTGGTTACCCTGGTGACAAACCATTAGC CACAATGTGGGAAAGTGTAGGTAAAGTTGTCTATATTGGTGGCGA GGAATTAAGATATGACCTAAGTACTGTAGGTGGAAACTCTGGATCT |
| | Forward primer | ACGATGCATG/iSpC3/TTGTGGGTCAAGAAATCATACCGT |
| | Reverse primer | /5Biosg/AGATCCAGAGTTTCCACCTACAGT |
| | DNA probe | CATGCATCGTTTTTT/3ThioMC6-D |

| | | |
|---|---|---|
| *M. smegmatis* | Amplicon (size: 292 bp) | GGCTCGATGTCACTGTCCTTCTCGGATCCGCGCTTCGACGAGGTC AAGGCCTCGGTCGACGAGTGCAAAGACAAGGACATGACGTACGC GGCCCCGCTGTTCGTCACGGCTGAGTTCATCAACAACAACACCGG CGAGATCAAGAGCCAGACGGTCTTCATGGGTGACTTCCCGATGAT GACCGAAAAGGGCACCTTCATCATCAACGGCACCGAGCGCGTCGT GGTGTCGCAGCTGGTCCGTTCGCCTGGCGTGTACTTCGACGAGAC CATCGACAAGTCCACCGAGAAGA |
| | Forward primer | AGTCTGATGGCAGCA/iSpC3/GGCTCGATGTCACTGTCCTTCTC |
| | Reverse primer | /5Biosg/TCTTCTCGGTGGACTTGTCGATG |
| | DNA probe | TGCTGCCATCAGACTTTTTT/3ThioMC6-D |
| *P. mirabilis* | Amplicon (size: 362 bp) | CGAGATGATTTTACGCTTTCCTCAAGGATATGAAACCCAAATTGGT GCTCACGGTGAAGGATTATCCGGCGGACAAAAACAACGTATTGCA TTAGCCAGAGCATTATATGGCGATCCCACGTTAGTTGTCTTAGATG AGCCAAATTCTAATTTAGATGATTTAGGTATTAGAGCGTTAACACA GGCGATCGAAACATTAAAACAGCATAAAAAAACCGTTATTTTAATT ACCCACCAAAAACAGCTACTTTCTGTCACTAATAAATTATTAGTGA TATTTGATGGAAACACCAAATTATTCGGTCCAACGGCGTCTGTTAT TGCTGAATTAAATAATCCGACTATCACAAAAACGGCAAACA |
| | Forward primer | TCTGCACCAATGTAC/iSpC3/CGAGATGATTTTACGCTTTCCTCA |
| | Reverse primer | /5Biosg/TGTTTGCCGTTTTTGTGATAGTCG |
| | DNA probe | GTACATTGGTGCAGATTTTTT/3ThioMC6-D |

Modifications are as indicated. iSpC3: internal modification with C3 spacer, 5Biosg: 5'-biotin modified, ThioMC6-D: 3'-thiol modifier C6 S-S (disulfide). The text in red represents the position of primers, and the text in blue highlights complementary sequence in primers and DNA probe nanotags.

**Table S2.** Reaction mixture for 4-plex PCR

| Reaction mixture | Volume (μL) |
|---|---|
| 5× MyTaq Reaction Buffer | 5 |
| *P. aeruginosa* F (10 μM) | 0.5 |
| *P. aeruginosa* R (10 μM) | 0.5 |
| *S. aureus* F (10 μM) | 0.5 |
| *S. aureus* R (10 μM) | 0.5 |
| *S. epidermidis* F (10 μM) | 0.5 |
| *S. epidermidis* R (10 μM) | 0.5 |
| *M. smegmatis* F (10 μM) | 0.5 |
| *M. smegmatis* R (10 μM) | 0.5 |
| DNA Polymerase (5 U/μL) | 0.3 |
| Input targets gDNA (5 ng/μL) | 2 |
| Nuclease-free water | 13.7 |
| **Total volume** | 25 |

