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Electronic Supplementary Information

Target DNA induced switches of DNA polymerase activity

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Experimental Section

Materials. All DNA oligonucleotides used in the present study were synthesized by Genotech Co. (Daejeon, Korea) and were purified by desalting, except for template DNA (purified by PAGE) and FAM and BHQ1 labeled TaqMan probe (purified by HPLC). The sequences of oligonucleotides are listed in Table S1. Ultrapure DNase/RNase-free distilled water was purchased from Bioneer® (Daejeon, Korea).[1] *Thermus aquaticus* DNA polymerase (Taq DNA polymerase) was purchased from NEB (New England Biolabs). The concentration of Taq DNA polymerase is 550 nM. All other chemicals were of analytical grade and used without further purification.[2]

General reaction conditions. The reaction mixtures were separately prepared as part A and part B. Part A (total volume of 20 μL), composed of 1X Taq reaction buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 4 mM $MgCl_2$), 500 μM dNTPs, 400 nM DNA aptamer, and complementary target DNA at varying concentrations, was heated at 90 °C for 5 min, cooled slowly to 25 °C (0.1 °C /s) and incubated at 25 °C for 30 min. Taq DNA polymerase (11 nM) was then added to each solution and incubated for 20 min. Part B (total volume of 20 μL), composed of 1X Taq reaction buffer, 600 nM template, 600 nM primer, and 500 nM TaqMan probe, was heated at 90 °C for 5 min, cooled slowly to 25 °C (0.1 °C /s) and incubated at 25 °C for 60 min. Part A and B were mixed and the fluorescence signal was measured on a C1000™ thermal cycler (Bio-Rad, CA, USA). The fluorescence signal arising from TaqMan probe during the primer extension reaction was monitored at 2 min intervals at 25 °C.

Melting curve analysis: Solutions (total volume of 40 μL) containing 100 nM DNA aptamer and 100 nM complementary target DNA in a 1X Taq reaction buffer, 250 μM dNTPs, 1X EvaGreen™ (Seoul, Korea) were heated at 90 °C for 5 min, cooled slowly to 25 °C (0.1 °C /s) and incubated at 25 °C for 30 min. The resulting fluorescence signal was measured on a C1000™ thermal cycler (Bio-Rad, CA, USA) as the temperature was increased from 25 °C to 60 °C with an increment of 0.5 °C. The first derivative plot [-d(RFU)/dT] was used to determine the melting temperature.[3]

Electrophoretic band shift experiment: Solutions (total volume of 40 μL) containing 100 nM DNA aptamer and 100 nM complementary target DNA in a 1X Taq reaction buffer were heated at 90 °C for 5 min, cooled slowly to 25 °C (0.1 °C /s) and incubated at 25 °C for 30 min. Taq DNA polymerase (110 nM) was then added to each solution and incubated for 20 min. The solutions were mixed with 6X loading buffer (Bioneer®, Daejeon, Korea) and subjected to electrophoresis analysis on a 15 % precast polyacrylamide gel (Bio-Rad, CA, USA). The analysis was carried out in 1X TBE (89 mM Tris, 89 mM Borate, and 2 mM EDTA, pH 8.3) at 100 V for 80 min. After SYBR gold (Invitrogen, CA, USA) staining, gels were scanned using a UV transilluminator. DNA polymerase is neither fluorescent itself nor stained by the dye.[4]

Polyacrylamide gel electrophoresis (PAGE). Solutions (total volume of 40 μL) were prepared as described under "General reaction conditions" except that TaqMan probe is excluded in Part B. The solutions obtained after the incubation for 200 min were mixed with

6X loading buffer (Bioneer®, Daejeon, Korea) and subjected to electrophoresis analysis on a 15% precast polyacrylamide gel (Bio-Rad, CA, USA). The analysis was carried out in 1X TBE (89 mM Tris, 89 mM Borate, and 2 mM EDTA, pH 8.3) at 120 V for 170 min. After SYBR gold (Invitrogen, CA, USA) staining, gels were scanned using a UV transilluminator.

Scheme 1 Schematic illustration of target DNA induced switching of DNA polymerase activity and monitoring using the TaqMan probe.

