A NOVEL PCR-FREE AND LABEL-FREE CLOTH-BASED DNA SENSOR FOR SENSITIVE AND RAPID DETECTION OF *ESCHERICHIA COLI*

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OUT LINE

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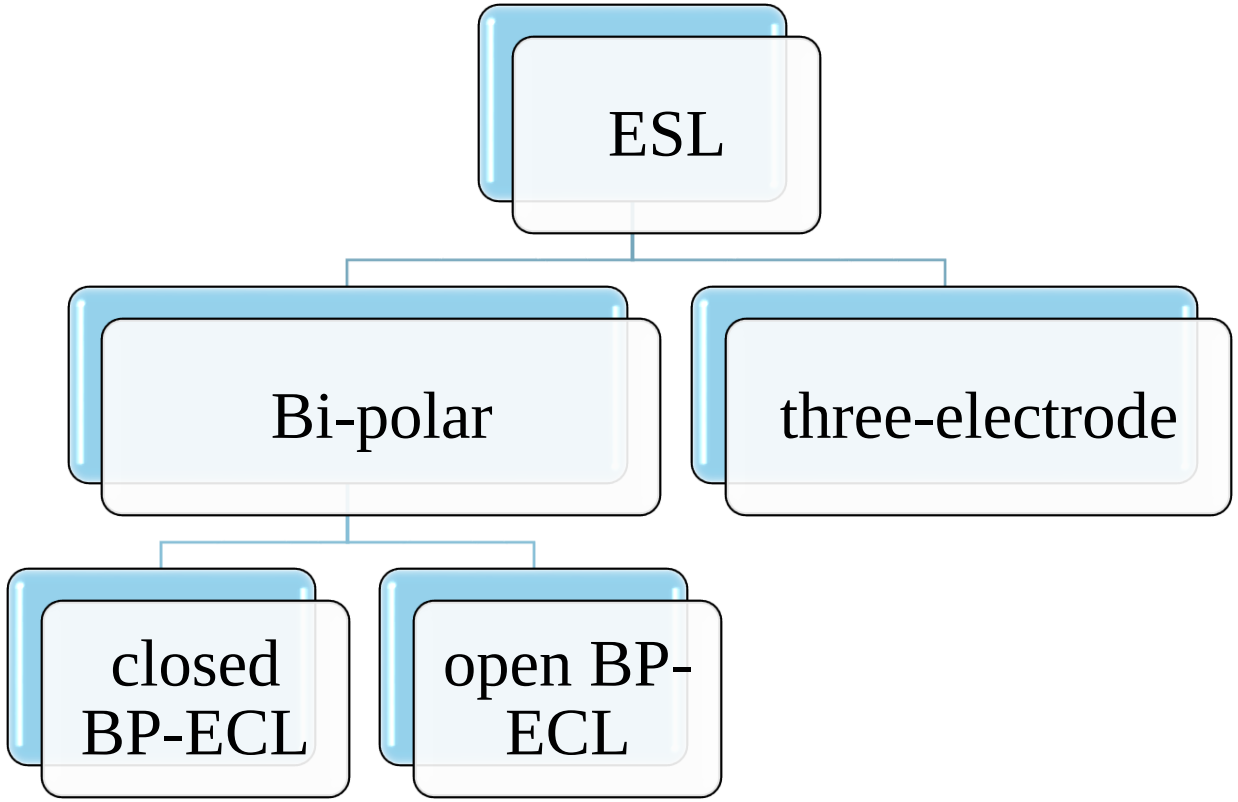
INTRODUCTION

- Bacterial detection methods are based on cultivation.
- electrochemiluminescence (ECL), electrochemical, colorimetry and fluorescence methods
- Biosensors can be used for the rapid and quantitative detection of bacteria.

INTRODUCTION



DNA SENSOR REMARKABLE FEATURES

1. low-cost and environmentally friendly
2. the target DNA (TD) can be quickly obtained
3. CP is immobilized onto C-BPE anode using CS and GA
4. $Ru(bpy)_3^{2+}$ is selected as the luminophore

EXPERIMENTAL SECTION

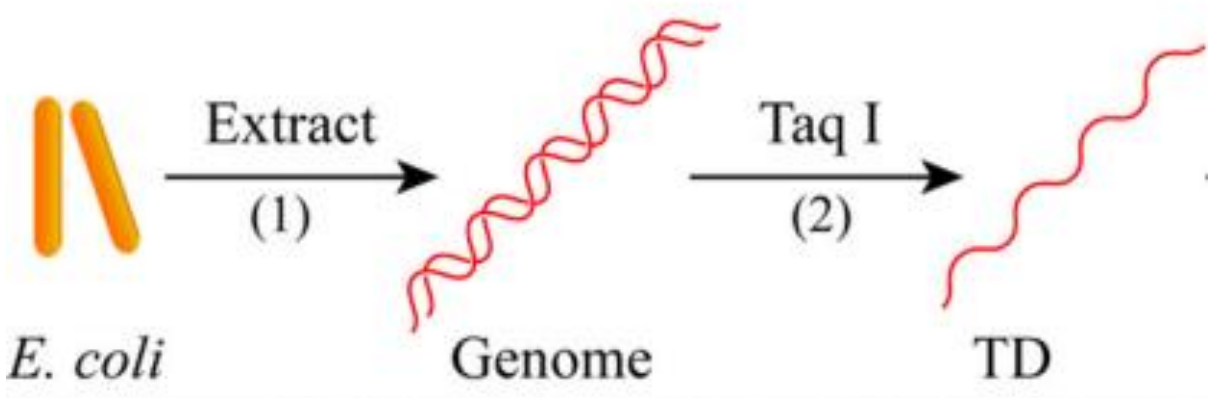
DESIGN AND PREPARATION OF DNA OLIGONUCLEOTIDES

- The synthetic CP, auxiliary probe (AP), and hairpin DNA (HD).

