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Article

# Electrochemical Detection of Genomic DNA Utilizing Recombinase Polymerase Amplification and Stem-Loop Probe

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**ABSTRACT:** Nucleic acid tests integrated into digital point-of-care (POC) diagnostic systems have great potential for the future of health care. However, current methods of DNA amplification and detection require bulky and expensive equipment, many steps, and long process times, which complicate their integration into POC devices. We have combined an isothermal DNA amplification method, recombinase polymerase amplification, with an electrochemical stem-loop (S-L) probe DNA detection technique. By combining these methods, we have created a system that is able to specifically amplify and detect as few as 10 copies/ $\mu$ L *Staphylococcus epidermidis* DNA with a total time to result of 70–75 min.

# INTRODUCTION

According to the criteria established by the World Health Organization (WHO) in 2014, a point-of-care (POC) diagnostic test should be rapid, equipment-free, affordable, and user-friendly as well as sensitive and specific.<sup>1</sup> Nucleic acid tests (NATs) that perform both nucleic acid amplification and detection represent an increasingly important class of diagnostic tools that could fulfil the WHO criteria. Isothermal amplification (RPA),<sup>2</sup> nucleic acid sequence-based amplification,<sup>3</sup> and loop-mediated isothermal amplification (LAMP)<sup>4</sup> have been proposed as alternative methods to the commonly used polymerase chain reaction (PCR)<sup>5</sup> in NATs. They are more suited to POC diagnostics because they eliminate the need for expensive and bulky equipment such as thermocyclers.

Among the various isothermal DNA amplification techniques developed so far, RPA has proven to be the most attractive option because it operates at low temperatures (37– 42 °C), and its primers are relatively simple to design and manufacture. LAMP, by comparison, operates at 60–65 °C with three sets of primers.<sup>6–9</sup> In this temperature range, the device can even be heated using the patient's own latent body heat, removing the need for any heating equipment.<sup>6</sup> RPA works through three proteins, a recombinase, a single-stranded DNA binding protein (SSB), and a strand-displacing polymerase available in a freeze-dried mixture, which is easy to transport to low resource settings and eliminates the need for cold chain storage.<sup>10,11</sup> The mechanism of DNA amplification by RPA has been published elsewhere<sup>12</sup> but will be briefly described here and is depicted in Figure 1A. Recombinase enzymes pair the primers with homologous DNA duplexes. Single-stranded DNA binding proteins bind to the displaced DNA, thereby preventing the displacement of the primers. A polymerase enzyme then begins DNA synthesis where the primer binds to the DNA. RPA has been shown to be able to amplify as few as 10 copies/ $\mu$ L DNA to detectable concentrations within 10–20 min.<sup>13,14</sup>

Stem-loop probe electrochemical DNA (E-DNA) detection has garnered some interest in the field of electrochemical diagnostics. An ssDNA probe molecule with a defined shape that holds a redox active tag in close proximity to the electrode surface is designed. Upon hybridization with a specific target sequence, which is complementary to the probe, the probe structure rearranges, and the redox tag is held further away from the electrode surface. This reduces the efficiency of the electron transfer between the electrode and the redox tag, which can be measured electrochemically. This is advantageous

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Figure 1. Schematic representation of the detection method showing how DNA was (A) amplified, (B) digested, and (C) detected. (A) Recombinase polymerase amplification (RPA) amplifies the DNA template at 38  $^{\circ}$ C. (B) Lambda exonuclease selectively digests the modified strand of the RPA product and relieves the single-stranded DNA (ssDNA), which contains the complementary sequence to the loop of the electrochemical probe. (C) Electrochemical detection of the unpurified ssDNA RPA target product by the stem-loop DNA probe labeled gold working electrode.



**Figure 2.** Gel electrophoresis image of RPA products and E-DNA sensor characterizations. (A) Gel electrophoresis image of unpurified RPA products with (10 copies/ $\mu$ L, 5 uL) of *S. epidermidis* genomic DNA and blank solutions before and after digestion by lambda exonuclease enzyme. (B) Cyclic voltammogram of the electropolished gold wire before and after labeling with stem-loop (S-L) probe in PBS (1×, pH 7.4) vs a silver pseudo-reference electrode at a scan rate of 50 mV/s. (C) Typical square wave voltammograms (SWV) of an electrode baseline in PBS and after incubating with 4  $\mu$ M of synthetic negative control and 4  $\mu$ M of synthetic target ssDNA. (D) Calibration plot showing the signal suppression (SS = ( $\Delta Ib - \Delta Iss$ )/ $\Delta Ib$ )), where  $\Delta Ib$  is the baseline current and  $\Delta I_{SS}$  is the suppressed current after hybridization of 0.2–8  $\mu$ M synthetic ssDNA target (*n* = 3). The inset shows the linear response of the E-DNA sensor equation in the presence of 0.2–1  $\mu$ M synthetic target sequence. The raw data can be found in the Supporting Information (Figure S1).

