Supporting Information for

Water-Enabled Visual Detection of DNA

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1. Materials, equipment, and measurement

3-(2-Aminoethylamino)propyltrimethoxysilane (EDAS, 96%) and *n*-hexanoic anhydride (HA, 98%) were purchased from Alfa Aesar. *N*-Succinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (SMCC, 98% HPLC) was from Meyer Chemical Technology Co. Ltd. 1-Hexanethiol (HT, 96%) and acetic acid (99.8%) were from J&K Chemical Co. Ltd. Dimethyl sulfoxide (DMSO, 99.0%), hydrogen peroxide (30 wt%, AR grade), pyridine (99.5%), and *N*,*N*'-dimethylformamide (DMF, 99.5%) were from Sinopharm Chemical Reagent Co. Ltd. Concentrated sulfuric acid (95-98%), hydrochloric acid (36-38 wt%), and ammonia solution (25 wt%) were from Nanjing Chemical Reagent Co. Ltd. Ethanol (99.5%) was from Shanghai Titanchem Co. Ltd. DMSO, pyridine, and DMF were distilled over CaH_2 prior to use, other reagents were used as received. ZipTip C18 pipette tip was from Millipore. *Taq* DNA ligase was from New England Biolabs. ϕ 29 DNA polymerase was from Thermo Scientific. T4 DNA ligase, exonuclease I, exonuclease III, 10× DNA loading buffer, 20 bp DNA ladder marker, and DL5000 DNA ladder marker were from TaKaRa Biotechnology (Dalian) Co. Ltd. dNTPs and SYBR green II were from Solarbio (Beijing) Co. Ltd. All DNAs were custom-synthesized by Sangon Biotech (Shanghai) Co. Ltd. Denaturing loading buffer was prepared in house containing 90% formamide, 10% glycerol, 0.5% EDTA, 0.1% xylene, and 0.1% bromophenol blue. Nano-pure water (18.2 MΩ•cm), purified by Sartorius Arium 611 system, was used throughout the experiment. All enzymes were used in the corresponding 1× reaction buffer supplied with the enzyme unless otherwise noted.

Ligase chain reaction was performed on a GeneAmp PCR system 9700 from Applied Biosystems. Contact angle measurement was performed on a KSV CVM200 optical contact angle meter. Gel electrophoresis was performed on a Bio-Rad system using 20% polyacrylamide or 1% agarose gel. UV-vis absorption spectra were obtained using a Perkin Elmer Lss 5 UV/vis spectrophotometer. Microscopic images were obtained from Shunyu (Ningbo) BH200 microscope system. Photographs were taken using a Nikon Coolpix P7000 digital camera.

2. DNA sequence information

Tables S1. DNA Symbol and sequence information[a]

DNA Symbol	Sequence
T-DNA1	5'-GGATTATTGTTAAATATTGATAAGGAT-3'
Palo-DNA2	5'-(P)-TTAACAATAATGGTCTGCCCTGACTGCCACTTCCTC GATGATCCTCGATGTCCTTATCAATAT-3'
RCA-DNA3	5'-HS-AAAAAAAAAATCATCGAGGAAGTGGCAGTC-3'
RCA-DNA3E	5'-TCATCGAGGAAGTGGCAGTC-3'
LCR-DNA4	5'-ATTATTGTTAA-3'
LCR-DNA5	5'-(P)-ATATTGATAAGG-3'
SBM1	5'-GGATTATTGTTAGATATTGATAAGGAT-3'
SBM2	5'-GGATTATTATTAAATATTGATAAGGAT-3'
SBM3	5'-GGATTATTGTTAAAGATTGATAAGGAT-3'
SBM4	5'-GGATTATTGTTAAGTATTGATAAGGAT-3'
SBM5	5'-GGATTATTGTTAATTATTGATAAGGAT-3'
SBM6	5'-GGATTATTGTTAAATACTGATAAGGAT-3'
T-DNA6	5'-TATGTTAGTATGATATAGGAATAGTTA-3'
Palo-DNA7	5'-(P)-TCATACTAACAGGTCTGCCCTCTGGCACGTGACATA TCTCCTCCTCGATGAACTATTCCTATA-3'
RCA-DNA8	5'-HS-AAAAAAAAAAGGAGATATGTCACGTGCCAG-3'
LCR-DNA9	5' –TGTTAGTATGA-3'
LCR-DNA10	5'-(P)-TATAGGAATAGT-3'

[a] (P) denotes the presence of a phosphate group. The part of the sequence marked in red denotes the mismatched base.

3. Ligation reaction

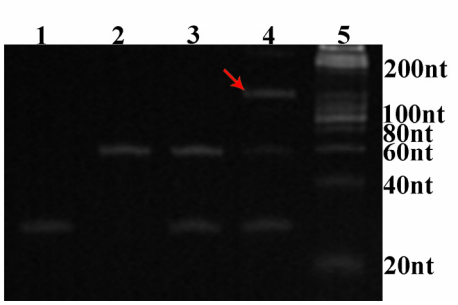

Figure S1. Verification of the ligation reaction by *Taq* DNA ligase. Gel electrophoresis diagram showing the ligation of **Palo-DNA2** by *Taq* DNA ligase in the presence of **T-DNA1**. Lane 1: **T-DNA1**; lane 2: **Palo-DNA2**; lane 3: mixture of **T-DNA1** and **Palo-DNA2**; lane 4: mixture of **T-DNA1** and **Palo-DNA2** after the ligation step; lane 5: DNA marker. The band marked in red represents the ligation product **Cir-Palo-DNA2**. **Palo-DNA2** (1 μM) was ligated using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (1 μM) at 45 ^{o}C for 4 h.