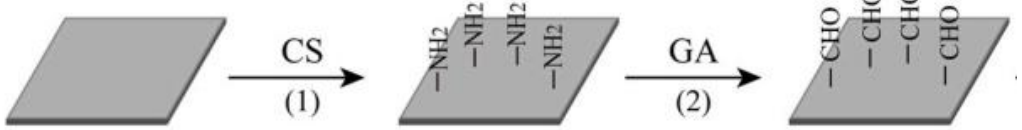
- HD1 and HD2 were heated at 95 ◦C for 2 min and then cooled at 4 ◦C for 1 h to form the hairpin structure.

PREPARATION OF TARGET DNA

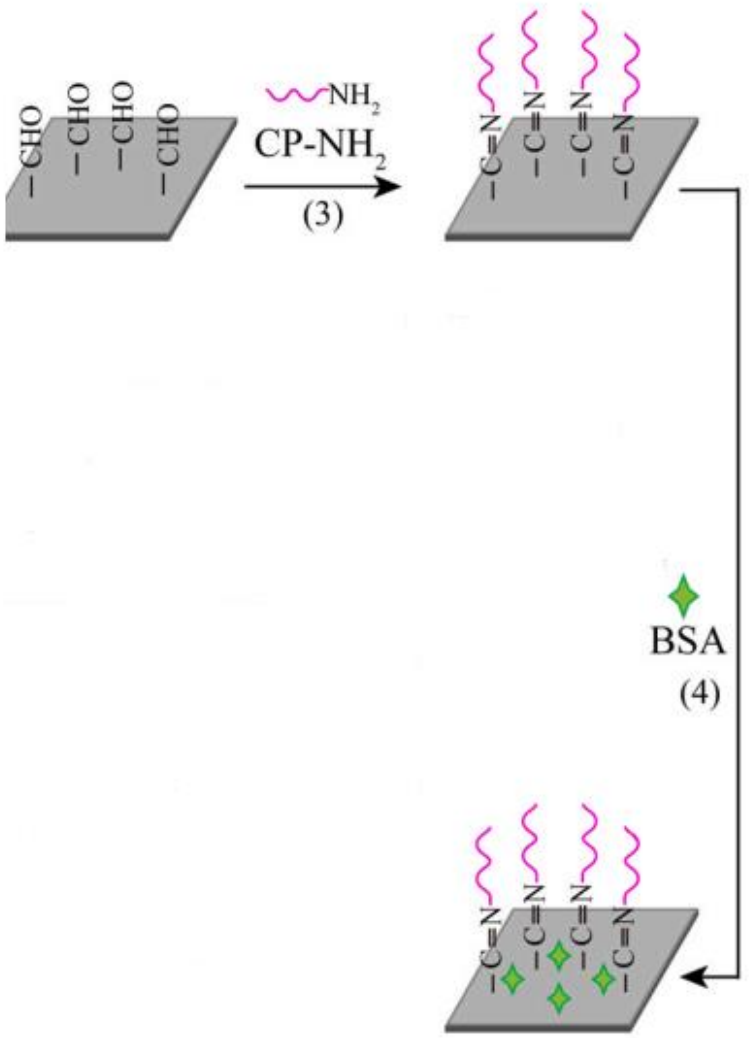
- *E. coli* strains revived in a 37°C water bath for 30 min
- Cultured in Luria-Bertani (LB) broth at
- Bacteria resuspended in PBS to obtain pure bacterial suspension
- Initial bacterial suspension diluted with PBS to form concentrations from 10^2 to 10^8 CFU/mL
- Genomic DNA digested at 65°C for 30 min in a enzyme reaction system



FABRICATION OF CLOTH-BASED DNA SENSOR



- Cloth-based device composed of electrodes and hydrophilic channels
- Fabricated through simple screen printing with carbon ink and crayon
- C-BPE anode (CBA) applied with CS (CS/CBA, step 1)
- CS/CBA dropped with , incubated at 37°C for 30 min to introduce the formyl group (-CHO) (GA/CS/CBA, step 2)

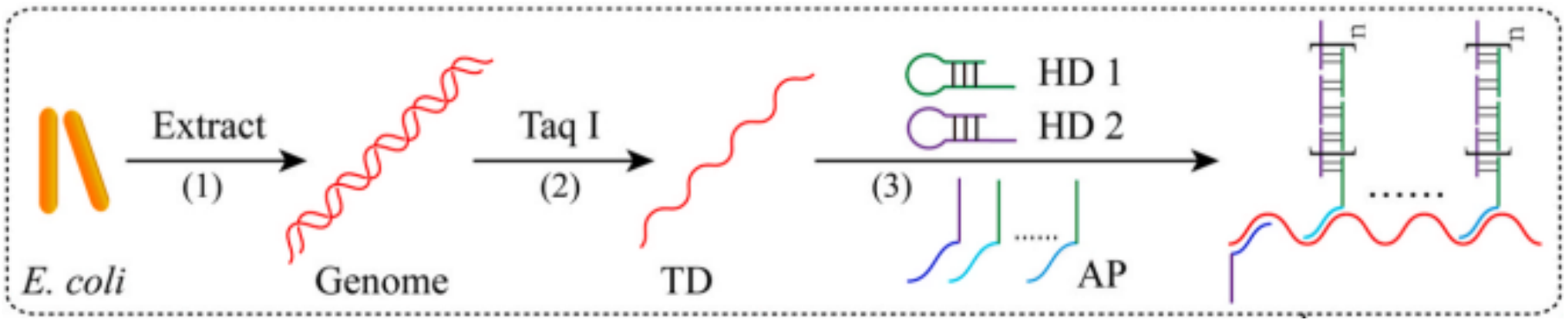


•CP covalently immobilized on GA/CS/CBA, incubated at 37°C for 30 min, anode washed with washing buffer three times to remove superfluous CP (CP/GA/CS/CBA, step 3)

•CP/GA/CS/CBA incubated with bovine serum albumin (BSA) at 37°C for 30 min to block nonspecific binding sites (BSA/CP/GA/CS/CBA, step 4)

•Desired DNA sensor obtained and stored in a 4°C refrigerator

- Before ECL measurement, a mixture of TD, AP1-8 , HD1, HD2 and TE buffer was prepared.
- The mixture was hybridized for 40 min in a 37 ◦C water bath to accomplish the ML-HCR for formation of the hybrid products of TD, AP and HD (i.e., T-ML-HCR complexes)