over other electrochemical sensing modalities where the redox molecules are added to the sensing solution. By having the redox tag bound to the probe, the sample matrix can be analyzed without any modification. There have been some recent successful attempts to electrochemically detect RPAamplified DNA.<sup>15–17</sup> These methods, however, all require multiple complex steps: the work by Lau et al.,<sup>15</sup> for example, requires gold nanoparticles, magnetic beads, heating, and wash

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Table 1. Sequences of the RPA Forward and	Reverse Primers,	the Stem-Loop (	S-L) Probe,	and Its Synthetic	Complementary
and Noncomplementary Targets					

RPA forward primer	5'-phosphate-TATAGGCTTAATTATCTCTGTTTTAGGAGCTT-3'
RPA reverse primer	5'-TGATAGGCACTATCTGTAAACAACATACTAAT-3'
S-L probe	5'-MeBlN/GCGAGGAAGCTCCGGTCAACGCTTCCTCGC/3'-ThioMC3-D
S-L complementary target	5'-TTTTTAAGCGTTGACCGGAGCTTCTCTTT-3'
S-L noncomplementary control	5'-CTTATAACCTATGTAGTATCCGTA-3'

steps, and the work by Sanchez-Salcedo et al.<sup>16</sup> requires multiple washing steps with different buffers. In order to create a point-of-care diagnostic device, having as few steps as possible is preferable as it cuts down on reagents, costs, and complexity. To address this, many in the field use probe molecules functionalized with redox reporters, such as stemloop probes. These have the advantage of not needing a redox agent added to the solution, which simplifies the system.<sup>18,19</sup>

Herein, we report for the first time the use of a stem-loop DNA probe to detect the RPA-amplified DNA of *Staphylococcus epidermidis* (Figure 1B,C), which we used as a model for nosocomial infections.<sup>20</sup> Previous works that combine isothermal amplification techniques with electrochemical detection do not achieve the same simplicity or sensitivity as has been achieved here.<sup>21,22</sup> The lack of a need for purification after amplification coupled with the low copy numbers of DNA required for RPA amplification makes this combination of methods suited for integration into POC devices.

### RESULTS AND DISCUSSION

RPA and Lambda Exonuclease. We first performed RPA using an "RPA target" solution, which contained all the RPA components and 10 copies/ $\mu$ L S. epidermidis DNA, and an "RPA blank" solution, which contained all the components for RPA but with no DNA (see the Experimental Section for more details). Gel electrophoresis confirmed the presence of the amplified target DNA (210 bp long) in the RPA target solution and nonspecific amplicons (~50 and 100 bp) in both the RPA blank and the target solutions (Figure 2A). Nonspecific amplification is a well-known weakness of low-temperature isothermal amplification methods; in RPA, for instance, the relatively low temperature with longer primers compared to PCR increases the possibility of primer-dimer formation and nonspecific amplification.  $^{14,23,24}$  Due to the presence of these nonspecific double-stranded amplicons, a detection protocol that is specific to the amplicon of interest is required. We, therefore, use stem-loop probes; however, these require the splitting of the dsDNA amplicons into ssDNA capable of binding to the probes.

To convert the dsDNA amplicons to ssDNA, we digested all the dsDNA RPA products using "lambda exonuclease" (see the Experimental Section).<sup>25</sup> The conversion of dsDNA to ssDNA is an essential step for any detection protocol where the probe sequence needs to hybridize via Watson–Crick base-pairing with the target. By designing the RPA forward primer with a phosphate group at the 5' end, it could be recognized by lambda exonuclease.<sup>26</sup> This enzyme selectively digests the phosphorylated strand of dsDNA (210 bp, target amplicon), generating ssDNA, which has a complementary sequence to the loop part of the probe. These ssDNAs are also 210 bases in length but migrate further in the agarose gel due to their higher electrophoretic mobility than dsDNA;<sup>27</sup> they, therefore, appeared at a lower band than dsDNA in the gel electrophoresis image (Figure 2A).

**Stem-Loop Probe Electrochemical Sensors.** We used a stem-loop-shaped single-stranded oligonucleotide with a methylene blue (MB) redox tag as our electrochemical DNA detection probe (Figure 1C). The stem-loop probes were covalently immobilized via the spontaneous formation of a Authiolate self-assembled monolayer on a gold wire, which was used as a working electrode. To achieve this, we designed and used the probe with a thiol modification on the 3' terminus (see Table 1 for the probe sequence).<sup>19</sup> The 5' terminus of the probe was labeled with the MB redox reporter. Upon binding of the target ssDNA, the stem-loop DNA probe undergoes a conformational change that positions the redox tag further away from the electrode surface (Figure 1C), leading to a reduction in the redox peak current of the tag.<sup>18,28,29</sup>