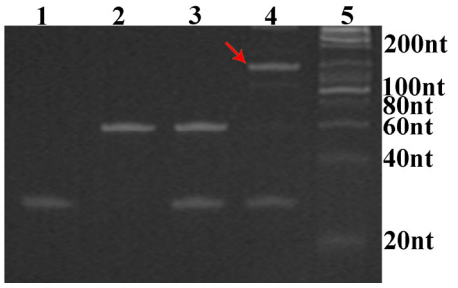

Figure S2. Verification of the ligation reaction by T4 DNA ligase. Gel electrophoresis diagram showing the ligation of **Palo-DNA2** by T4 DNA ligase in the presence of **T-DNA1**. Lane 1: **T-DNA1**; lane 2: **Palo-DNA2**; lane 3: mixture of **T-DNA1** and **Palo-DNA2**; lane 4: mixture of **T-DNA1** and **Palo-DNA2** after the ligation step; lane 5: DNA marker. The band marked in red represents the ligation product **Cir-Palo-DNA2**. **Palo-DNA2** (1 μM) was ligated using T4 DNA ligase (17.5 U/μL) in the presence of **T-DNA1** (1 μM) at 45 ^{o}C for 4 h.

4. Melting curve

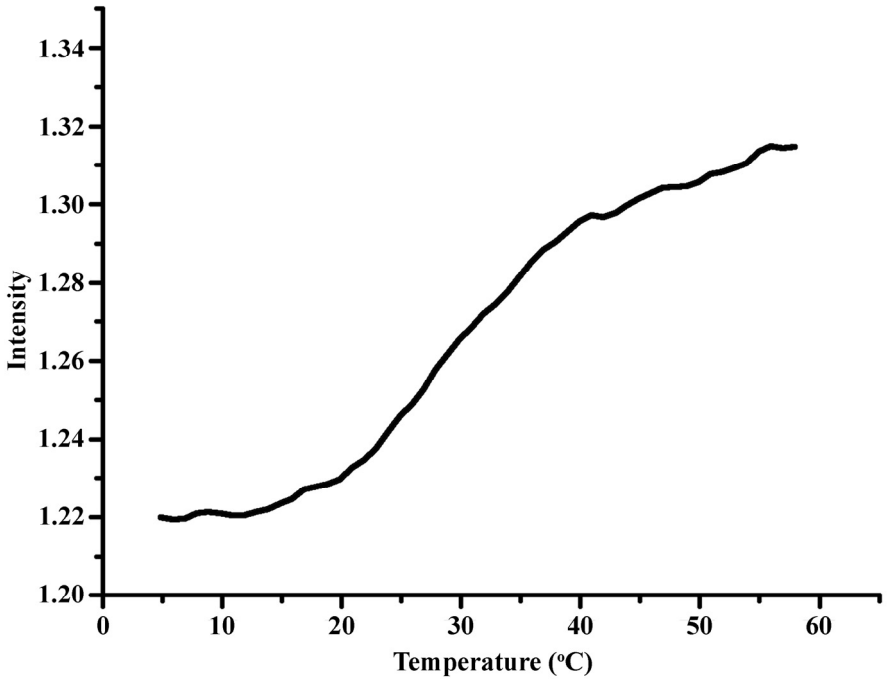

Figure S3. Melting curve, as measured by the recording of temperature-dependent UV-vis absorption at 260 nm, of 10 μM **T-DNA1** and 10 μM **Palo-DNA2** in a 0.1 M phosphate-buffered saline (PBS) solution (0.1 M NaCl, 10 mM phosphate buffer, pH 7.0).

5. Purification of ligation product

After the ligation reaction, the ligation mixture (10 μL solution containing **T-DNA1**, **Palo-DNA2**, and **Cir-Palo-DNA2**) was subjected to enzymatic digestion in a final 11.6 μL solution (exonuclease I 0.2 U/μL, exonuclease III 1.6 U/μL, 0.5 μL each of the two 10× nuclease reaction buffer) at 37 ^{o}C for 1 h for the removal of single-strand DNA, **T-DNA1** and **Palo-DNA2**. The exonucleases were heat inactivated by incubation at 80 ^{o}C for 15 min.

Cir-Palo-DNA2 was then extracted with ZipTip C18 pipette tip according to its user guide: repeated aspiration and dispensing of the sample, washing of the pipette tip with water, aspiration and dispensing of 10 μL 40% acetonitrile through the pipette tip; drying of the eluent under vacuum.

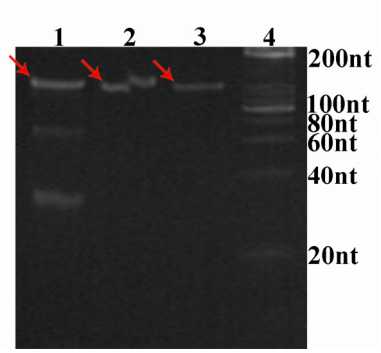

Figure S4. Purification of **Cir-Palo-DNA2** through enzymatic digestion and ZipTip extraction. Gel electrophoresis diagram showing the ligation of **Palo-DNA2** and purification of **Cir-Palo-DNA2**. Lane 1: ligation mixture before the enzymatic digestion; lane 2: ligation mixture after the enzymatic digestion; lane 3: ligation mixture after the enzymatic digestion and ZipTip extraction; lane 4: DNA marker. The band marked in red represents the ligation product **Cir-Palo-DNA2**. **Palo-DNA2** (1 μM) was ligated using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (1 μM) at 45 ^{o}C for 4 h. Then the ligation mixture was subjected to the enzymatic digestion and further the ZipTip extraction process for the purification of **Cir-Palo-DNA2**.

6. Ligation-rolling circle amplification in solution

The purified **Cir-Palo-DNA2** was used as a template for the RCA reaction with **RCA-DNA3E** (**RCA-DNA3E**, 10^{-9} M; ϕ29 DNA polymerase, 0.5 U/μL; dNTPs, 2 mM in the 1× ϕ29 DNA polymerase reaction buffer). The mixture was incubated overnight at 37 °C.