ECL ASSAY PROCEDURE

1. Measurement procedures involved applying T-ML-HCR complexes to the DNA sensor.

2. T-ML-HCR complexes captured onto C-BPE anode surface through base complementation of CP and AP1

3. C-BPE anode incubated with $Ru(bpy)_3^{2+}$ at 37°C for 30 min and washed three times with washing buffer

4. Cloth-based device fixed on a homemade bracket, driving electrodes stuck with conductive tapes

5. PBS and tripropylamine (TPA) added to supporting and reporting channels

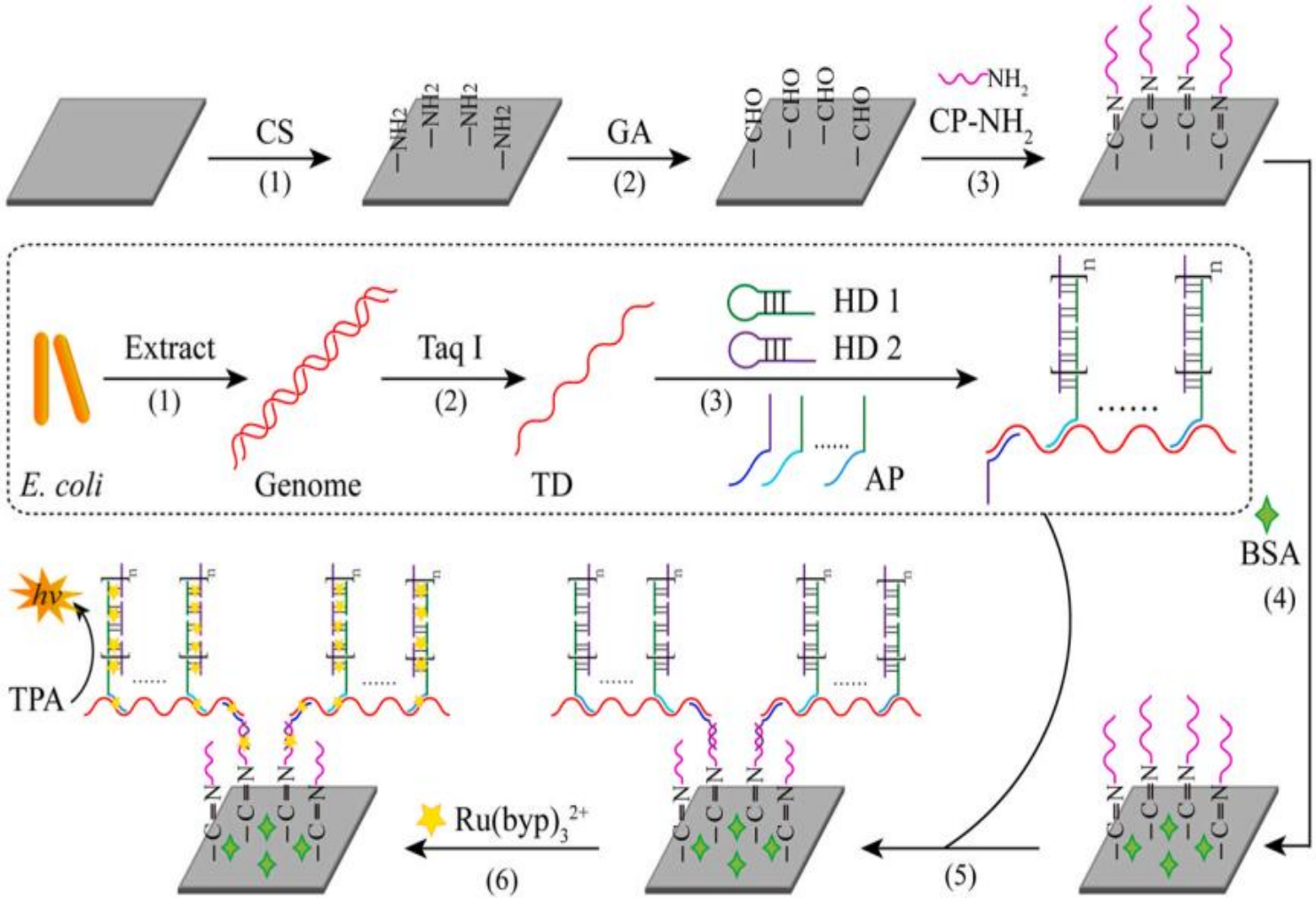


Fig. 1. Schematic illustration of the PCR-free and label-free DNA sensor for detection of *E. coli* (CS-chitosan; GA-glutaraldehyde; CP–NH_2–amino modified capture probe; TD-target DNA; HD1-harirpin DNA1; HD2-harirpin DNA2; AP-auxiliary probe; and TPA-tripropylamine).