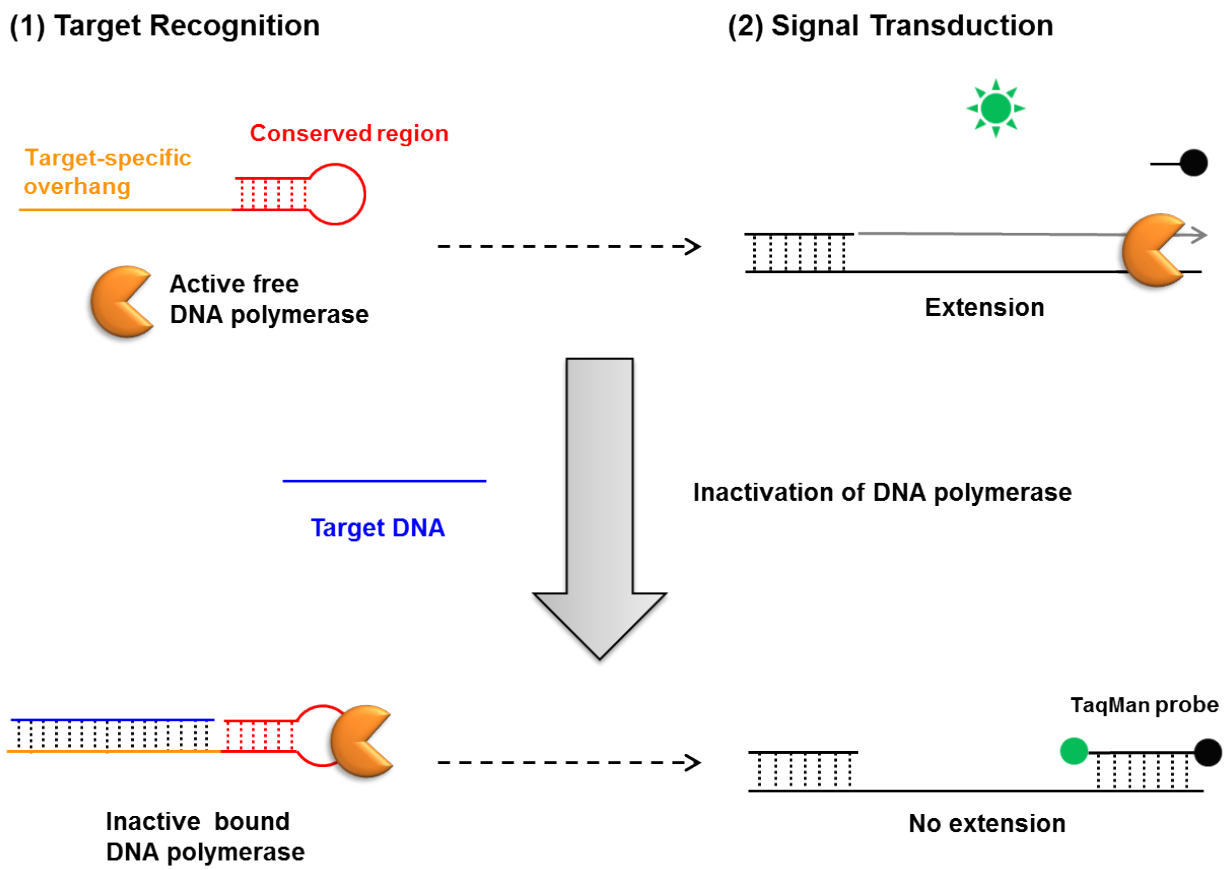
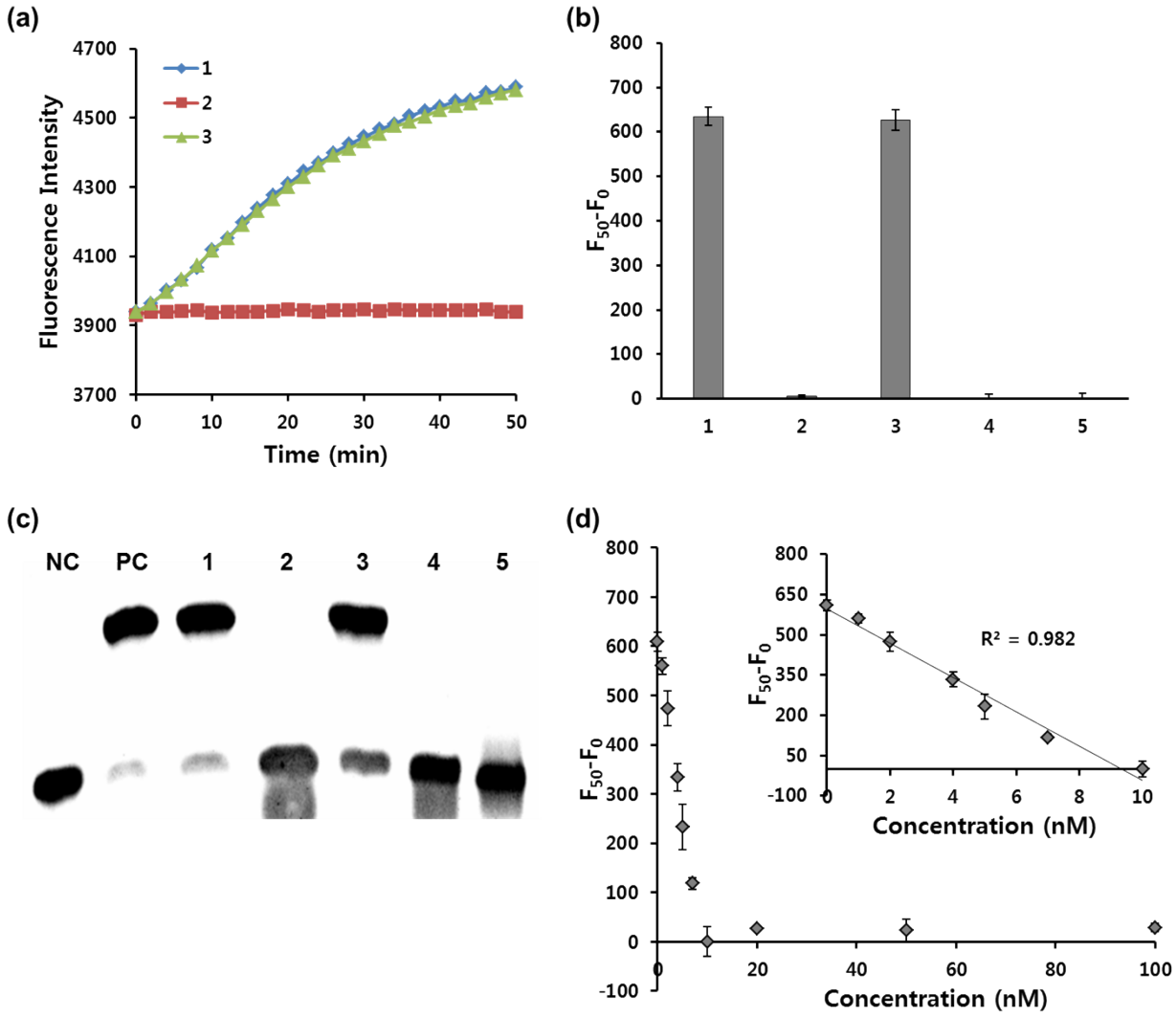


Fig. 1 Detection of target DNAs associated with urea. Fluorescence signal change (F_{50}-F_0), where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively, after introduction of reagents for the primer extension reaction including TaqMan probe. (a) Time dependent fluorescence intensities from TaqMan probe during primer extension reactions. (b) Fluorescence signal change (F_{50}-F_0) (c) Polyacrylamide gel electrophoresis image. Negative control (NC) and positive control (PC) are samples in the absence and presence of DNA polymerase, respectively. Free urea-specific DNA aptamer (1), urea-specific DNA aptamer in the presence of 25-nt (2), 35-nt (4), or 45-nt (5) complementary urea target DNA, or non-complementary chlamydia target DNA (3) was additionally applied to the solutions containing DNA polymerase. (d) Fluorescence signal change (F_{50}-F_0) in the presence of urea target DNA at varying concentrations. Inset in (d): Linear range between (F_{50}-F_0) and urea target DNA concentration (0-10 nM). Final concentrations of DNA polymerase and DNA aptamer are 5.5 nM and 200 nM, respectively.


(a)
Fluorescence Intensity
Time (min)
1
2
3
(b)
F50-F0
(c)
NC
PC
1
2
3
4
5
(d)
F50-F0
Concentration (nM)
R² = 0.982

Scheme 2 Schematic illustration of target DNA induced switching of DNA polymerase activity and monitoring using TaqMan probe. The switching-on sensing system is created by introducing blocker DNA that specifically recognizes complementary target DNA.