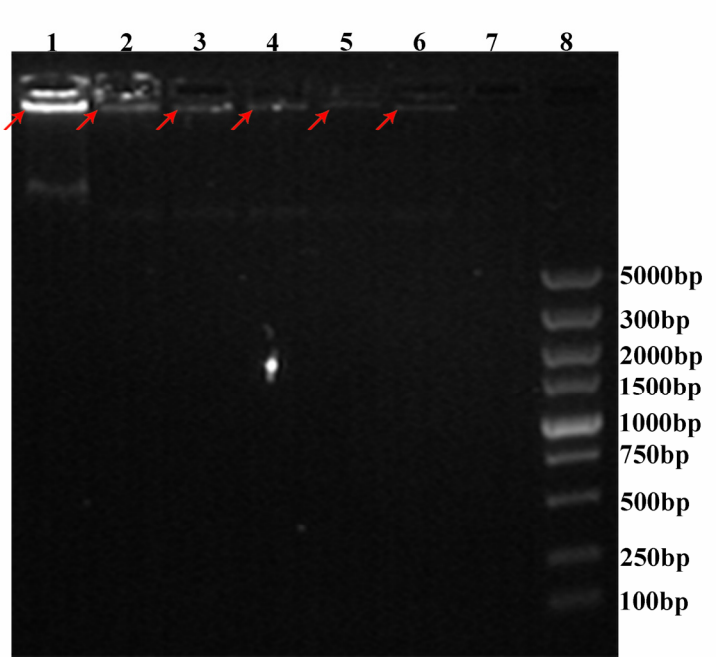

Figure S5. Verification of the ligation-RCA reaction in solution through gel electrophoresis. Gel electrophoresis diagram showing the ligation-RCA reaction of **RCA-DNA3E** on **Cir-Palo-DNA2** template. From lane 1 to lane 7: **T-DNA1** concentration of 0.1 μM, 10 nM, 1 nM, 0.1 nM, 10 pM, 1 pM, 0 M; lane **8**: DNA marker. The band marked in red represents the long and tandem single-strand DNA product after the ligation-RCA reaction. **Palo-DNA2** (0.1 μM) was ligated using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (various concentrations) at 45 °C for 4 h. **Cir-Palo-DNA2** was purified and used as a template for the RCA reaction with **RCA-DNA3E**.

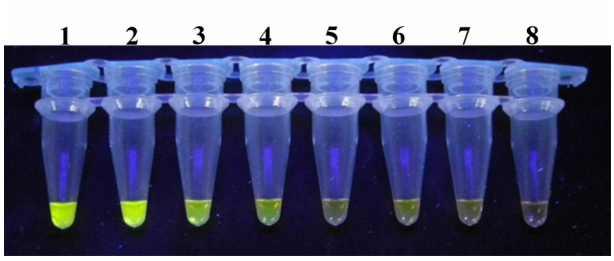

Figure S6. Verification of the ligation-RCA reaction in solution through fluorescent staining in solution. Macroscopic fluorescence image (excited with 254 nm UV light) showing the ligation-RCA reaction of **RCA-DNA3E** on **Cir-Palo-DNA2** template. From sample 1 to sample 8: **T-DNA1** concentration of 0.1 μM, 10 nM, 1 nM, 0.1 nM, 10 pM, 1 pM, 0.1 pM, 0 M. **Palo-DNA2** (0.1 μM) was ligated using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (various concentrations) at 45 ^{o}C for 4 h. **Cir-Palo-DNA2** was purified and used as a template for the RCA reaction with **RCA-DNA3E**. The RCA reaction mixture was subjected to SYBR Green II staining (100×, 1 μL).

7. Surface modification of silicon chip with primer DNA

The surface modification of silicon chip with primer DNA was carried out according to a slightly modified literature procedure.[S1] Specifically, a silicon chip was first treated with a Piranha solution (sulfuric acid:hydrogen peroxide = 3:1, v/v, Caution! Piranha solution is highly corrosive) at 95 ^{o}C for 2 h, followed by sequential incubation in a basic solution (ammonia:hydrogen peroxide:water = 1:1:5, v/v/v) and an acidic solution (hydrochloric acid:hydrogen peroxide:water = 1:1:5, v/v/v) at 75 ^{o}C for 30 min each. The chip was then immersed in an EDAS solution (EDAS:1 mM acetic acid = 1:100, v/v) at room temperature for 30 min and baked at 120 ^{o}C for 30 min. For the above steps, the chip was washed with copious amount of water between each step and dried with nitrogen flow before the 120 ^{o}C baking step. For the following steps, the chip was washed with copious amount of ethanol and water and dried with nitrogen flow between each step. The chip was immersed overnight in 1 mg/mL DMSO solution of SMCC at 37 ^{o}C, followed by immersion in a solution of HA:pyridine:DMF (1:1:8, v/v/v) at ~15 ^{o}C for 2 h. The chip was then spotted with 10 μM **RCA-DNA3** (0.1 M PBS buffer, 3 μL) and the reaction was allowed to proceed at 37 ^{o}C for 6 h in a humidity chamber. The chip was finally immersed overnight in an ethanol solution of 1 mM HT at room temperature. The assay-ready chip was stored at 4 ^{o}C prior to use.

8. DNA detection through the on-chip ligation-rolling circle amplification protocol

Palo-DNA2 (0.1 μM) was ligated using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (various concentrations) at 45 ^{o}C for 4 h. The ligation mixture (10 μL) was subjected to an identical purification process (enzymatic digestion and ZipTip extraction) as described above.

The purified **Cir-Palo-DNA2** was dissolved in 9 μL solution containing 0.5 U/μL ϕ29 DNA polymerase and 2 mM dNTPs (in the 1× ϕ29 DNA polymerase reaction buffer). The solution was then divided into three equal portions (each containing 3 μL), each of which was aliquotted onto a **RCA-DNA3** spot on a chip (the triplicate experiment was used to examine the reproducibility of the result). The reaction was allowed to proceed overnight in a humidity chamber at 37 ^{o}C. The chip was then washed with water and dried under nitrogen flow. The signal was then visualized by placing the pre-cooled chip (on ice) in a 30 ^{o}C, 45±5% RH atmosphere for 2 min.