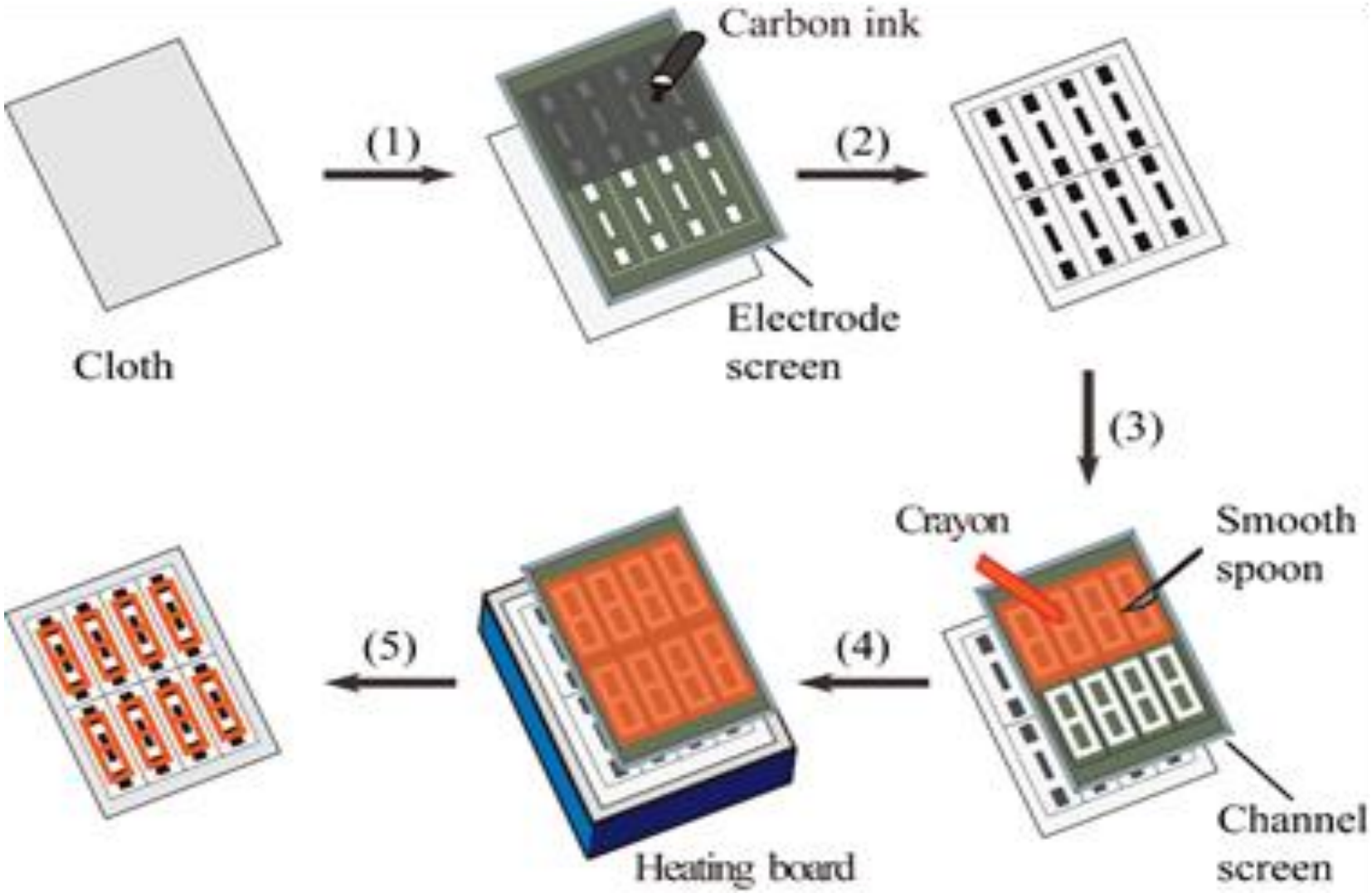
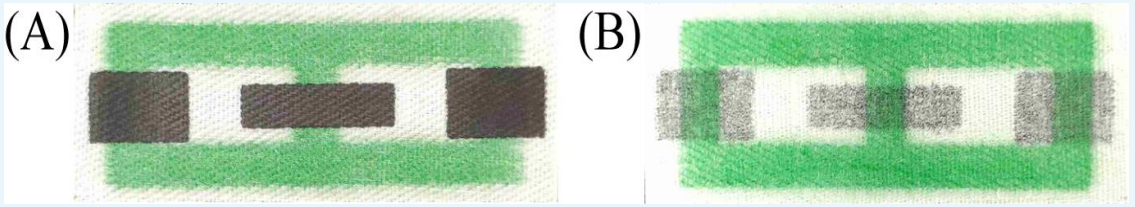
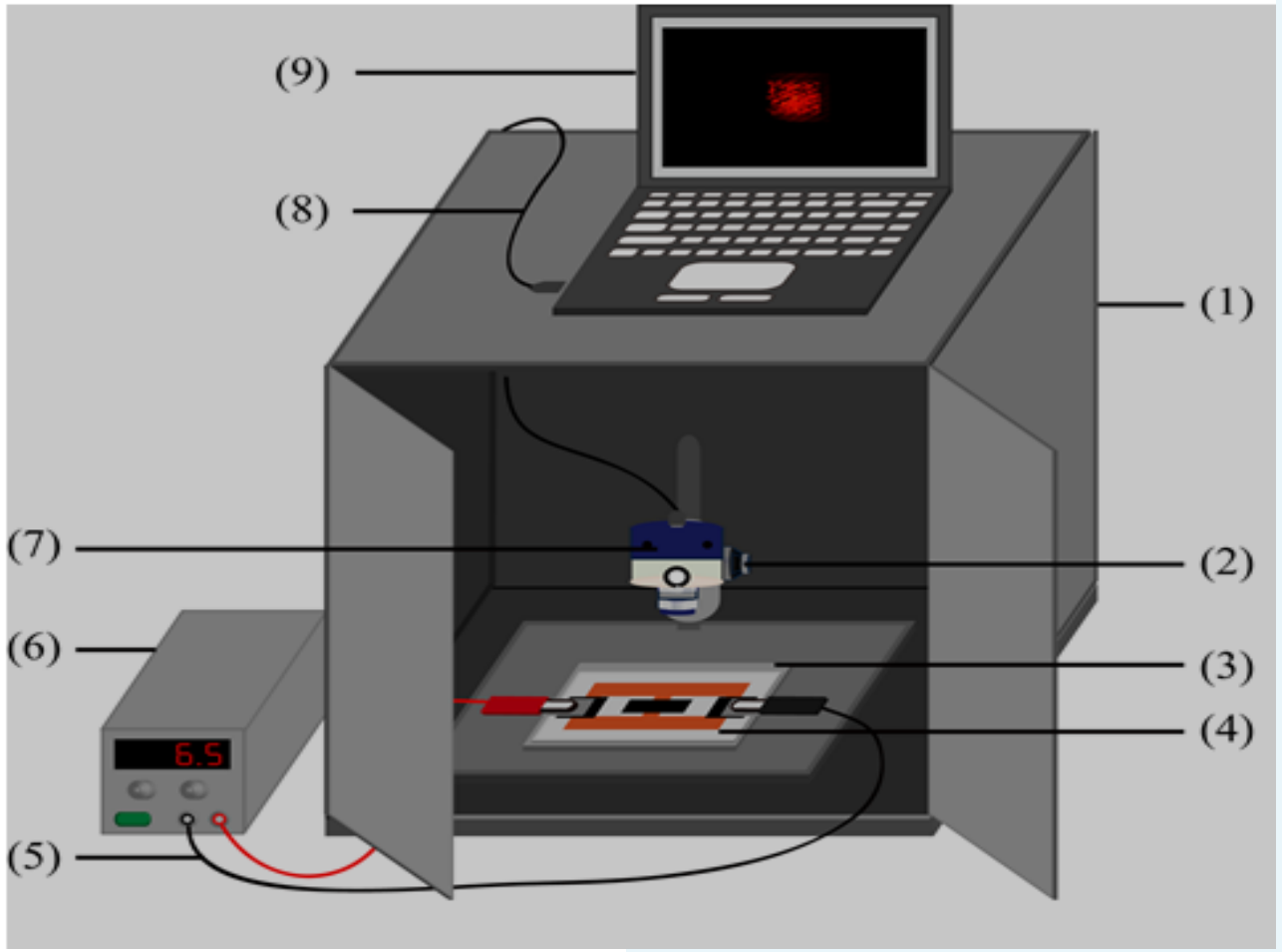

