

Electronic Supplementary Information for

Direct detection of unamplified genomic DNA based on photo-induced silver ion reduction by DNA molecules

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Experimental Section

Materials: Silver nitrate (AgNO_3) was purchased from Sigma-Aldrich. DNA oligonucleotides were synthesized and HPLC-purified by Genotech Co. (Daejeon, Korea). Streptavidin-coated magnetic beads (2.8 μm diameter) were purchased from Invitrogen (Dynabeads[®] M-280 Streptavidin, CA, USA). Biotinylated rabbit polyclonal antibodies of *Salmonella typhimurium* were purchased from Abcam (Cambridge, UK). *S.typhimurium* and *Escherichia coli* cells were obtained from Korean Collection for Type Cultures (KCTC, Daejeon, Korea) and were grown overnight in 5 mL LB broth at 37 °C with shaking at 200 rpm.

Magnetic separation of *S.typhimurium* cell: Biotinylated anti-*S.typhimurium* antibodies (Ab) were conjugated to streptavidin-coated magnetic beads using the following procedure. Magnetic beads (3 mg, *ca.* 2×10^8) was washed and resuspended in 300 μL of PBS buffer (pH 7.4). Ab (60 μg) was added to the bead solution and the mixture was incubated for 1 h at room temperature with gentle rotation. The Ab-conjugated beads were then blocked by using PBS containing 1% BSA and stored at 2-8 °C prior to use.

The suspension of equal concentrations (*ca.* 10^8 CFU) of cultured *E.coli* and *S.typhimurium* cells with a volume of 340 μL was added to 360 μL of the anti-*S.typhimurium* Ab-conjugated magnetic beads. After allowing the bacteria to bind to the beads for 1 h, the bead-bacteria complexes were separated by using a magnet and washed 3 times with PBS to remove unbound cells and other contaminants. The bead-bacteria complexes were suspended in 100 μL of dissociation buffer and incubated at room temperature for 5 min to dissociate the bound cells from the beads. Finally, the bead vial was placed on a magnet for 2 min and the supernatant containing the isolated bacteria was subjected to genomic DNA extraction and PCR amplification.

Genomic DNA extraction and PCR identification of the isolated bacteria: Genomic DNA was extracted from the isolated bacteria by using a bacterial genomic DNA extraction kit (iNtRON Biotechnology, Korea) according to the manufacturer's protocol. Briefly, cultured bacterial cells,

harvested by centrifugation at 13,000 rpm for 1 min, were mixed with lysis buffer containing RNase A and Proteinase K and incubated at 65 °C for 15 min for complete cell lysis. The cell lysates were then loaded on column and centrifuged at 13,000 rpm for 1 min followed by two washings with washing buffer. Finally, the genomic DNA bound to column was eluted in distilled water. After extraction of genomic DNA, the purity of DNA was assessed by measuring the ratios of absorbance at 260 nm and 280 nm (A_{260}/A_{280}) and 260 nm and 230 nm (A_{260}/A_{230}). The ratios of our sample were determined to be 1.87 for A_{260}/A_{280} and 2.18 for A_{260}/A_{230} which show the high purity of the extracted genomic DNA used in the assay.¹

PCR was performed with the extracted genomic DNA by using species-specific PCR primers to confirm the isolation of target *S.typhimurium* from (*S.typhimurium* + *E.coli*) cell mixture. A 314 bp PCR product was amplified for the *ipaB* gene of *S.typhimurium* encoding the invasion plasmid antigen B (Forward primer: 5'-GGACTTTTAAAAGCGGCGG-3', Reverse primer: 5'-GCCTCTCCCAGAGCCGTCTGG-3').² Amplification for the *uidA* gene of *E.coli* encoding β -D-glucuronidase (Forward primer: 5'-AAAACGGCAAGAAAAAGCAG-3', Reverse primer: 5'-ACGCGTGGTTACAGTCTT-GCG-3' for 147 bp amplicon)³ was also performed to examine the presence of non-target bacteria (*E.coli*) after the magnetic separation. PCR amplification was carried out on a DNA engine-Peltier thermo cycler (Bio-Rad, Hercules, CA) in a 50 μ L solution containing 1 μ L of template, 0.25 μ M of each primer, 1X PCR reaction buffer (50 mM Tris-HCl, 10 mM KCl, 5 mM $(\text{NH}_4)_2\text{SO}_4$, 2 mM MgCl_2), 0.2 mM dNTPs, and 1.25 U FastStart *Taq* DNA polymerase (Roche, Germany). PCR was programmed for 5 min at 95 °C, followed by 40 cycles of 30 s at 95 °C, 45 s at 62 °C, and 1 min at 72 °C, and then a final extension at 72 °C for 7 min. The amplified products were electrophoresed on 2% agarose gel in 1X TBE buffer (Fig. S4).