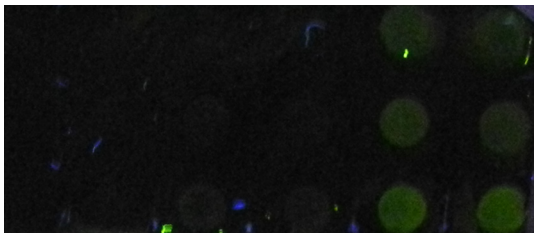

Figure S7. Verification of the ligation-RCA reaction on chip through fluorescent staining. Macroscopic fluorescence image (excited with 254 nm UV light) showing the ligation-RCA reaction of **RCA-DNA3** on **Cir-Palo-DNA2** template. From leftmost column to rightmost column (each column containing 3 spots for triplicate experiment): **T-DNA1** concentration of 0 M, 10 pM, 0.1 nM, 1 nM, 10 nM, 0.1 μM. For fluorescent staining, to the chip was added 1 μL 100× SYBR Green II and the SYBR Green II solution was thoroughly mixed using a pipette tip. The chip was then washed with water and dried under nitrogen flow. The macroscopic fluorescence was visualized by the placement of the chip in water. The detection limit through fluorescent staining is merely 10 nM under our experimental condition.

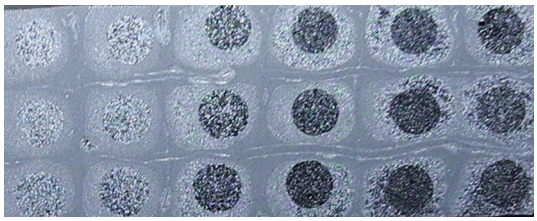

Figure S8. DNA detection with a silicon chip based on ligation-RCA protocol. Macroscopic image showing the detection of DNA through the WEVD strategy. From leftmost column to rightmost column (each column containing 3 spots for triplicate experiment): **T-DNA1** concentration of 0 M, 10 pM, 0.1 nM, 1 nM, 10 nM, 0.1 μM.

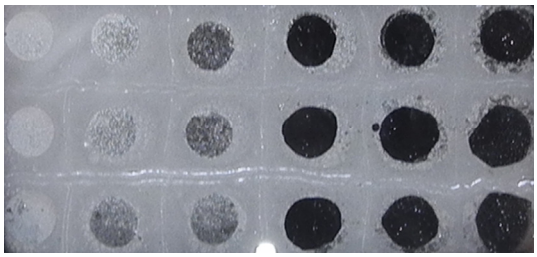

Figure S9. DNA detection with a glass slide (against a black plastic backdrop) based on ligation-RCA protocol. Macroscopic image showing the detection of DNA through the WEVD strategy. From leftmost column to rightmost column (each column containing 3 spots for triplicate experiment): **T-DNA1** concentration of 0 M, 10 pM, 0.1 nM, 1 nM, 10 nM, 0.1 μM.

9. Water contact angle measurement

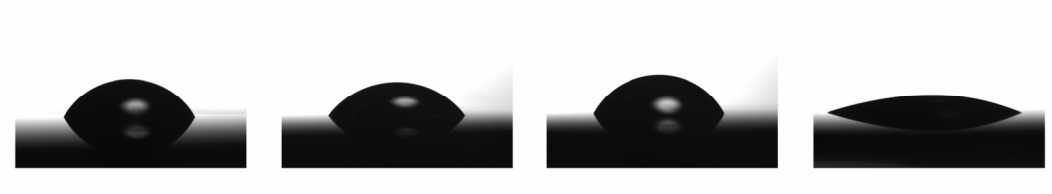

Figure S10. Change of the water contact angle before and after the ligation-RCA reaction. Macroscopic image of water droplet showing the water contact angle at different locations of the silicon chip and before and after the ligation-RCA reaction. From leftmost image to rightmost image: primer **RCA-DNA3**-free area, primer **RCA-DNA3**-derivatized area, primer **RCA-DNA3**-derivatized area after the ligation-RCA reaction in the absence of target **T-DNA1**, primer **RCA-DNA3**-derivatized area after the ligation-RCA reaction in the presence of target **T-DNA1**.

Table S2. Water contact angle measured at different locations of the silicon chip and before and after the ligation-RCA reaction (ref. Figure S10)

	Primer-free area	Primer-derivatized area	Primer-derivatized area after ligation-RCA reaction in the absence of target DNA	Primer-derivatized area after ligation-RCA reaction in the presence of target DNA
Contact angle (^{o})	64	57	66	22