Carbon ink
(1)
(2)
Cloth
Electrode screen
(3)
Crayon
Smooth spoon
(5)
(4)
Heating board
Channel screen

Fig. S2. Photos of the cloth-based device. (A) Front of the cloth-based device; (B) Back of the cloth-based device.





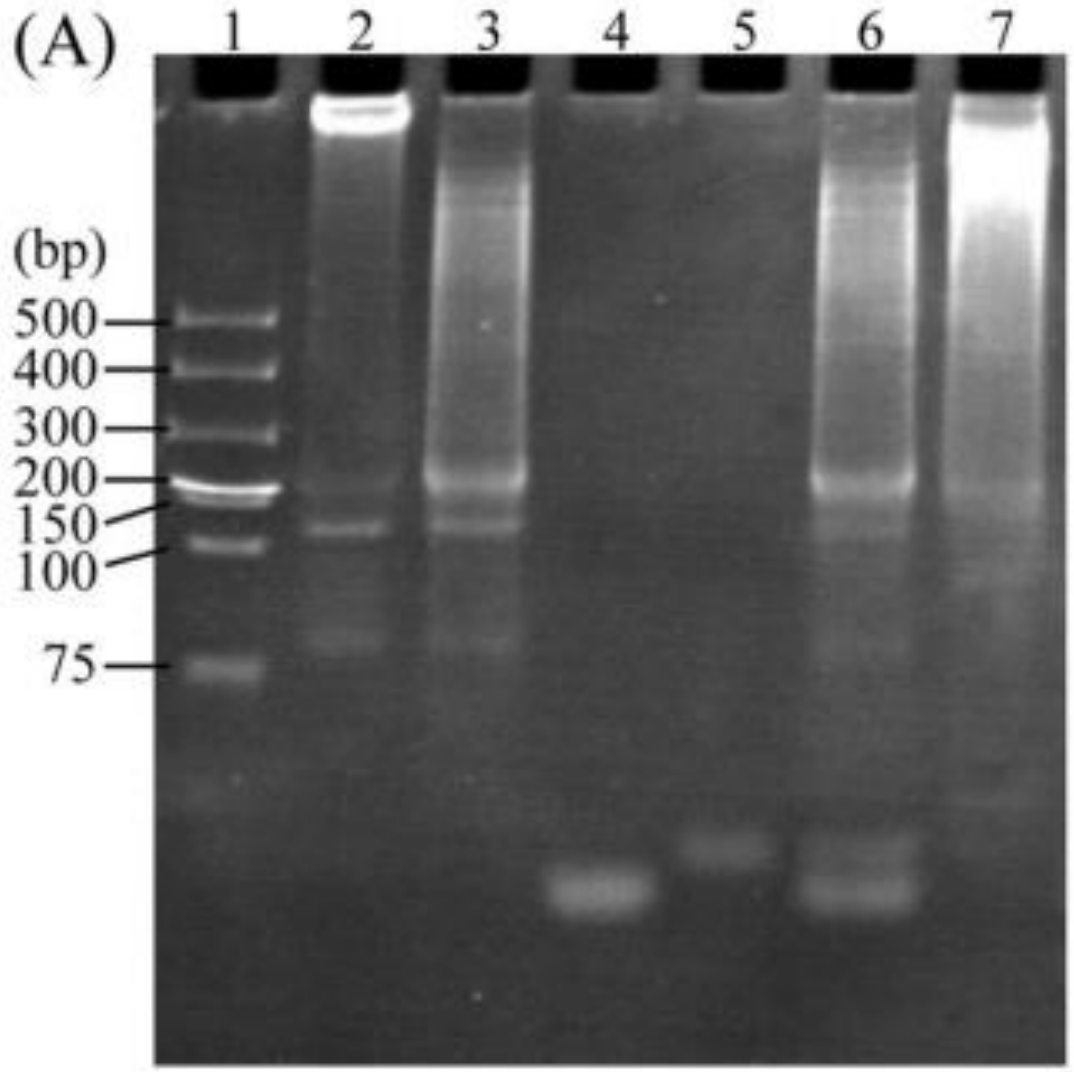
RESULTS AND DISCUSSION

CHARACTERIZATIONS OF THE PROPOSED ML-HCR PROTOCOL AND DNA SENSOR

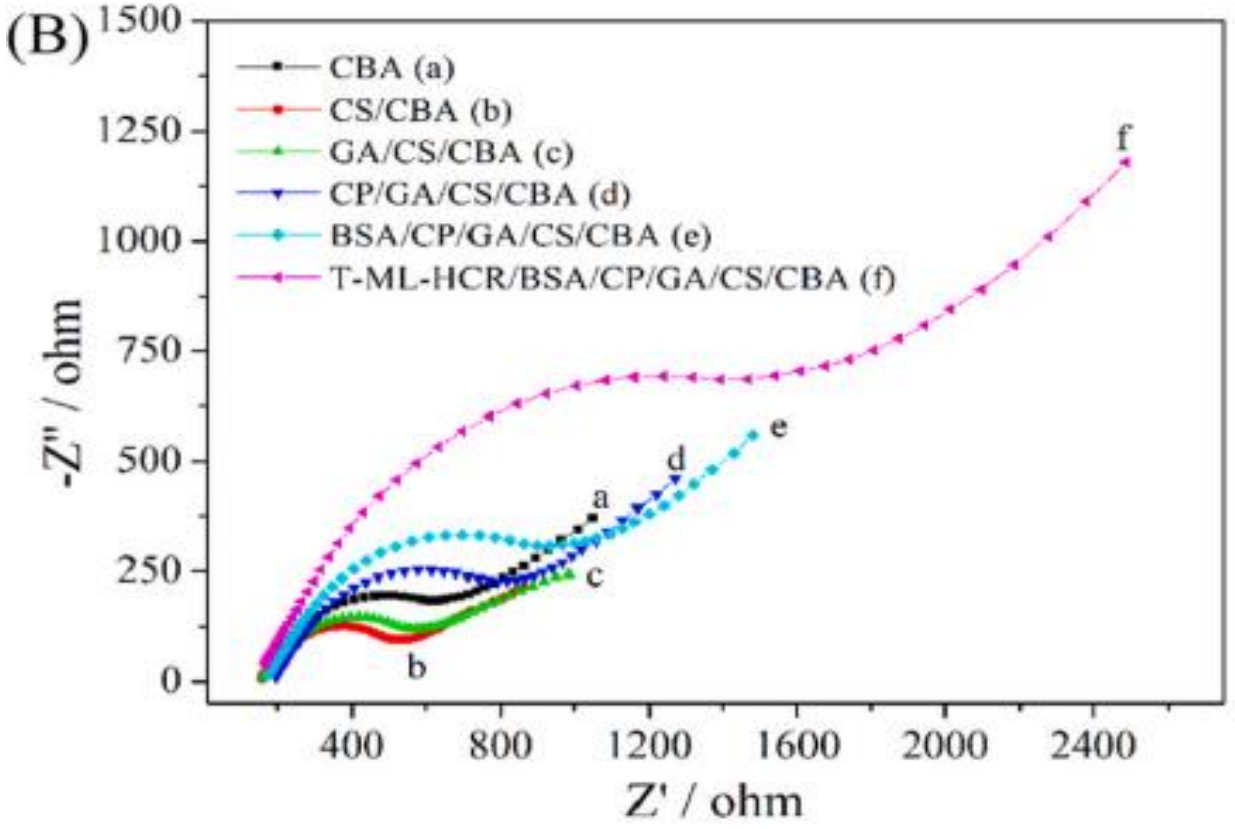
• A legible DNA band near the loading slot (lane 2)

•Lane 3 shows Taq I restriction enzyme digestive product, genomic DNA band disappears, and 200 bp band (TD) is obvious.

•HD1 and HD2 not opened or hybridized with TD, but triggered by AP2 in presence of digestive products (lane 7).



- CS/CBA has a smaller Ret value, indicating an improved electron transfer rate