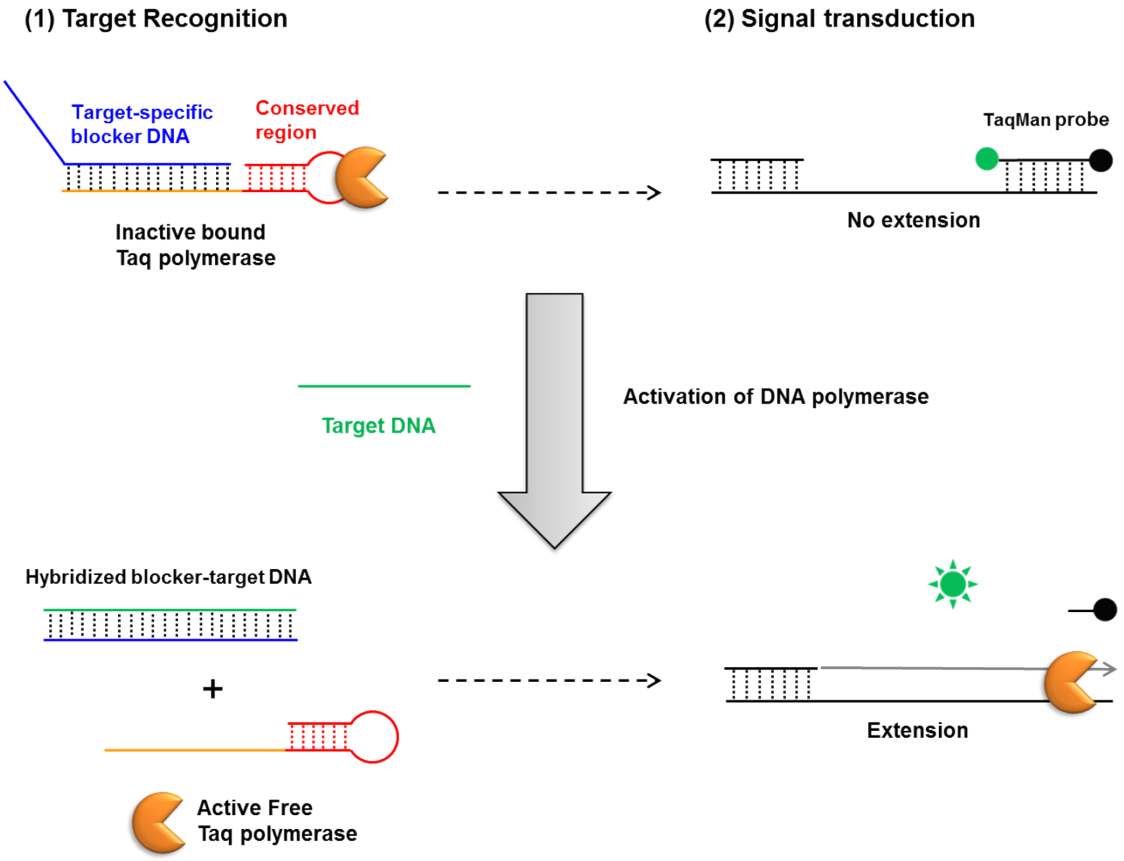


Fig. 2 Detection of target DNAs associated with urea in a switching-on manner. Fluorescence signal change (F_{50}-F_0), where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively, after introduction of reagents for the primer extension reaction including TaqMan probe. (a) Urea-specific blocker DNAs at varying concentrations were additionally applied to the solutions containing DNA polymerase and DNA aptamer. (b) Free DNA aptamer (1), DNA aptamer in the presence of urea-specific blocker DNA (2), or DNA aptamer in the presence of urea-specific blocker DNA supplied with complementary urea target DNA (3) or non-complementary chlamydia DNA (4) was additionally applied to the solutions containing DNA polymerase. (c) Time-dependent fluorescence intensities from TaqMan probe during primer extension reactions. Urea target DNAs at varying concentrations were applied to the solutions containing DNA polymerase and DNA aptamer hybridized with urea-specific blocker DNA. (d) Fluorescence signal change (F_{50}-F_0) in the presence of urea target DNA at varying concentrations. Inset in (d): Linear range between (F_{50}-F_0) and urea target DNA concentration (0-20 nM). Final concentrations of DNA polymerase, DNA aptamer, and urea-specific blocker DNA are 5.5 nM, 200 nM, and 20 nM, respectively.