10. Ligase chain reaction

(1) Verification of DNA amplification by LCR

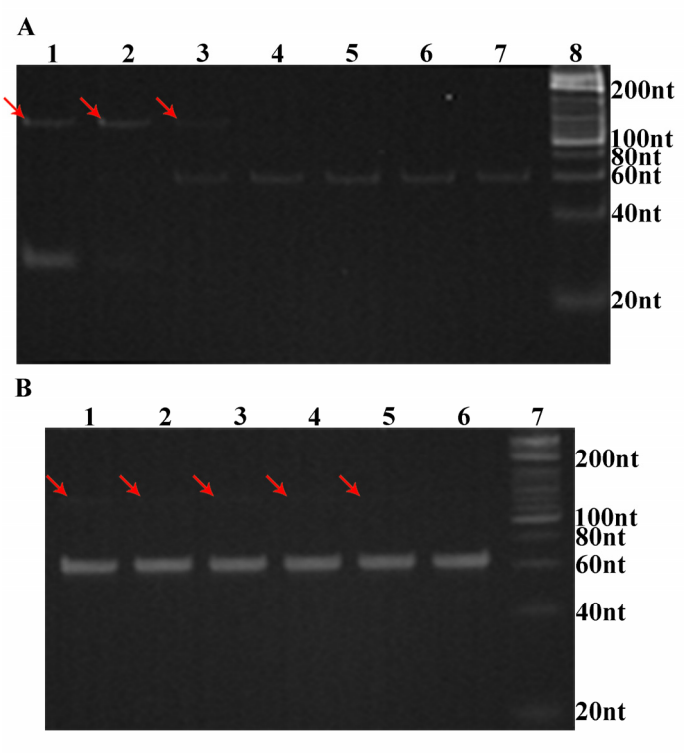

Figure S11. Verification of DNA amplification by LCR. (A) Gel electrophoresis diagram showing the ligation of **Palo-DNA2** in the presence of **T-DNA1**. From lane 1 to lane 7: **T-DNA1** concentration of 1 μM, 0.1 μM, 10 nM, 1 nM, 0.1 nM, 10 pM, 0 M; lane 8: DNA marker. The band marked in red represents the ligation product **Cir-Palo-DNA2**. **Palo-DNA2** (0.1 μM) was ligated using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (various concentrations) at 45 ^{o}C for 4 h. (B) Gel electrophoresis diagram showing the amplified ligation of **Palo-DNA2** by LCR in the presence of **T-DNA1**. From lane 1 to lane 6: **T-DNA1** concentration of 0.1 nM, 10 pM, 1 pM, 0.1 pM, 10 fM, 0 M; lane 7: DNA marker. The band marked in red represents the LCR product **Cir-Palo-DNA2**. **Palo-DNA2** (1 μM), **LCR-DNA4** (1 μM), and **LCR-DNA5** (1 μM) were ligated by *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (various concentrations) under LCR condition: 65 ^{o}C, 5 min/20 cycles of (65 ^{o}C, 30 sec/25 ^{o}C, 2 min/45 ^{o}C, 3 min)/65 ^{o}C, 7 min.

(2) Optimization of ligase concentration for LCR

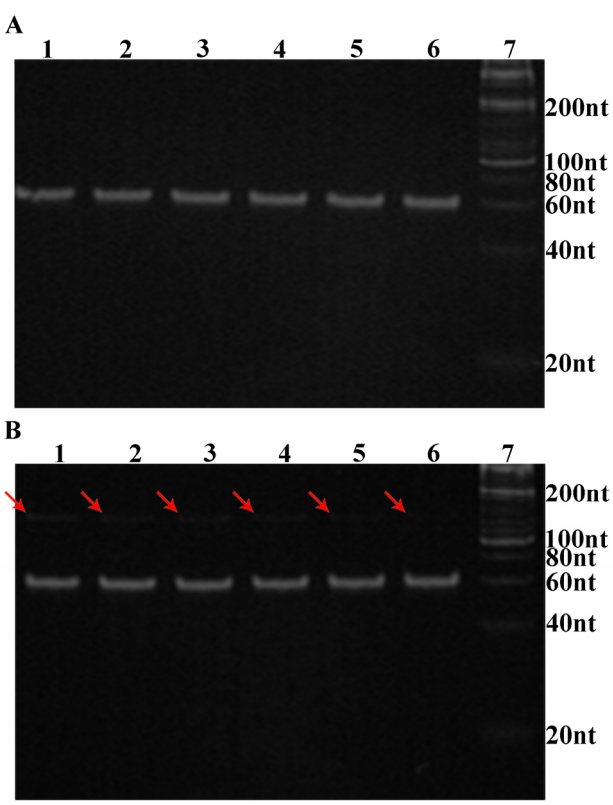

Figure S12. Optimization of ligase concentration for LCR. Gel electrophoresis diagram showing the LCR of **Palo-DNA2** with different ligase concentrations in the presence of **T-DNA1**. (A) 0.4 U/μL *Taq* DNA ligase. (B) 2.0 U/μL *Taq* DNA ligase. From lane 1 to lane 6: **T-DNA1** concentration of 0.1 nM, 10 pM, 1 pM, 0.1 pM, 10 fM, 0 M; lane 7: DNA marker. The band marked in red represents the LCR product **Cir-Palo-DNA2**. **Palo-DNA2** (1 μM), **LCR-DNA4** (1 μM), and **LCR-DNA5** (1 μM) were ligated by *Taq* DNA ligase (various concentrations) in the presence of **T-DNA1** (various concentrations) under LCR condition: 65 ^{o}C, 5 min/20 cycles of (65 ^{o}C, 30 sec/25 ^{o}C, 2 min/45 ^{o}C, 3 min)/65 ^{o}C, 7 min.

(3) Optimization of LCR cycles

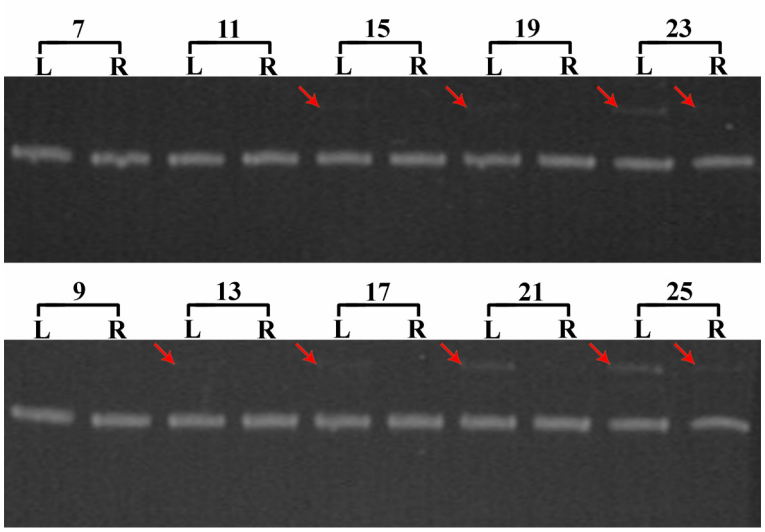

Figure S13. Optimization of LCR cycles. Gel electrophoresis diagram showing the LCR of **Palo-DNA2** with different LCR cycles in the presence and absence of **T-DNA1**. L: 0.1 nM **T-DNA1**; R: 0 M **T-DNA1**. The band marked in red represents the LCR product **Cir-Palo-DNA2**. **Palo-DNA2** (1 μM), **LCR-DNA4** (1 μM), and **LCR-DNA5** (1 μM) were ligated by *Taq* DNA ligase (0.8 U/μL) in the presence and absence of **T-DNA1** under LCR condition: 65 ^{o}C, 5 min/20 cycles of (65 ^{o}C, 30 sec/25 ^{o}C, 2 min/45 ^{o}C, 3 min)/65 ^{o}C, 7 min. At the end of each LCR cycle, the LCR mixture was quenched by 10 μL of denaturing loading buffer.