- BSA and T-ML-HCR complexes dropped layer by layer, leading to a continuous increase in Ret (curves e and f)
- Increase in Ret suggests hindrance of electron transfer due to protein and nucleic acid macromolecules



CONDITION OPTIMIZATION

DRIVING VOLTAGE (ETOT):

•Etot of 6.5 V selected for optimal signal-to-background ratio (SBR).

CP (CAPTURE PROBE):

•Signal decreased beyond 0.7 μM due to decreased electron transfer rate.

AP (Auxiliary Probe) and HD (Helper DNA):

•Optimal [AP] chosen as 20 nM.

•Lack of HD resulted in low ECL intensity.

$RU(BYP)_3^{2+}$ AND TPA:

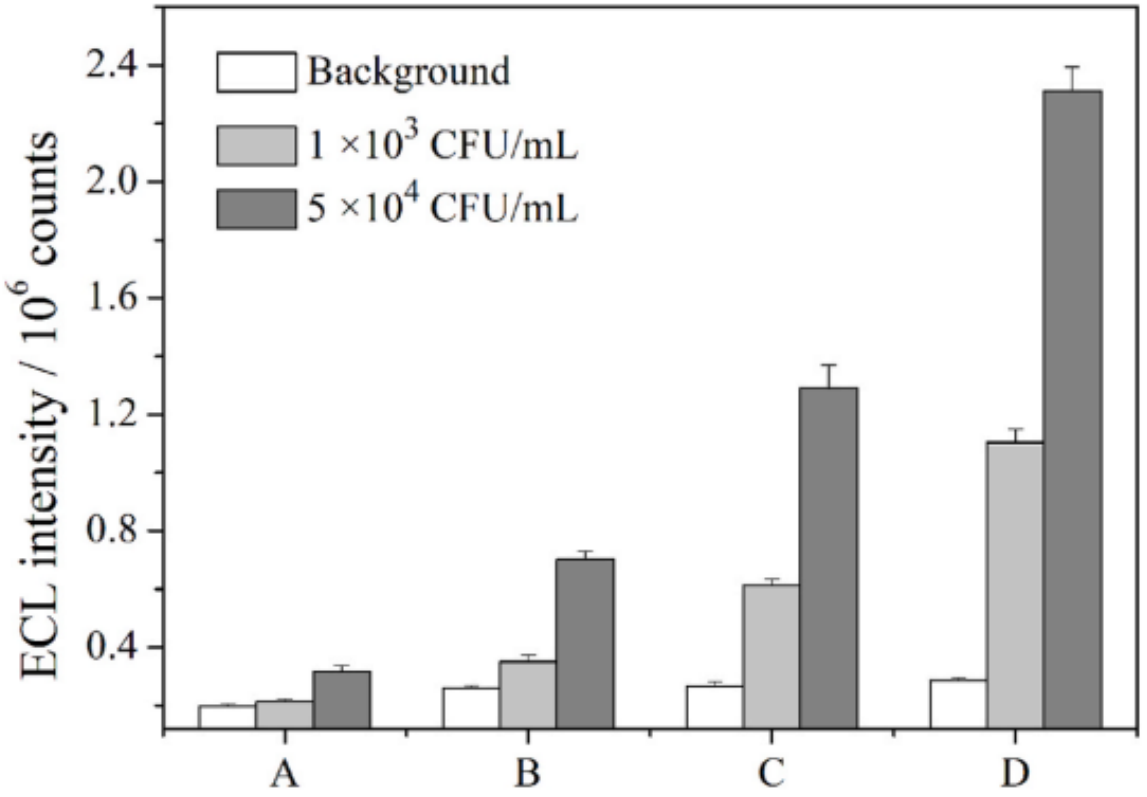
- Optimal [TPA] chosen as 25 mM.
- Optimal [$Ru(byp)_3^{2+}$] chosen as 2 mM.