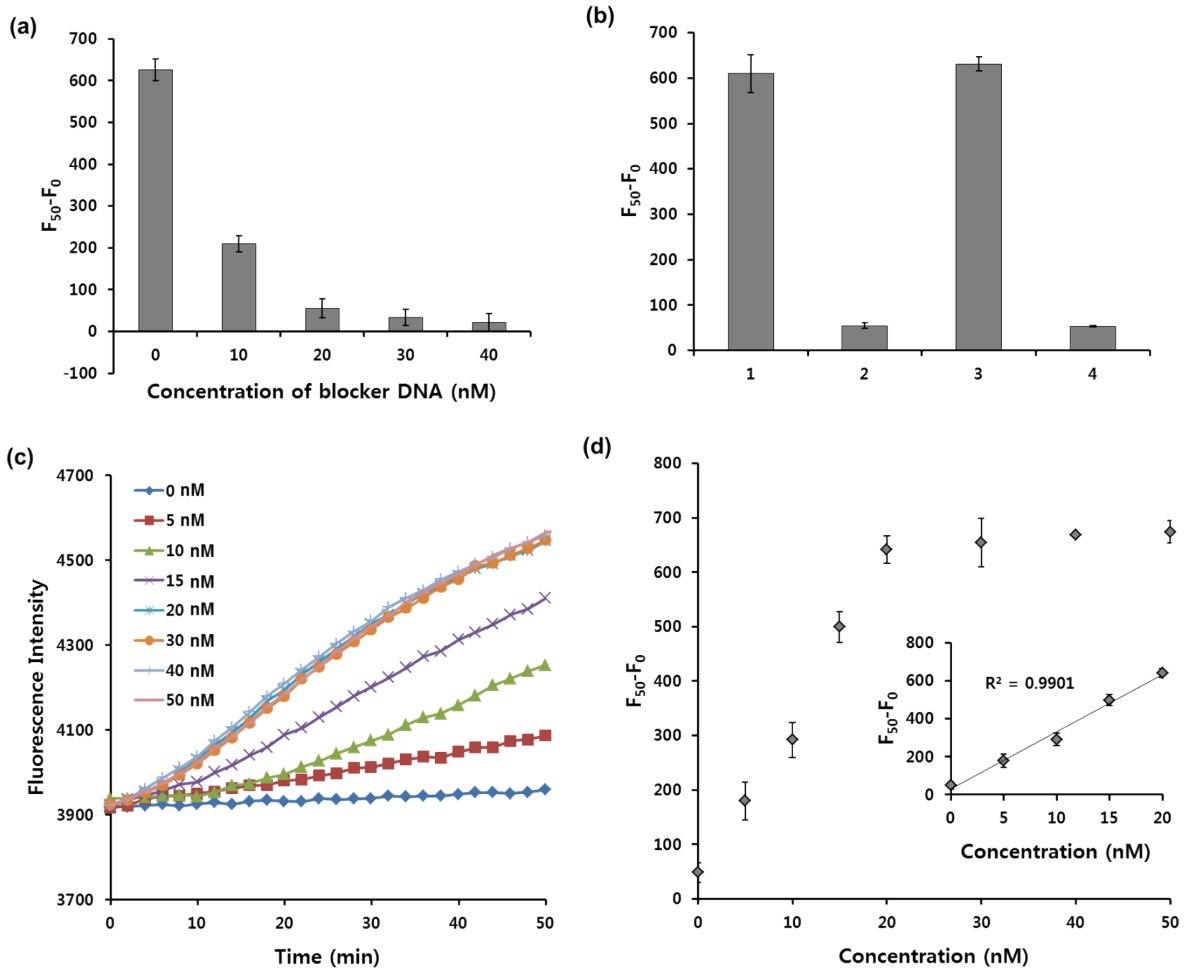
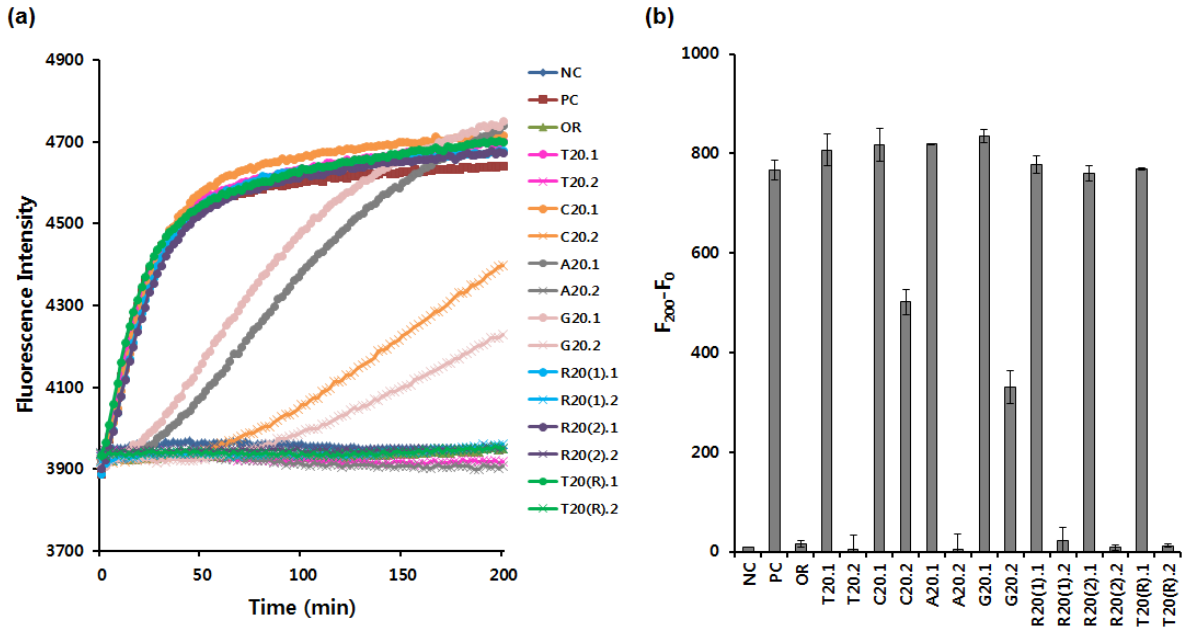

(a)
700
600
500
400
300
200
100
0
-100
F_{50}-F_0
0
10
20
30
40
Concentration of blocker DNA (nM)
(b)
700
600
500
400
300
200
100
0
F_{50}-F_0
1
2
3
4
(c)
4700
4500
4300
4100
3900
3700
Fluorescence Intensity
0 nM
5 nM
10 nM
15 nM
20 nM
30 nM
40 nM
50 nM
0
10
20
30
40
50
Time (min)
(d)
800
700
600
500
400
300
200
100
0
F_{50}-F_0
0
10
20
30
40
50
Concentration (nM)
R^2 = 0.9901
0
5
10
15
20
Concentration (nM)

Table S1 DNA sequences employed in this study.

Strand name	**DNA sequence (5'→ 3')**
Template DNA	CAGA AATC TCAG GGAC TCTA AAGC TCAA CTTG CATA AACT TCTG AGGA
Primer DNA	TCCT CAGA AGTT TATG CA
TaqMan probe	FAM-TAGA GTCC CTGA GATT TCTG-BHQ1
Original DNA aptamer (OR)	TTCT CGGT TGGT CTCT GGCG GAGC AAGA CCAG ACAA TGTA CAGT ATTG GCCT GATC TTGT GTAT GATT CGCT TTTC CC
T8 aptamer (T8)	TTTT TTTT CAAT GTAC AGTA TTG
A8 target	AAAA AAAA
T12 aptamer (T12)	TTTT TTTT TTTT CAAT GTAC AGTA TTG
A12 target	AAAA AAAA AAAA
T16 aptamer (T16)	TTTT TTTT TTTT TTTT CAAT GTAC AGTA TTG
A16 target	AAAA AAAA AAAA AAAA
T20 aptamer (T20)	TTTT TTTT TTTT TTTT TTTT CAAT GTAC AGTA TTG
A20 target	AAAA AAAA AAAA AAAA AAAA
C20 aptamer (C20)	CCCC CCCC CCCC CCCC CCCC CAAT GTAC AGTA TTG
G20 target	GGGG GGGG GGGG GGGG GGGG
A20 aptamer (A20)	AAAA AAAA AAAA AAAA AAAA CAAT GTAC AGTA TTG
T20 target	TTTT TTTT TTTT TTTT TTTT
G20 aptamer (G20)	GGGG GGGG GGGG GGGG GGGG CAAT GTAC AGTA TTG
C20 target	CCCC CCCC CCCC CCCC CCCC
Random20(1) aptamer (R20(1))	AGTC AGTC AGTC AGTC AGTC CAAT GTAC AGTA TTG
Random20(1) target	GACT GACT GACT GACT GACT
Random20(2) aptamer	ACTG ACTG ACTG ACTG ACTG CAAT GTAC AGTA TTG