11. DNA detection through the on-chip ligase chain reaction-rolling circle amplification protocol

In a typical DNA detection experiment, a 10 μL LCR solution containing **Palo-DNA2** (1 μM), **LCR-DNA4** (1 μM), **LCR-DNA5** (1 μM), *Taq* DNA ligase (0.8 U/μL), and **T-DNA1** (target to be detected, various concentrations) was subjected to a thermal cycling treatment: 65 ^{o}C, 5 min/16 cycles of (65 ^{o}C, 30 sec/25 ^{o}C, 2 min/45 ^{o}C, 3 min)/65 ^{o}C, 7 min. Then the LCR mixture (10 μL) containing **Cir-Palo-DNA2** was subjected to enzymatic digestion in a final 11.6 μL solution (exonuclease I 0.2 U/μL, exonuclease III 1.6 U/μL, 0.5 μL each of the two 10× nuclease reaction buffer) at 37 ^{o}C for 1 h for the removal of single-strand DNA, **T-DNA1**, **Palo-DNA2**, **LCR-DNA4** and **LCR-DNA5**, and ligation product from **LCR-DNA4** and **LCR-DNA5**. The exonucleases were heat inactivated by incubation at 80 ^{o}C for 15 min. To the mixture was further added several components, with a final 14.1 μL solution (ϕ29 DNA polymerase, 0.35 U/μL; dNTPs, 0.7 mM; 1 μL 10× ϕ29 DNA polymerase reaction buffer). The solution was then divided into three equal portions, each of which was aliquotted onto a **RCA-DNA3** spot on a chip (the triplicate experiment was used to examine the reproducibility of the result). The reaction was allowed to proceed in a humidity chamber at 37 ^{o}C for 5 h. The chip was then washed with water and dried under nitrogen flow. The signal was then visualized by placing the pre-cooled chip (on ice) in a 30 ^{o}C, 45±5% RH atmosphere for 2 min.

12. Single-base discrimination

(1) Demonstration of single-base discrimination by isothermal ligation

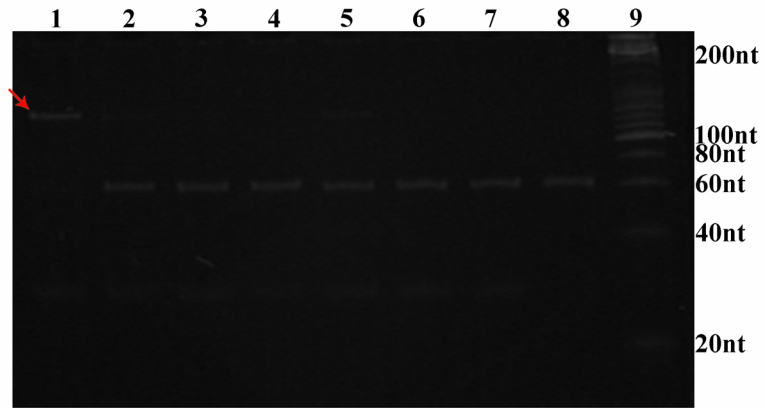

Figure S14. Confirmation of the single-base discrimination specificity of isothermal DNA ligation process. Gel electrophoresis diagram showing the generation of ligation product **Cir-Palo-DNA2** exclusively in the presence of **T-DNA1**. The band marked in red represents the ligation product **Cir-Palo-DNA2**. Lane 1: **T-DNA1**; lane 2: **SBM1**; lane 3: **SBM2**; lane 4: **SBM3**; lane 5: **SBM4**; lane 6: **SBM5**; lane 7: **SBM6**; lane 8: no DNA; lane 9: DNA marker. The ligation of **Palo-DNA2** (0.1 μM) was attempted using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (0.1 μM) or a single-base mismatched sequence (**SBM1** through **SBM6**, 0.1 μM) at 45 ^{o}C for 4 h.

(2) Demonstration of single-base discrimination by LCR

T-DNA1 (0.1 nM) or a single-base mismatched sequence (**SBM1** through **SBM6**, 0.1 nM) underwent a typical DNA detection procedure except with a different thermal cycling condition: 65 ^{o}C, 5 min/16 cycles of (65 ^{o}C, 30 sec/45 ^{o}C, 5 min)/65 ^{o}C, 7 min.

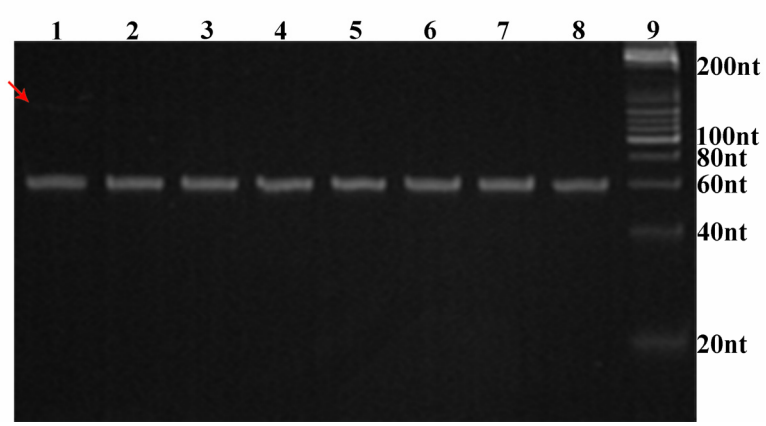

Figure S15. Confirmation of the single-base discrimination specificity of LCR process. Gel electrophoresis diagram showing the generation of LCR product **Cir-Palo-DNA2** exclusively in the presence of **T-DNA1**. Lane 1: **T-DNA1**; lane 2: **SBM1**; lane 3: **SBM2**; lane 4: **SBM3**; lane 5: **SBM4**; lane 6: **SBM5**; lane 7: **SBM6**; lane 8: no DNA; lane 9: DNA marker. The band marked in red represents the LCR product **Cir-Palo-DNA2**. The LCR of **Palo-DNA2** was attempted using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** or a single-base mismatched sequence (**SBM1** through **SBM6**).

