Supplementary Information

Using Surface-Enhanced Raman Spectroscopy and Electrochemical Driven Melting to Discriminate *Yersinia Pestis* from *Y. Pseudotuberculosis* Based on Single Nucleotide Polymorphisms within Unpurified Polymerise Chain Reaction Amplicons.

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Experimental Details

Extraction of DNA. Genomic DNA from *Y. pestis* CO92 and *Y. pseudotuberculosis* NCTC 824 was extracted from overnight cultures using a QIAamp DNA mini-prep kit (Qiagen, UK) according to the manufacturer's instructions.

Generation of template PCR amplicons containing the *groEL* and *metH* SNPs. PCR was performed in 25 µl reaction volumes on a GeneAmp® PCR System 9700 (Applied Biosystems, UK). Reactions contained: 0.2 µM of forward and reverse primer, 1 × PCR buffer without Mg^{2+}, 3 mM $MgCl_2$ (Sigma-Aldrich, UK), 80 mM each dNTP (Roche, UK), 0.04 U µl-1 Taq polymerase (Roche) and 18.4 µl molecular grade dH_2O (Sigma-Aldrich, UK). PCR conditions were 94 ^oC for 5 min; 35 cycles of 95 ^oC for 30 s, 58 ^oC for 30 s and 72 ^oC for 30s; 72 ^oC 7 min. E-gels® (Invitrogen, Life Technologies, UK) were used to visualize amplicons. E-gels® were used according to the manufacturer's instructions. Visualization was done using a Chemi HR410 BioSpectrum® Imaging System (Ultraviolet Products Ltd, UK).

Asymmetric PCR Amplification to generate DNA amplicons labelled with Texas Red. Template DNA was further amplified and labelled with Texas Red using asymmetric PCR. The PCR conditions were optimized for each assay by varying the concentration of the template amplicons, the number of cycles and the concentration of the primers (Figures S1 and S2). The PCR reactions were performed using a thermocycler (Eppendorf Mastercycler Gradient) and they were carried out in 20 µl volumes each containing 10 µl of 2x BioRad SoFast super-mix PCR master-mix and the optimized concentrations of primers and target. The amplification was monitored by adding 0.6 µl of Sybr green (Biorad) to the reaction volume. For the *groEL* SNP assay, 0.005 pg/µl of double stranded DNA amplicon generated

from a standard PCR amplification of the gene fragment from either *Y. pestis* or *Y. pseudotuberculosis* was used as the optimized target concentration. The primer's concentrations were 0.5 μM for the Texas Red labelled forward primer and 0.05 μM for the reverse primer. PCR conditions were 95 °C for 2 min, followed by 40 cycles of 95 °C for 1 s and 55 °C for 1 s. For the *metH* SNP assay, 0.5 pg/μl of the amplicon was used as the optimized target concentration and the primer concentrations were 1 μM for the Texas Red labelled forward primer and 0.1 μM for the reverse primer. PCR conditions were 95 °C for 2 min, followed by 45 cycles of 95 °C for 1 s and 55 °C for 1 s.

UV Melting Studies. UV melting experiments were performed on a Varian Cary 400 Scan UV-visible spectrophotometer. All oligonucleotides were prepared in 10 mM Tris buffer (pH 7.2) containing 1 M NaCl. The probe and target was mixed in a 1:1 ratio and filtered into Hellma SUPRASIL synthetic quartz cuvettes using Kinesis regenerated cellulose 13 mm, 0.45 μm filters. The duplexes were initially denatured by heating to 85 °C at 10 °C/min and then cooled to 18 °C at a slow rate of 1 °C/min. The temperature was maintained at 18 °C for 2 min before starting the melting experiment by heating to 85 °C at 1.0 °C/min, holding at 85 °C for 2 min then cooling to 18 °C at 1.0 °C/min. Eight melting curves were measured by following the change in absorbance at 260 nm. Tm values were calculated from the first derivatives using Cary Win UV thermal application software. The reported melting temperatures are an average of the eight melting curves measured.

Preparation of Sphere Segment Void (SSV) Substrates. A gold-chrome coated microscope slide was prepared by thermal vapor deposition of a 10 nm chromium adhesion layer followed by approximately 200 nm of gold onto a standard glass microscope slide. A monolayer template of 600 nm of polystyrene spheres (Fisher Scientific as a 1 % wt aqueous suspension) was formed at the surface using a convective assembly method.42 Gold was deposited through the template to a height of 480 nm at -0.72 V vs. SCE from commercial gold plating solution (ECF 60, Metalor) containing 100 μL brightener (E3, Metalor) in 20 ml of plating solution. After deposition the polystyrene spheres were removed by immersion in DMF (Rathburn, HPLC) for thirty minutes, and the substrates were rinsed in deionized water before immediate use. An SEM picture of the SSV substrate is shown if Figure S4.