For the experiment to detect the PCR product, *S.typhimurium* PCR products obtained from the above procedures were purified with NucleoSpin™ (Macherey-Nagel, Duren, Germany) and then subjected to the colorimetric assay. The concentrations of PCR products were determined by using a

Nanodrop[®] ND-1000 (Wilmington, DE) spectrophotometer based on the generally accepted extinction coefficient of double-stranded DNA (50 ng·cm/μL).

Photoinduced Ag⁺ reduction: AgNO₃ was added to solutions containing PCR product and genomic DNA and mixed thoroughly. The final concentration of AgNO₃ was 1.5 mM. Photoinduced reduction was performed by placing the solutions under UV light (λ=254 nm, by Stratalinker[®] UV Crosslinker (Stratagene, USA)) for 20 min. UV–vis absorption spectra were recorded after irradiation by using an Infinite[®] 200 PRO microplate reader (Tecan Group Ltd., Switzerland).

TEM analysis: Field emission transmission electron microscopy (Tecnai, FEI, Netherlands), operated at an accelerated voltage of 300 kV, was employed to characterize the detailed structure of the synthesized silver particles. Samples were prepared by applying solutions to a carbon-coated copper TEM grid followed by drying at room temperature. The EDS spectrum was measured using an element analyzer (FlashEA 1112, Thermo Finnigan, Italy) connected to the microscope.

Investigation of the effects of AgNO₃ concentration and UV irradiation time on Ag⁺ reduction mediated by DNA molecules

The effects of Ag⁺ concentration and UV irradiation time on color change of DNA solution resulting from Ag⁺ reduction were examined. The absorption intensities were measured at 420 nm after reduction of solutions containing varying Ag⁺ concentrations and varying UV irradiation times (Fig. S1). The absorption intensities increase as the concentration of AgNO₃ increases at fixed DNA concentration (2.5 μM of 15 bp) and UV irradiation time (20 min) (Fig. S1a). In the same manner, absorbance increases as UV irradiation time increases at fixed DNA (2.5 μM of 15 bp) and AgNO₃ (1.5 mM) concentrations (Fig. S1b). Based on the results, the conditions of 1.5 mM AgNO₃ and 20 min UV irradiation were employed for DNA detection experiments in this study.