13. DNA detection sensitivity

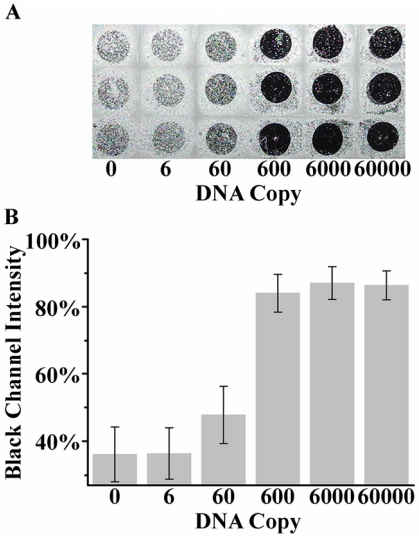

Figure S16. WEVD-based on-chip DNA detection with the LCR-RCA protocol. (A) Macroscopic image of the spot in the presence of **T-DNA1**. From left to righ (each column containing 3 spots for triplicate experiment): **T-DNA1** (10 μL) concentration of 0 M, 1 aM, 10 aM, 0.1 fM, 1 fM, 10 fM. The corresponding color-scale image is presented as Figure 3. (B) The average black channel intensity of 3 spots in each column of (A) as a function of the quantity of **T-DNA1**. The black channel intensity has been normalized (completely black: 255, 100%; completely white: 0, 0%). The error bar represents the standard deviation.

14. DNA detection with single-base discrimination specificity

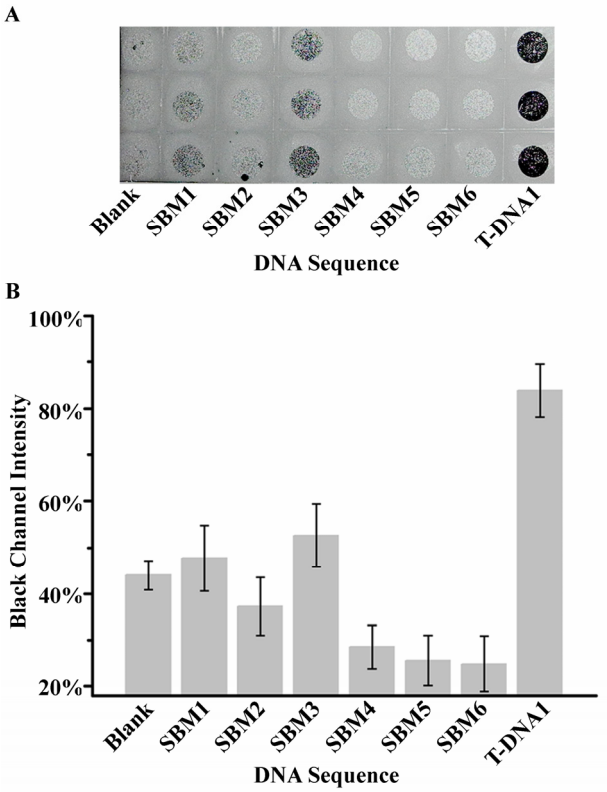

Figure S17. WEVD-based on-chip single-base discrimination with the LCR-RCA protocol. (A) Macroscopic image of the spot in the presence of **T-DNA1** (0.1 nM) or a single-base mismatched sequence (**SBM1** through **SBM6**, 0.1 nM), or in the absence of any DNA (blank) The corresponding color-scale image is presented as Figure 4. (B) The average black channel intensity of 3 spots in each column of (A) as a function of the DNA sequence.

15. Two-target system

(1) Demonstration of the absence of cross-ligation

The ligation of **Palo-DNA2** (0.1 μM) and/or **Palo-DNA7** (0.1 μM) were attempted using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (0.1 μM) and/or **T-DNA6** (0.1 μM) at 45 °C for 4 h to verify that no non-specific ligation occurs.

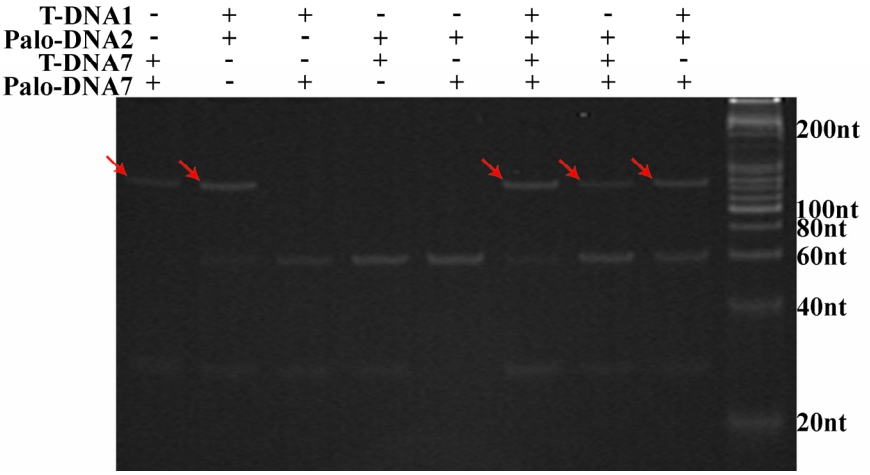

Figure S18. Demonstration of the absence of isothermal cross-ligation of the two-target system. Gel electrophoresis diagram showing the generation of ligation products **Cir-Palo-DNA2** and **Cir-Palo-DNA7** exclusively in the presence of respective targets **T-DNA1** and **T-DNA6**. The band marked in red represents the ligation product **Cir-Palo-DNA2** or/and **Cir-Palo-DNA7**. The plus (+) and minus (-) signs on top of each column indicate the presence and absence of a particular component, respectively.