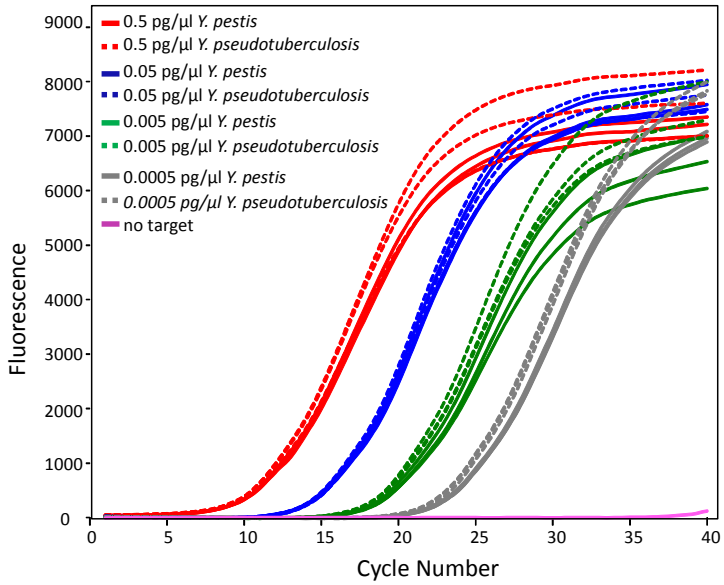

Fig. S1. Amplification curves for different concentrations of the *Y. pestis* and *Y. pseudotuberculosis* amplicons of the *groEL* assay as marked in the figure. PCR was performed over a wide concentration range of the *Y. pestis* and *Y. pseudotuberculosis* amplicons, from 0.5 pg/µl to 0.0005 pg/µl.

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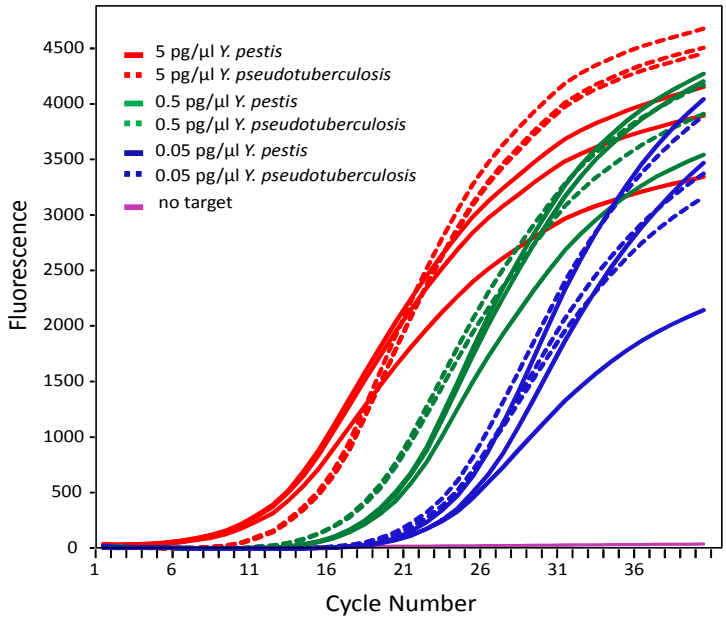

Fig. S2. Amplification curves for different concentrations of the *Y. pestis* and *Y. pseudotuberculosis* amplicons of the *metH* assay as marked in the figure. PCR was performed over a concentration range of the amplicons, 5 pg/µl to 0.05 pg/µl.

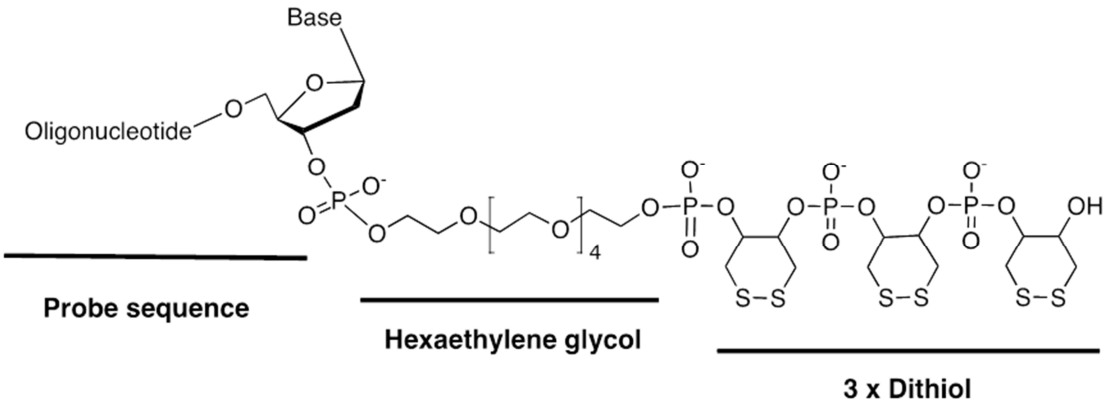

Fig. S3. Structure of disulphide linker attached at the 3’ end of the capture probes.

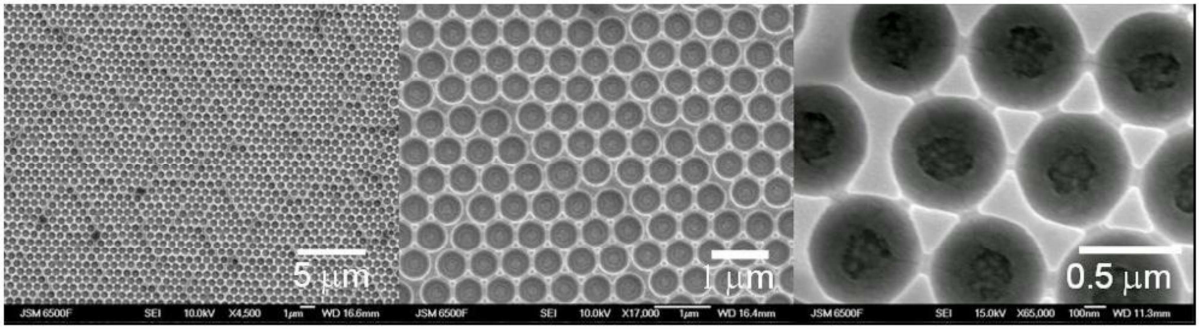

Fig. S4. SEM pictures of a 0.8*D* (*D* = 600 nm) SSV substrate at different resolutions