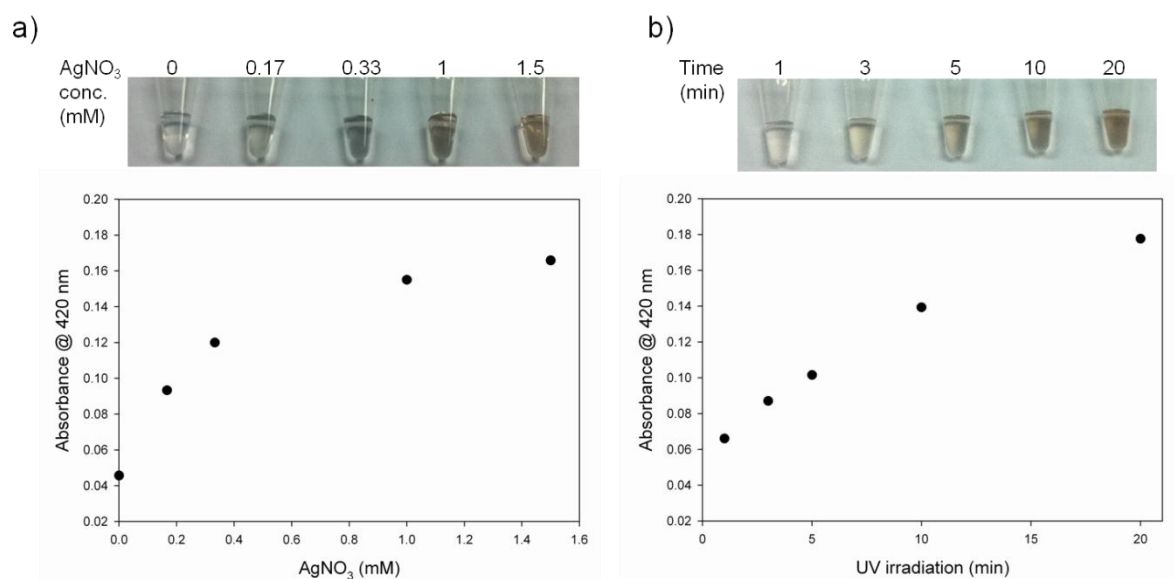


Fig. S1 Absorption intensities at 420 nm after photo-reduction of DNA solution (2.5 μ M of 15 bp) at varying concentrations of AgNO₃ (a) and UV irradiation times (b).

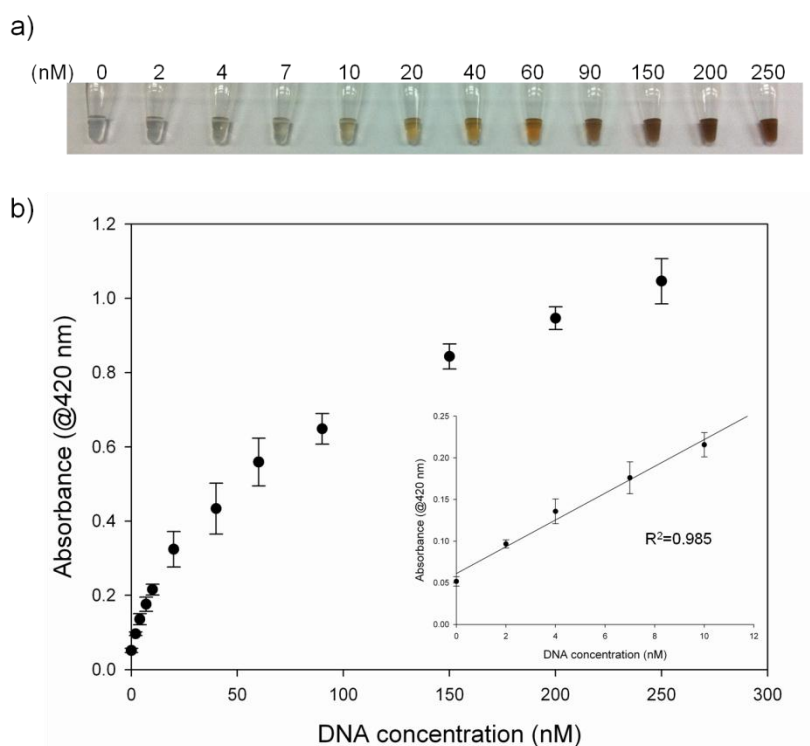


Fig. S2 a) Colorimetric responses of solutions containing the PCR product of *S.typhimurium* and AgNO₃ and after photoirradiation. b) Calibration curve of absorbance at 420 nm. Data are averages of three independent experiments.

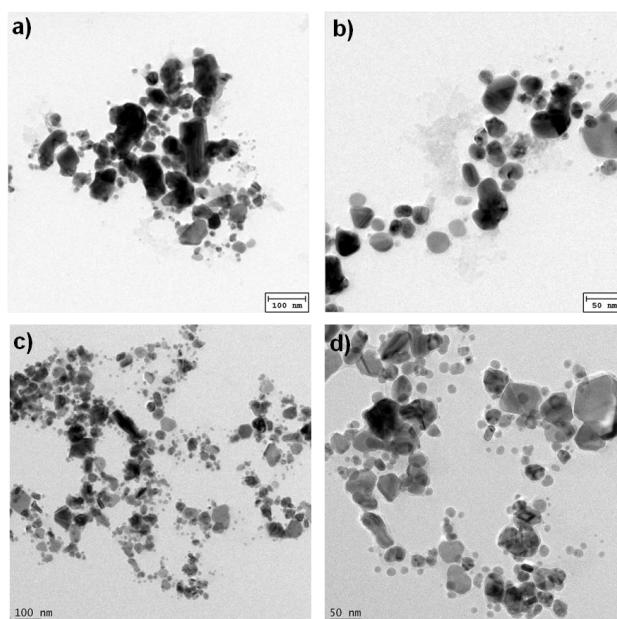


Fig. S3 TEM images of AgNPs synthesized from PCR product (a, b) and genomic DNA (c, d) of *S.typhimurium*.