(R20(2))	
Random20(2) target	CAGT CAGT CAGT CAGT CAGT
T20 aptamer (Reverse) (T20(R))	CAAT GTAC AGTA TTGT TTTT TTTT TTTT TTTT TTT
Signal-off sensing system	
Urea-specific aptamer	TAGG ACGG TCAC CAGT ATTT TTAA TCAA TGTA CAGT ATTG
25-nt urea target	ATTA AAAA TACT GGTG ACCG TCCT A
35-nt urea target	CCAT CATT AAAA ATAC TGGT GACC GTCC TAGG AGA
45-nt urea target	ATAT TCCA TCAT TAAA AATA CTGG TGAC CGTC CTAG GAGA CAAA A
Chlamydia-specific aptamer	TACA AGCT GCAA TCCC TTTT AAGA TCAA TGTA CAGT ATTG
25-nt chlamydia target	ATCT TAAA AGGG ATTG CAGC TTGT A
35-nt chlamydia target	GGGT TATC TTAA AAGG GATT GCAG CTTG TAGT CCT
45-nt chlamydia target	GTGC GGGG TTAT CTTA AAAG GGAT TGCA GCTT GTAG TCCT GCTT G
Signal-on sensing system	
Urea-specific aptamer	AAAT ACTG GTGA CCGT CCTA CAAT GTAC AGTA TTG
Urea-specific blocker	TAGG ACGG TCAC CAGT ATTT TTAA TGCT GATT ACTT TTGC
Urea target	GCAA AAGT AATC AGCA TTAA AAAT ACTG GTGA CCGT CCTA
Chlamydia target	AAAA GGGA TTGC AGCT TGTA GTCC TGCT TGAG AGAA CGTG
The conserved region is indicated in red color.	

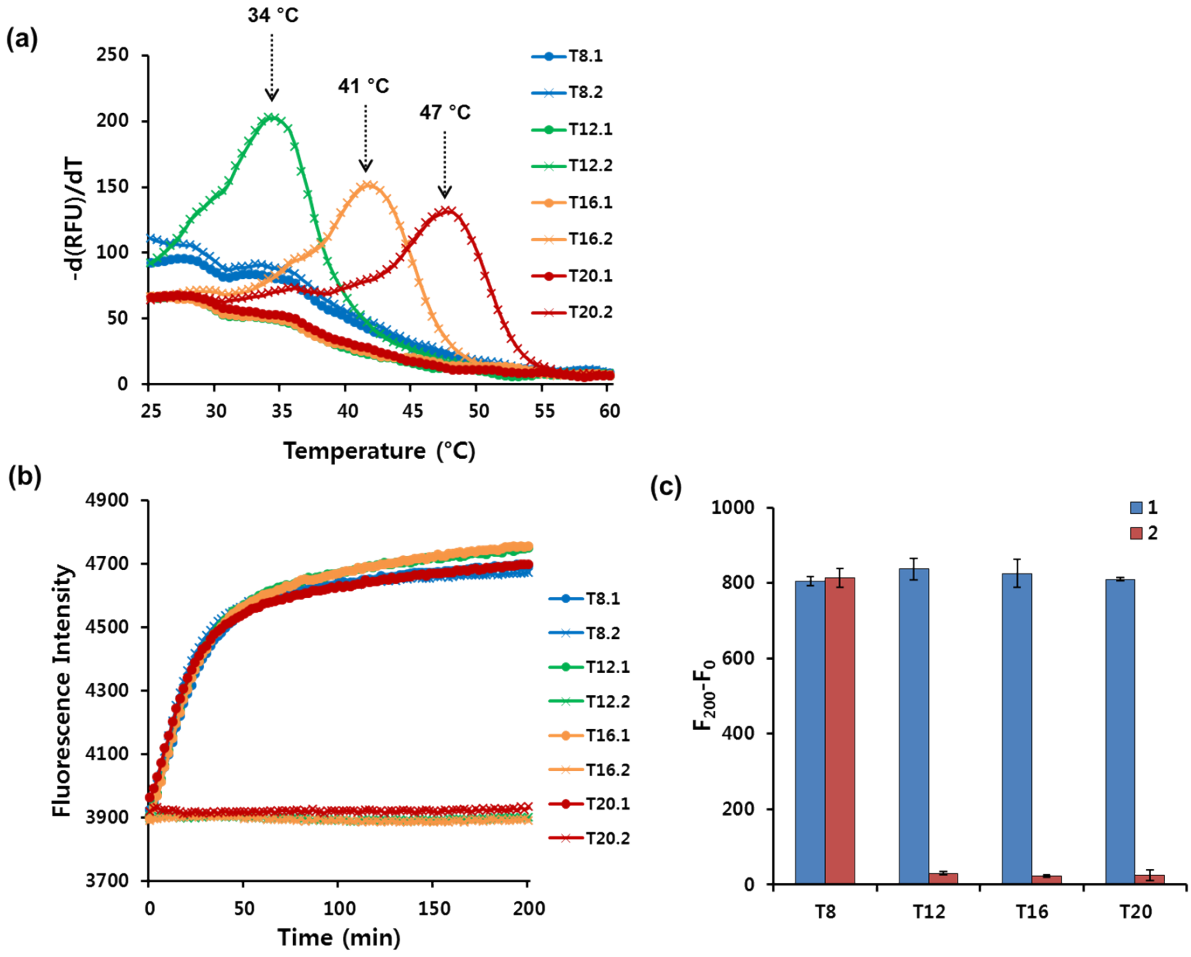
Fig. S1 Feasibility study for target DNA induced switching of DNA polymerase activity. (a) Time dependent fluorescence intensities from TaqMan probe during primer extension reactions. (b) Fluorescence signal change (F_{200}-F_0), where F_{200} and F_0 are the fluorescence intensities from TaqMan probe measured at 200 min and 0 min, respectively, after introduction of reagents for the primer extension reaction including TaqMan probe. Negative control (NC) and positive control (PC) are samples in the absence and presence of DNA polymerase, respectively. Original DNA aptamer (OR) or overhang sequence-containing DNA aptamers (T20, C20, A20, G20, R20(1), R20(2), and T20(R)) in the absence (1) and presence (2) of complementary target DNA was additionally applied to the solutions containing DNA polymerase. Final concentrations of DNA polymerase, DNA aptamer, and target DNA are 5.5 nM, 100 nM, and 100 nM, respectively.