(2) Demonstration of the absence of cross-interference under LCR condition

The ligation of **Palo-DNA2** (1 μM) and/or **Palo-DNA7** (1 μM) were attempted using *Taq* DNA ligase (0.8 U/μL) in the presence of **T-DNA1** (0.1 nM) and/or **T-DNA6** (0.1 nM) and in the presence of **LCR-DNA4**/**LCR-DNA5** (1 μM each) or **LCR-DNA9**/**LCR-DNA10** (1 μM each) through a thermal cycling treatment: 65 ^{o}C, 5 min/16 cycles of (65 ^{o}C, 30 sec/45 ^{o}C, 5 min)/65 ^{o}C, 7 min.

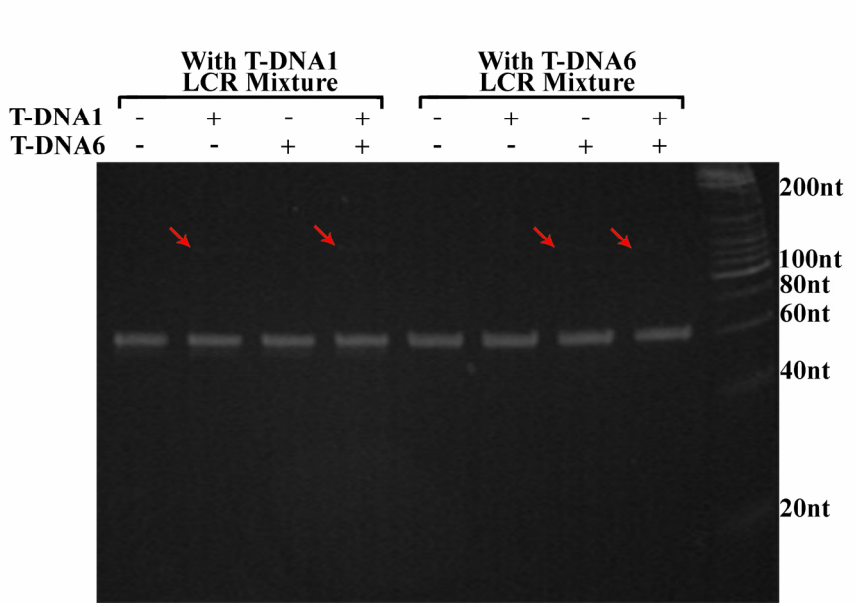

Figure S19. Demonstration of the absence of LCR cross-interference of the two-target system. Gel electrophoresis diagram showing the generation of LCR products **Cir-Palo-DNA2** and **Cir-Palo-DNA7** exclusively in the presence of respective target LCR mixtures (**T-DNA1**/**LCR-DNA4**/**LCR-DNA5**) and (**T-DNA6**/ **LCR-DNA9**/**LCR-DNA10**). The band marked in red represents the LCR product **Cir-Palo-DNA2** or **Cir-Palo-DNA7**. The plus (+) and minus (-) signs on top of each column indicate the presence and absence of a particular component, respectively.

16. Two-target detection

T-DNA1 (0.1 nM) and **T-DNA6** (0.1 nM) underwent a typical DNA detection procedure, in the presence of **Palo-DNA2**/**LCR-DNA4**/**LCR-DNA5** (1 μM each) or **Palo-DNA7**/**LCR-DNA9**/**LCR-DNA10** (1 μM each) on the respective primer (**RCA-DNA3** or **RCA-DNA8**)-derivatized spot, except with a different thermal cycling condition: 65 ^{o}C, 5 min/16 cycles of (65 ^{o}C, 30 sec/45 ^{o}C, 5 min)/65 ^{o}C, 7 min.

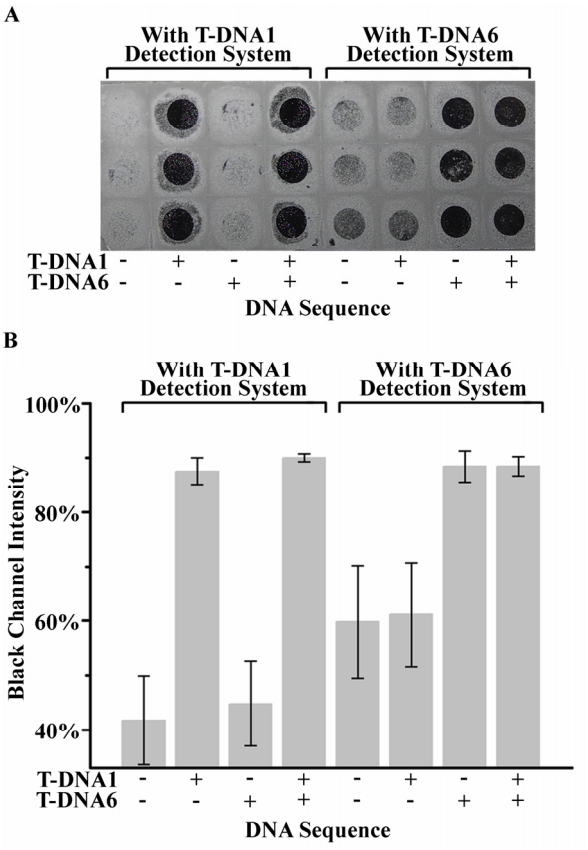

Figure S20. WEVD-based on-chip two-target detection with the LCR-RCA protocol. (A) Macroscopic image of the spot in the presence of **T-DNA1** (0.1 nM) and/or **T-DNA6** (0.1 nM). The plus (+) and minus (-) signs at the bottom of each column indicate the presence and absence of a particular target, respectively. The corresponding color-scale image is presented as Figure 5. (B) The average black channel intensity of 3 spots in each column of (A) as a function of the DNA sequence.

17. References

(S1) Zhou, X.; Xia, S.; Lu, Z.; Tian, Y.; Yan, Y.; Zhu, J. *J. Am. Chem. Soc.* **2010**, *132*, 6932-6934.