Supplementary Information

Investigation of the signaling mechanism and verification of the performance of an electrochemical real-time PCR system based on the interaction of methylene blue with DNA

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Genomic DNA isolation. A 30 mL sample of a urine specimen, collected from a patient infected by *Chlamydia trachomatis* after prostatic massage, was centrifuged at 14,000 ×g for 2 min. The centrifuged pellet was then washed twice with 1 mL of PBS (0.2 M phosphate, 1.5 M sodium chloride, pH 7.4) and resuspended in 400 μL of PBS. Genomic DNA was extracted by using an Accuprep^{TM} Genomic DNA Extraction Kit (Bioneer, Korea) according to the manufacturer's protocol and was stored at -20°C until use.

Preparation of template DNA. Amplification of the specific sites of *Chlamydia trachomatis* gene encoding virulence proteins was performed on a PCT-0200 (Bio-rad, Hercules, CA) thermo cycler in a 50 μL solution containing 1 μL of template, 0.25 μM of each primer (forward: 5'-TTAATG GAAAAGTTGTTTCA-3' and reverse: 5'-TCCATATCTTTGATACGACG-3'), 5 μL of 10× PCR reaction buffer (500 mM Tris-HCl, 100 mM KCl, 50 mM $(NH_4)_2SO_4$, 20 mM $MgCl_2$), 0.2 mM dNTPs, and 1.25 U FastStart Taq DNA polymerase. PCR was programmed for 4 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 30 s at 55°C, and 1 min at 72°C, and finalized with 7 min at 72 °C. The resulting 1800 bp-long PCR product after the purification (NucleoSpin^{TM}, Macherey-Nagel, Duren, Germany) was used as a template in this work.

Preparation of DNA sample by secondary PCR. dsDNA was obtained from the secondary PCR of the template employing the same procedure described above but different primers (forward: 5'-CCATCTTCTTTGAAGCGTTGT and reverse: 5'-ACAGGATGACTCAAGGAATAG). The resulting 612 bp-long dsDNA was used in fluorescent or electrochemical titration experiments.

Conventional real-time PCR with TaqMan probe. Conventional real-time PCR amplification was carried out in a microfuge tube containing 10 μL of two-fold master mix buffer containing DNA polymerase and dNTPs (FINNZYMES, Finland), 0.25 μM of each primer (forward: 5'–AGGCGTTTGTACTCCGTCAC–3' and reverse: 5'–TGGTGGGGTTAAGGCAAATCG–3'), 0.25 μM of TaqMan probe (5'-/FAM/CCGCACGTTCTCTCAAGCAGGACTACA/BHQ_1) and deionized water in a total volume of 20 μl with C1000 thermal cycler in the combination with CFX96 real-time system (Bio-rad). After denaturing at 95 °C for 15 min, the reaction was carried out during 40 cycles at 95 °C for 15 s and 60 °C for 1 min. The florescent signal was recorded after every extension step.

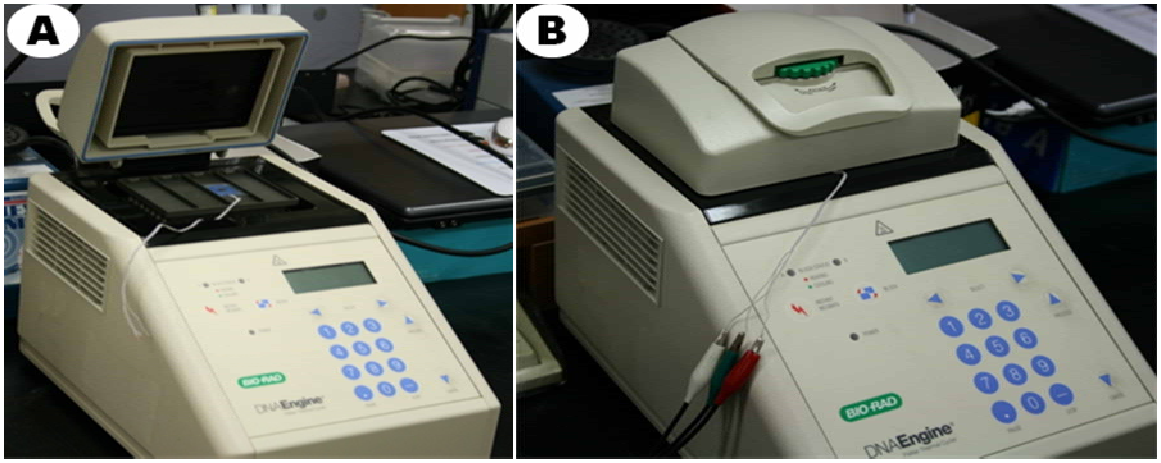

Figure S1. Pictures of the PCR device for the electrochemical real-time PCR. (A) PCR chip in thermocycler and (B) Link with electrochemical analyzer.