Two DNA aptamers (A20 and G20) containing the overhang sequence composed of homopurine nucleobase (homoadenine or homoguanine) promote formation of fluorescence signals with slightly reduced intensities even in the absence of the corresponding target

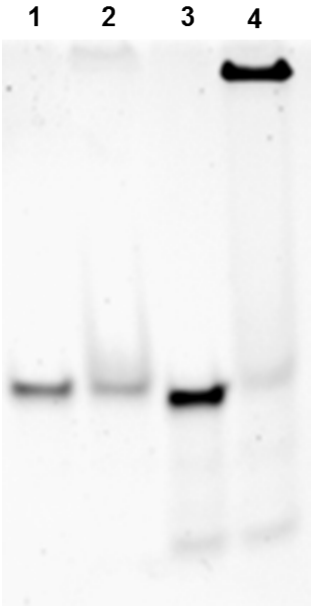
DNAs (A20.1 and G20.1, Fig. S1). This phenomenon is likely a consequence of the fact that the more hydrophobic purine nucleobases within DNA aptamers[5, 6] cause larger interactions with DNA polymerase in a manner that is dependent on the affinity between DNA polymerase and homo-oligonucleotides ($(A)_n = (G)_n > (T)_n > (C)_n$).[7, 8] These interactions bring about a slight inhibition of DNA polymerase activity. In addition, C20 and G20 DNA aptamers do not completely inhibit DNA polymerase in the presence of the respective complementary homoguanine and homocytosine (C20.2 and G20.2, Fig. S1). This observation may be a consequence of the fact that guanine nucleobases are susceptible to formation of G-quartet secondary structures, which hinder perfect hybridization of DNA aptamer with complementary target DNA and prevent formation of the conformation of the DNA aptamer that is required to inhibit DNA polymerase.[9, 10] Importantly, DNA aptamers**,** which contain random overhang sequences that resemble those of natural target DNA completely inhibit DNA polymerase when the corresponding complementary target DNAs are present, while DNA aptamers alone do not influence DNA polymerase activity (R20(1) and R20(2), Fig. S1, ESI†). In addition, the DNA aptamer containing an overhang sequence at the 3’ instead of the 5’ end also effectively inactivates DNA polymerase in the presence of complementary target DNA (T20(R), Fig. S1, ESI†). This finding indicates that the inhibitory capabilities of DNA aptamers are not influenced by the location of overhang regions.

Fig. S2 Effect of the length of the overhang sequence on the inhibitory capability of DNA aptamer against DNA polymerase. (a) Melting curve analysis of DNA aptamers (T8, T12, T16, and T20 aptamers) in the absence (1) and presence (2) of complementary target DNAs. (b) Time-dependent fluorescence intensities from TaqMan probe during primer extension reactions. DNA aptamers (T8, T12, T16, and T20 aptamers) in the absence (1) or presence (2) of complementary target DNAs were additionally applied to the solutions containing DNA polymerase. (c) Fluorescence signal change (F_{200}-F_0) in the absence (1) or presence (2) of complementary target DNAs, where F_{200} and F_0 are the fluorescence intensities from TaqMan probe measured at 200 min and 0 min, respectively, after introduction of reagents for the primer extension reaction including TaqMan probe. Final concentrations of DNA polymerase, DNA aptamer, and target DNA are 5.5 nM, 100 nM, and 100 nM, respectively.



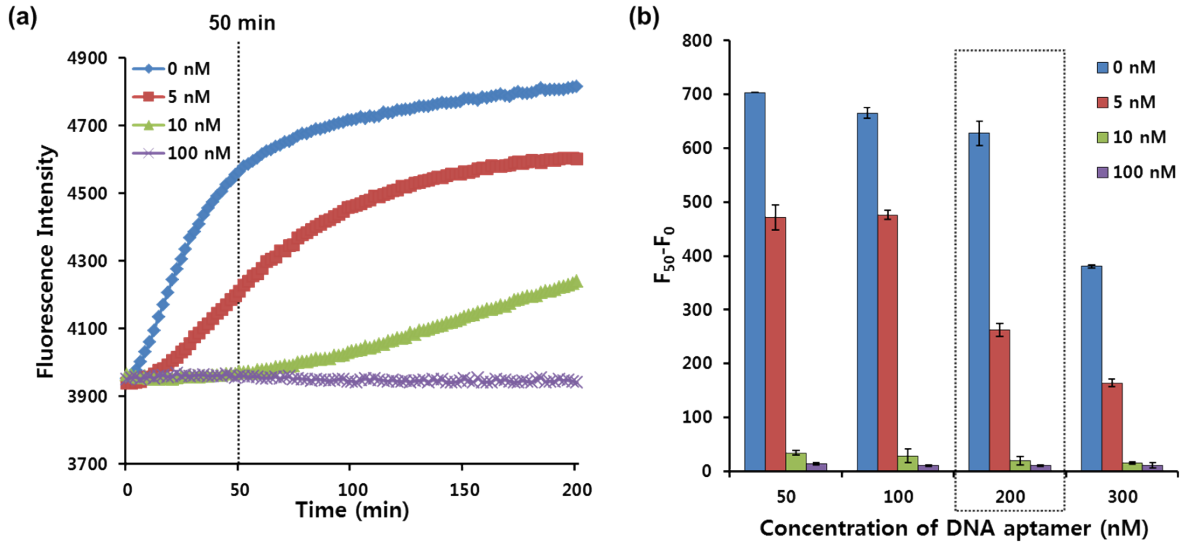
The effect of the length of the overhang sequence on the inhibitory capability of DNA aptamers was investigated by evaluating DNA aptamers that have homothymine overhang sequence lengths from 8 to 20-nt. As the results displayed in Fig. S2 show, the DNA aptamer containing an overhang sequence comprised of eight thymine nucleobases does not inhibit DNA polymerase even when the complementary homoadenine sequence is present. This finding is a result of the fact that the DNA aptamer does not hybridize with the complementary target DNA owing to its low melting temperature (< 25 °C). On the other hand, other DNA aptamers that contain longer homothymine overhang sequences and have melting temperatures higher than 25 °C effectively inhibit DNA polymerase activity upon hybridization with complementary target DNAs (Fig. S2). The combined results demonstrate that at least 12-nt hybridization between complementary target DNA and the DNA aptamer containing an overhang sequence is required for the effective inhibition of DNA polymerase.