PCR-based identification of target bacteria separated by using anti-*S.typhimurium* antibody-coated magnetic beads

Immunomagnetic separation method has been shown to be efficient for purification as well as concentration of targets in a wide range of analytes and sample types.⁴ It has also been reported that this procedure can be employed to separate selectively target bacterium from other, nontarget microorganisms in heterogeneous sample matrices.⁵ In our assay, specific capture of the target pathogen, *S.typhimurium* by using a magnetic separation process was confirmed by analysis of the PCR product obtained using the target-specific PCR primers. Lanes 1 and 2 in Fig. S4 show PCR products amplified from the genomic DNA of bead-captured bacteria using *E.coli*- and *Salmonella*-specific primers, respectively. Only the PCR product corresponding to *Salmonella* was observed (lane 2) while no PCR product was obtained from *E.coli*-specific primer (lane 1). In order to further verify the specificity of the primers, each primer was tested by performing PCR for *E.coli* and

Salmonella genomic DNA using their corresponding primers (lane 3 and 4; positive controls). Lanes 5 and 6 show negative controls amplified from *E.coli* genomic DNA using *Salmonella* specific primer and from *Salmonella* genomic DNA using *E.coli* specific primer. PCR template and primer set employed in each lane was summarized in Table S1. These analyses indicated that the target nucleic acids was obtained only when both the target bacteria and the target-specific primers were employed. From the above results, we could confirm that target bacteria were successfully isolated by the magnetic separation process.

Table S1. The PCR template and primer set employed in each PCR process shown in Fig. S4.

Lane	Template	Primer specific for
1	Genomic DNA from bead-captured bacteria	<i>E.coli</i>
2	Genomic DNA from bead-captured bacteria	<i>Salmonella</i>
3	<i>E.coli</i> genomic DNA	<i>E.coli</i>
4	<i>Salmonella</i> genomic DNA	<i>Salmonella</i>
5	<i>E.coli</i> genomic DNA	<i>Salmonella</i>
6	<i>Salmonella</i> genomic DNA	<i>E.coli</i>

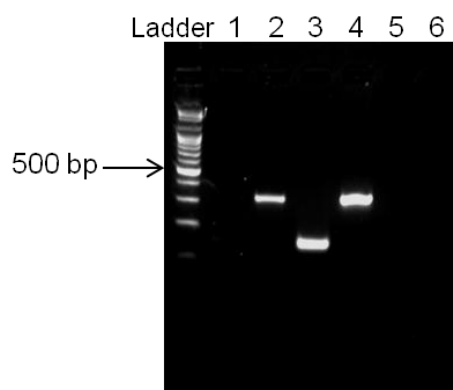


Fig. S4 Gel electrophoresis analyses of PCR products obtained from the PCR template and primer set as summarized in Table S1.

A control experiment to examine the specificity of the assay using bovine serum albumin (BSA)

In the new detection system, genomic DNA extracted from target bacterial cells may contain protein contaminants as a result of incomplete cell lysis. To determine if the presence of protein contaminants has an effect on the accuracy of the assay, a control experiment for photoinduced Ag^+ reduction was carried out using bovine serum albumin (BSA) as a model protein contaminant. The results show that UV irradiation in the presence of 10 ng/ μL BSA does not result in any observable color change or the generation of the absorption peak indicating the synthesis of AgNPs (Fig. S5). In contrast, the presence of 10 ng/ μL *S.typhimurium* genomic DNA led to generation of an absorption peak at *ca.* 420 nm indicating the formation of AgNPs, as described in the manuscript. These results clearly demonstrate that DNA specifically facilitates the reduction of Ag^+ to form AgNPs by absorbing 254 nm UV light more efficiently than protein. Finally, we conclude that the most probable protein contamination that might be encountered in the assay procedures does not diminish the detection reliability.