Hybridization Time (th) and Incubation Time (ti):

- Signal value increased with ti, stabilizing after 30 min.
- Optimal th and ti selected as 40 min and 30 min, respectively.

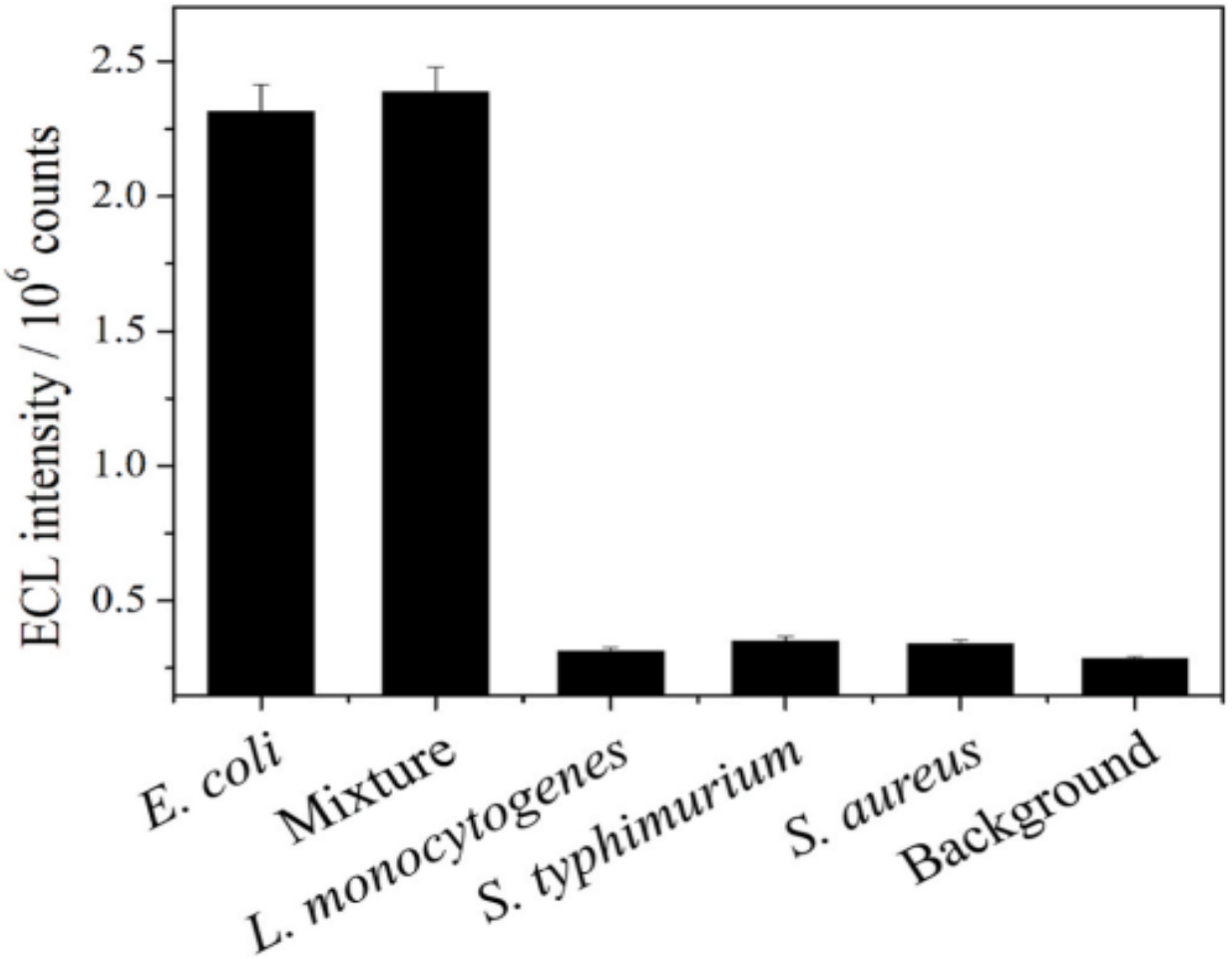
ECL CHARACTERIZATION OF THE ML-HCR PROTOCOL

- Non-HCR (scheme A),
- Single linear HCR (scheme B),
- Triplex linear HCR (scheme C)
- Septuple linear HCR (scheme D, i.e., the proposed ML-HCR protocol).