Fig. S3 Gel shift assay in polyacrylamide gel electrophoresis to prove binding of DNA aptamer with DNA polymerase. 1) T20 aptamer, 2) T20 aptamer + DNA polymerase, 3) T20 aptamer + A20 target, 4) T20 aptamer + A20 target + DNA polymerase. Final concentrations of DNA polymerase, DNA aptamer and target DNA are 110 nM, 100 nM and 100 nM, respectively.



Binding of DNA aptamer to DNA polymerase was confirmed by using electrophoretic band shift experiments (Fig. S3).[4] As shown in Fig. S3, a DNA aptamer possessing an overhang sequence of twenty thymine nucleobases in the absence of complementary target DNA is seen as an electrophoresis band at a bottom position regardless of whether or not DNA polymerase is present (lane 1 and 2, Fig. S3). On the other hand, when complementary target DNA is present, the same DNA aptamer in the presence of DNA polymerase is seen as a strong band that is shifted to an upper position (lane 4, Fig. S3), indicating that complexation of the DNA aptamer with DNA polymerase takes place. Overall, these results unambiguously demonstrate that complementary target DNA stabilizes a DNA aptamer containing an overhang sequence and enables it to bind to and effectively inhibit DNA polymerase.

Fig. S4 Optimization of reaction conditions. (a) Time-dependent fluorescence intensities from TaqMan probe during primer extension reactions. (b) Fluorescence signal change (F_{50}-F_0), where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively, after introduction of reagents for the primer extension reaction including TaqMan probe. Free urea-specific DNA aptamers (blue) or urea-specific DNA aptamers in the presence of complementary urea target DNA (red: 5 nM, green: 10 nM, and violet: 100 nM) were additionally applied to the solutions containing DNA polymerase. The final concentration of DNA polymerase is 5.5 nM.



The concentration of a DNA aptamer containing an urea-specific overhang sequence (urea-specific DNA aptamer) and the extension time needed to detect target DNA were chosen to be 200 nM and 50 min, respectively (Fig. S4).

Fig. S5 Sensitivity of the system to detect target DNAs associated with urea. Time-dependent fluorescence intensities from TaqMan probe during primer extension reactions. Urea-specific DNA aptamers in the presence of varying concentrations of urea target DNA were additionally applied to the solutions containing DNA polymerase. Final concentrations of DNA polymerase and DNA aptamer are 5.5 nM and 200 nM, respectively.

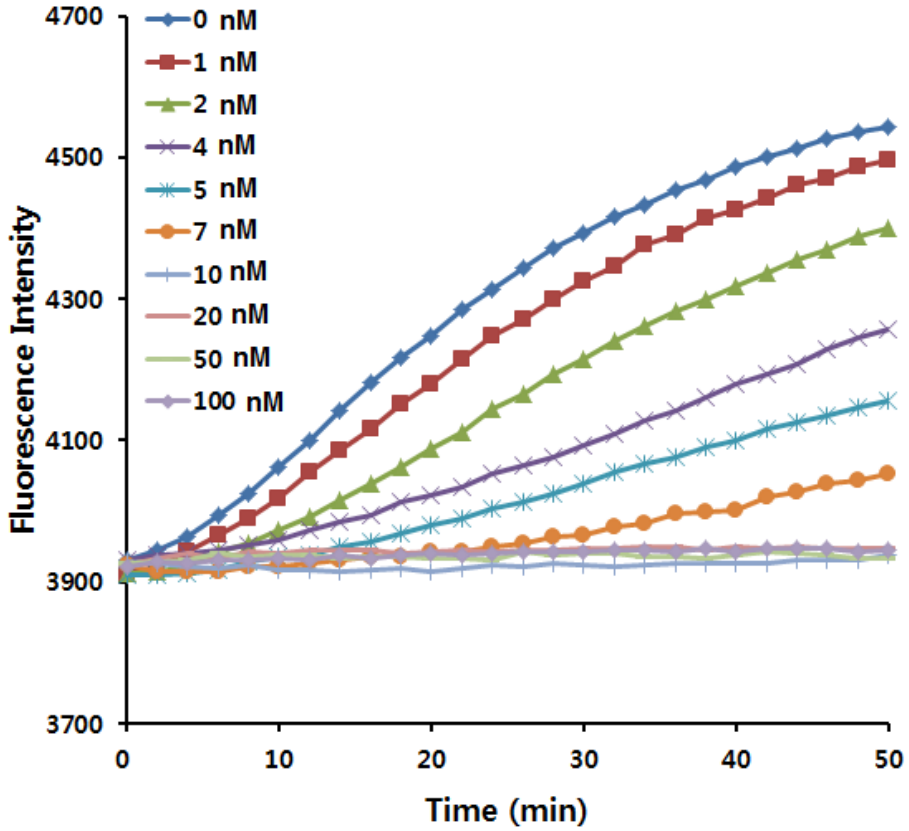


Fig. S6 Detection of target DNAs associated with chlamydia. (a) Time-dependent fluorescence intensities from TaqMan probe during primer extension reactions. (b) Fluorescence signal change (F_{50}-F_0), where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively, after introduction of reagents for the primer extension reaction including TaqMan probe. (c) Polyacrylamide gel electrophoresis image. Negative control (NC) and positive control (PC) are solutions in the absence and presence of DNA polymerase, respectively. Free chlamydia-specific DNA aptamer (1), chlamydia-specific DNA aptamer in the presence of 25-nt (2), 35-nt (4), or 45-nt (5) complementary chlamydia target DNA, or non-complementary urea DNA (3) was additionally applied to the solutions containing DNA polymerase. Final concentrations of DNA polymerase, DNA aptamer, and target DNA are 5.5 nM, 200 nM, and 100 nM, respectively.