F: Forward primer, R: Reverse primer

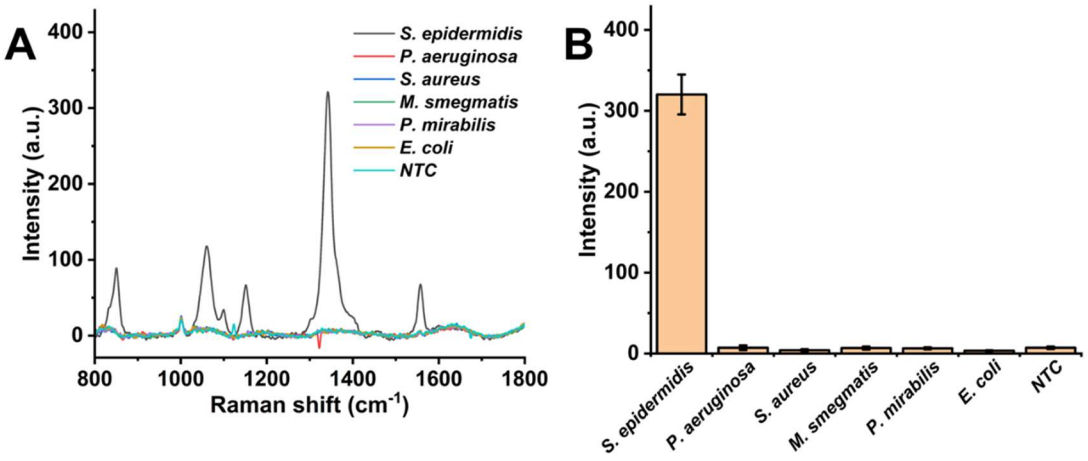


**Fig. S1** Specific identification of *S. epidermidis* gDNA amplicons by SERS nanotags. (A) Typical raw Raman spectra and (B) bar graph of average SERS intensities at 1343 $cm^{-1}$. NTC is the no-template control.

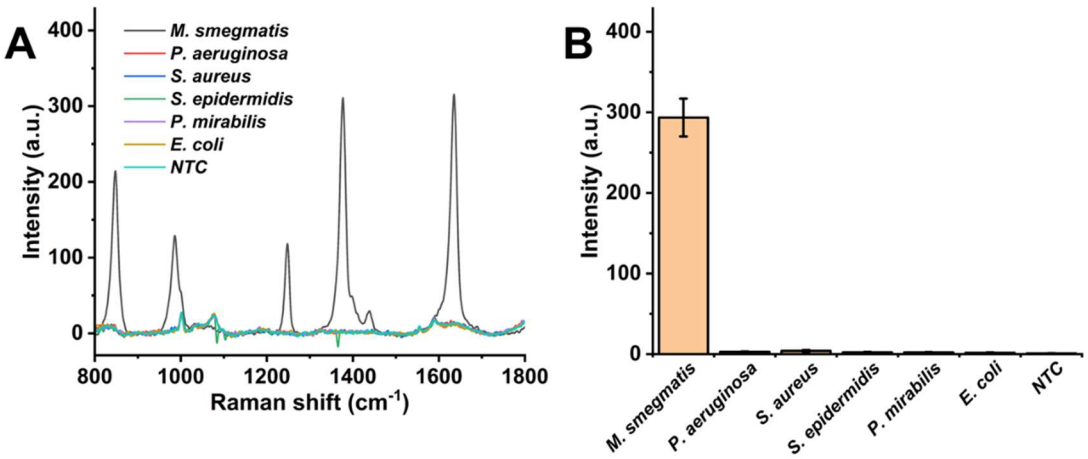


**Fig. S2** Specific identification of *M. smegmatis* gDNA amplicons by SERS nanotags. (A) Typical raw Raman spectra and (B) bar graph of average SERS intensities at 1380 $cm^{-1}$. NTC is the no-template control.