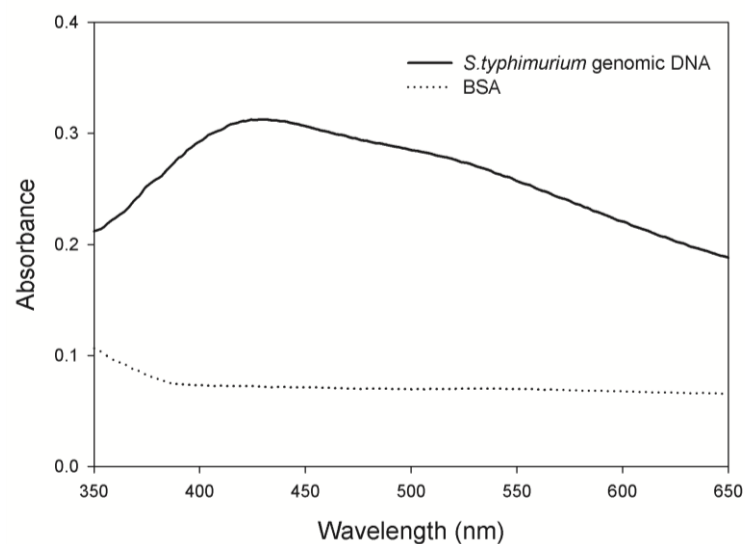


Fig. S5 UV-vis absorption spectra of solutions containing 10 ng/ μL of *S.typhimurium* genomic DNA (solid line) and BSA (dotted line) after photoinduced Ag^+ reduction.

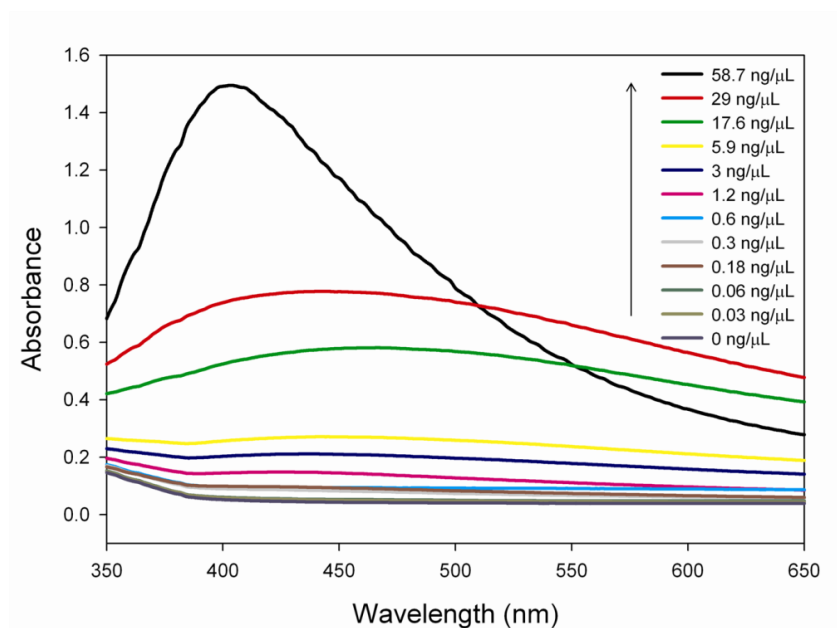


Fig. S6 UV-vis absorption spectra of solutions containing *S.typhimurium* genomic DNA after photoinduced Ag^+ reduction.

References

- 1 <http://www.nanodrop.com/Library/T042-NanoDrop-Spectrophotometers-Nucleic-Acid-Purity-Ratios.pdf>
- 2 M. N. Momba, V. K. Malakate, J. Theron, *J. Water Health* 2006, **4**, 289.
- 3 Y. L. Tsai, C. J. Palmer, L. R. Sangermano, *Appl. Environ. Microbiol.* 1993, **59**, 353.
- 4 O. Olsvik, T. Popovic, E. Skjerve, K. S. Cudjoe, E. Hornes, J. Ugelstad and M. Uhlen, *Clin. Microbiol. Rev.* 1994, **7**, 43.
- 5 I. Safarik and M. Safarikova, *J. Chromatogr. B* 1999, **722**, 33.