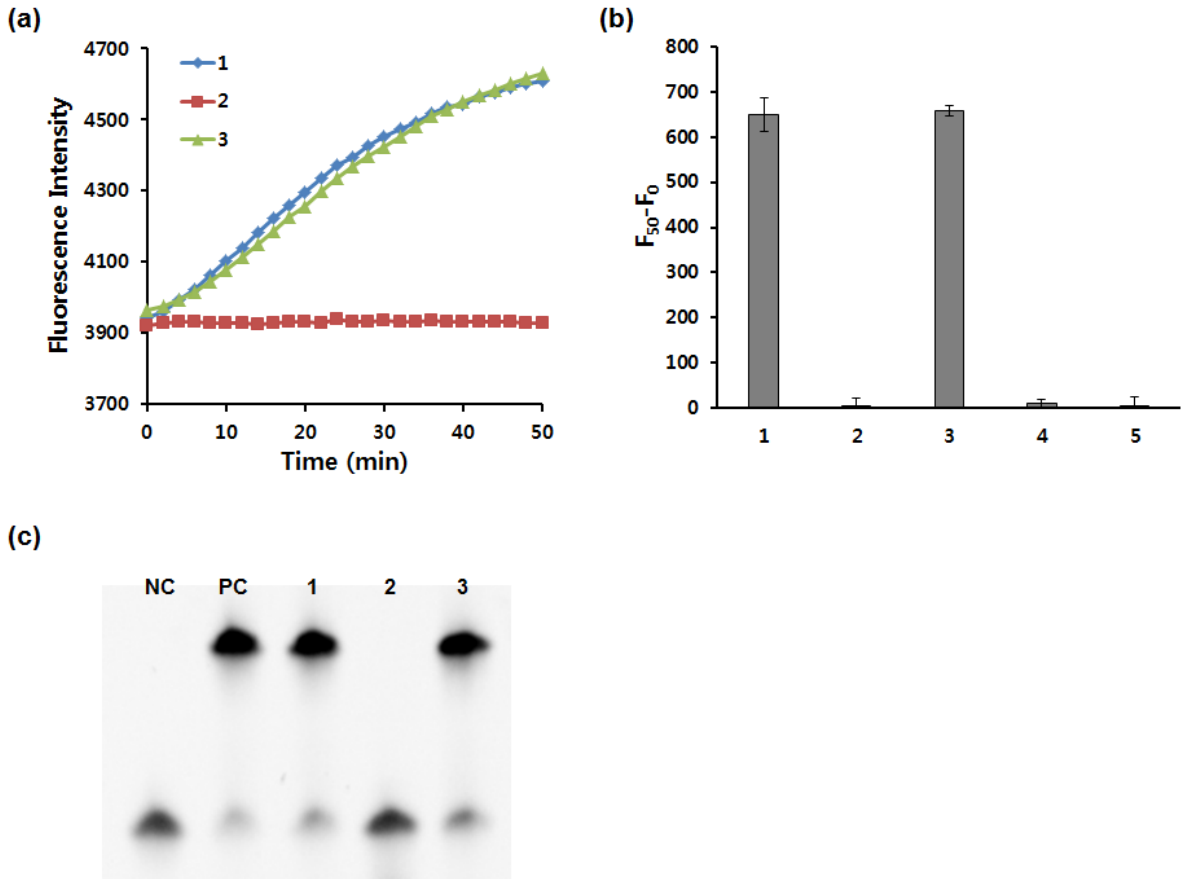
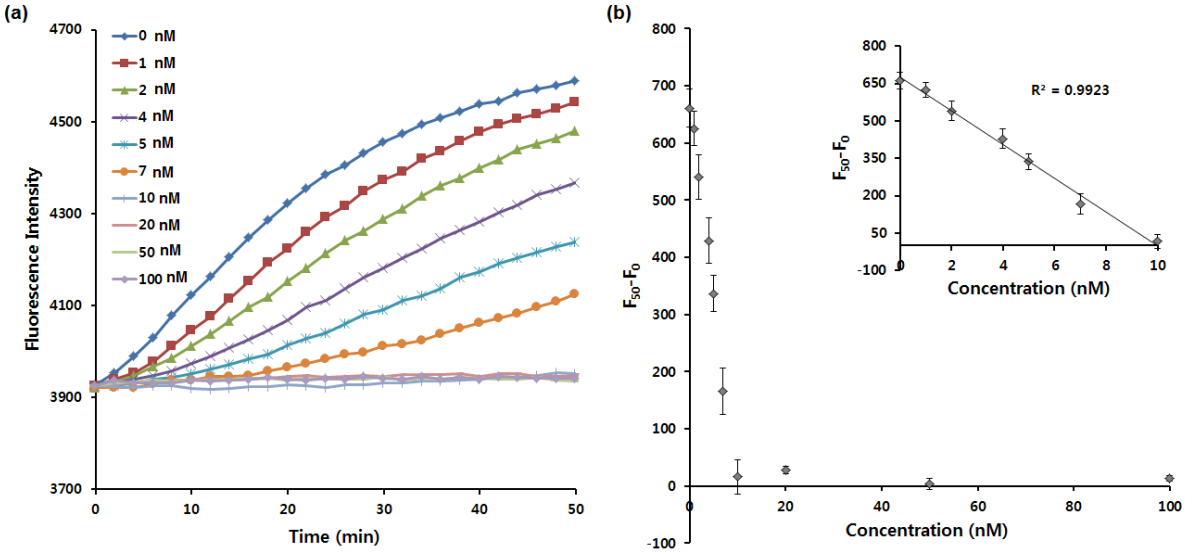


Fig. S7 Sensitivity of the system to detect target DNAs associated with chlamydia. (a) Time-dependent fluorescence intensities from TaqMan probe during primer extension reactions. Chlamydia-specific DNA aptamers in the presence of chlamydia target DNA at varying concentrations were additionally applied to the solutions containing DNA polymerase. (b) Fluorescence signal change (F_{50}-F_0) in the presence of chlamydia target DNA at varying concentrations, where F_{50} and F_0 are the fluorescence intensities from TaqMan probe measured at 50 min and 0 min, respectively, after introduction of reagents for the primer extension reaction including TaqMan probe. Inset in (b): Linear range between (F_{50}-F_0) and chlamydia target DNA concentration (0-10 nM). Final concentrations of DNA polymerase and DNA aptamer are 5.5 nM and 200 nM, respectively.



How to improve the detection sensitivity of this system

This detection sensitivity could be improved by coupling this method with isothermal nucleic acid amplification techniques such as rolling circle amplification,[11] nicking endonuclease signal amplification,[12] and exonuclease amplification.[13] In addition, highly sensitive detection modules such as electrochemical and chemiluminescence assays can be integrated to monitor target DNA recognition in the form of the activity of DNA polymerase.

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