SENSITIVITY AND SPECIFICITY OF THE PROPOSED DNA SENSOR

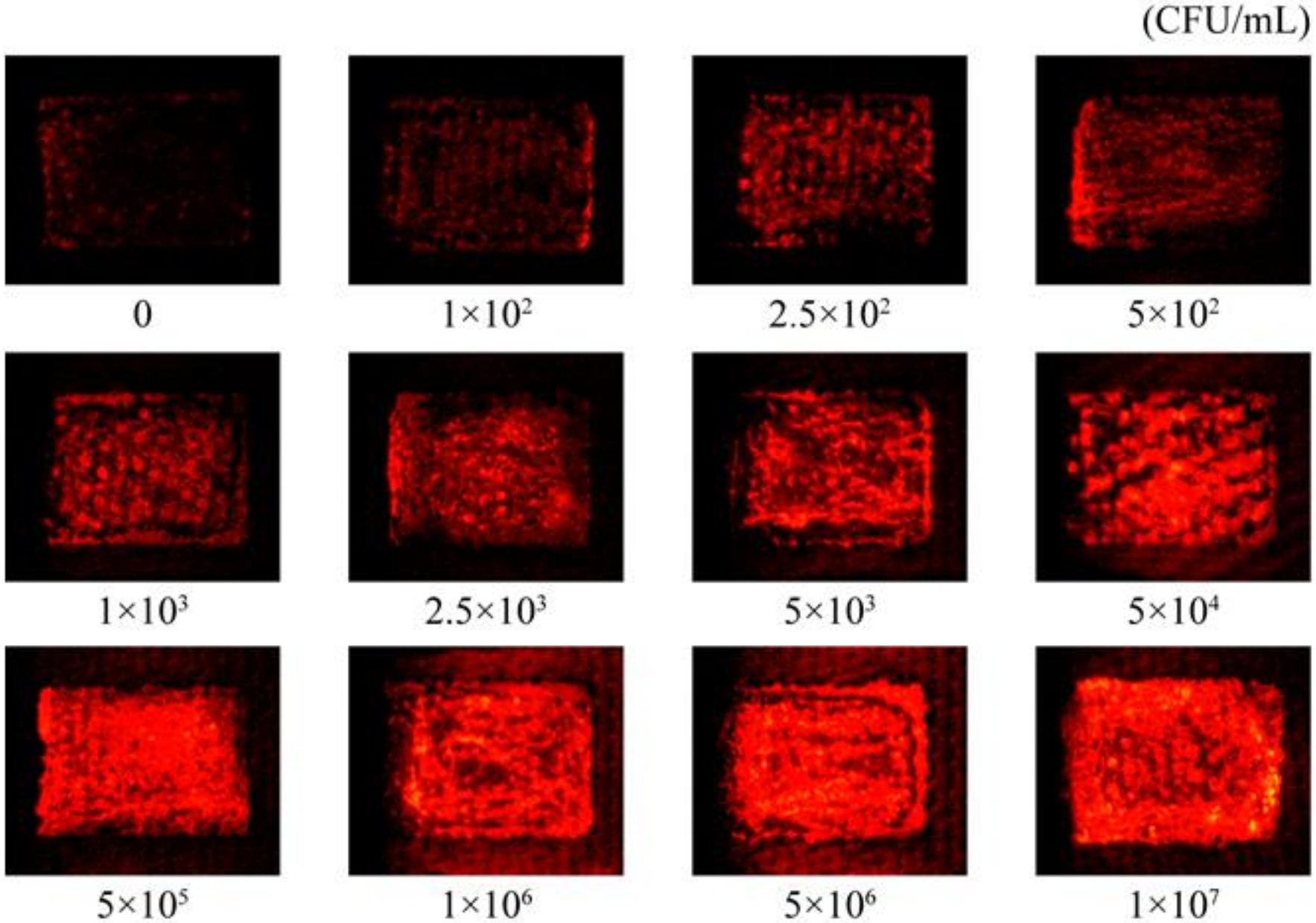
- ECL images for various *E. coli* concentrations were presented, with a detection limit of 38 CFU/mL .
- The ECL intensity from the mixture of the three bacteria was almost identical to that of *E. coli*, indicating the sensor's ability to distinguish *E. coli* from other bacteria.



APPLICATION IN REAL SAMPLES

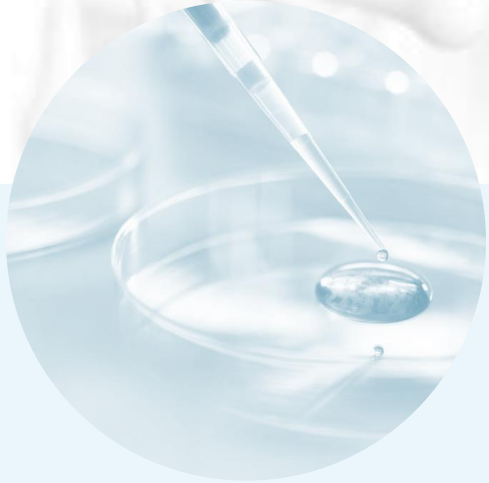
Table S2. Bacteria detection performance comparison of the proposed DNA sensor and other detection methods.

Method	Analyte	Signal amplification	detection range (CFU/mL)	LOD (CFU/mL)	Application
Colorimetry	*Salmonella*	Linear-HCR	1.0×10^4-1.0×10^7	3×10^3	*N*
Colorimetry	*Salmonella*	Gold &platinum nanocatalyst	3.5×10^2-3.5×10^5	350	Chicken and milk
Fluorescence	*Salmonella*	PCR and linear-HCR	4.2×10^1-4.2×10^7	42	Milk
Fluorescence	*E. coli*	Linear-HCR	4.9×10^1-4.9×10^6	35	Milk
EC	*S. aureus*	*N*	3×10^2-3×10^7	21.9	Tap water
	E. coli			25.1	
EC	*Enterobacteriaceae*	PCR and Exonuclease III-assisted target recycling	4.0×10^1-4.0×10^8	40	Milk
ECL	*S. aureus*	LSP	10^2-10^8	52	Clinical blood samples
ECL	*E. coli*	Direct format	5.0×10^2-5.0×10^5	120	*N*
		Sandwich format	1.0×10^3-5.0×10^5	230	
ECL	*E. coli*	ML-HCR	1.0×10^2-1.0×10^7	38	Milk


(CFU/mL)
0
1×10²
2.5×10²
5×10²
1×10³
2.5×10³
5×10³
5×10⁴
5×10⁵
1×10⁶
5×10⁶
1×10⁷

CONCLUSION

- The sensor has advantages such as easy preparation of cloth-based devices, elimination of complex PCR process, isothermal DNA amplification, and direct insertion of signal molecules into the DNA.
- However, the sensor requires further improvements, including the development of automated fabrication, simplification of detection processes, improvement of detection sensitivity, and miniaturization of the analysis system.



THANK YOU