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The functionalized gold wire showed reversible redox peaks at +0.1 and -0.1 V versus a silver pseudo-reference electrode when cycled in a PBS solution in the CV, assigned to the oxidation and reduction of the methylene blue (Figure 2B). The presence of these redox peaks proved that the probe was bound to the surface in an orientation that allowed good reversible electron transfer from the methylene blue.<sup>30,31</sup> The average coverage of the stem-loop probe on the three labeled gold electrodes in the cyclic voltammogram was  $1.1 \pm 0.5 \times 10^{13}$  molecules/cm<sup>2</sup>. This surface coverage is in good agreement with other publications.<sup>31,32</sup> We determined the probe coverage using the charge under the methylene blue oxidation peak in CVs collected for three individual E-DNA sensors at scan rates of 50, 20, and 10 mV/s through eq 1

$$\Gamma = Q/nFA \tag{1}$$

where  $\Gamma$  is the surface coverage in molecules/cm<sup>2</sup>, O is the charge passed in coulombs, obtained by integrating the area under the oxidation peak in the CV, n is the number of electrons transferred per redox event (n = 2 for methylene)blue), F is Faraday's constant, and A  $(cm^2)$  is the active surface area of the cleaned gold electrode. The obtained average probe density and variability make it reasonable to assume that the probe packing and conformation and thus target accessibility are similar for each replicate. We first characterized the functionalized Au wires in PBS solution for 20 min with SWV recorded every 5 min to establish a baseline response (see Figure S2). We then performed control experiments by spiking the solution with a noncomplementary DNA sequence (4  $\mu$ M, Figure 2C and Figure S2). The specificity of the probe to S. epidermidis was confirmed by comparing with the noncomplementary sequence (Table 1). The noncomplementary DNA sequence did not cause a signal suppression as significant as the complementary target, evident of the specificity of the probe to homologous DNA sequences. The wires were assayed by making the PBS solution to increasing concentrations of complementary target DNA between 0.1 and 8  $\mu$ M. The resulting signal suppression (the percentage decrease of the methylene blue peak in the SWV after 10 min of binding) for each concentration averaged over the three devices tested is



**Figure 3.** Electrochemical DNA sensor characterization of unpurified RPA products. (A) Typical SWV scans for an electrode incubated with RPA blank (the same components as target solution but with nuclease-free water instead of genomic DNA). (B) Typical SWV scans from an electrode in the RPA target product (10 copies/ $\mu$ L *S. epidermidis* genomic DNA). (C) Signal suppression of the stem-loop DNA probe in the presence of RPA blank and RPA target over time. The results are from three electrodes exposed to RPA blank and three with the RPA target. The results are based on three individual labeled electrodes with three replicates of SWV. \*\* significant at p < 0.05 (n = 3).

shown in Figure 2D. Here, we can see a linear response between 0.2 and 1  $\mu$ M after which point the sensor is saturated by the target. The noncomplementary response produced a minor (6%) signal suppression (see Figure S2). Given that the equal concentration of complementary target produced a 63  $\pm$ 13% signal suppression under the same conditions, this small response from the noncomplementary DNA is postulated to be from the nonspecific binding of the DNA to the electrode interface, which could theoretically impede the methylene blue electron transfer. It is satisfying that the noncomplementary response is so low compared to the specific response of probetarget hybridization. The observed potential shift (Figure 2C) is attributed to the use of a silver wire as a pseudo-reference electrode, which could be fouling over time in the DNA-spiked PBS buffer. A pseudo-reference electrode was used as opposed to a formal reference electrode as this is more suited to POC applications. The technical limit of detection of the sensor is 0.5  $\mu$ M. However, RPA is able to amplify as few as 10 copies/  $\mu$ L S. epidermidis genomic DNA. Therefore, the limit of detection of the whole system is 10 copies/ $\mu$ L, which is lower than those of other isothermal amplification methods.<sup>16,25</sup>

Detecting RPA Products Directly. Having established that we can perform the RPA and digest the amplicons to relieve ssDNA as well as establishing that we can functionalize a Au wire with a probe that is capable of detecting our target sequence in ideal conditions, the next step was to combine the two and directly detect RPA amplicons using the functionalized Au wires. Figure 3A,B shows the SWV scans of an E-DNA sensor in the presence of unpurified RPA products. The digested RPA mixture consists of proteins, genomic DNA, polymerase and recombinase enzymes, lambda enzyme, and specific and nonspecific amplicons. These are all capable of fouling the electrode surface nonspecifically. We, therefore, tested the fouling of the unpurified RPA products by measuring the electrochemical response of a labeled wire in the RPA blank solution (which contains all of the RPA reagents and impurities except for the target amplicons) over a period of 20 min after incubation and compared that to the RPA target solution (where the target sequence has been amplified by RPA) (Figure 3C). In theory, there will be nonspecific fouling of the sensor surface as well as specific binding from probe-target hybridization in the RPA target solution. We found that the fouling in RPA blank solutions decreased the SWV signal by  $17 \pm 12$  and  $31\% \pm 11\%$  after 5

and 10 min of incubation times, respectively. Meanwhile, in the RPA target case, we recorded decreases of  $56 \pm 13$  and  $75 \pm 12\%$  after the corresponding incubation times, respectively. We can, therefore, conclude that the E-DNA sensor showed a significant difference between the RPA target and RPA blank samples after 5 and 10 min of incubation (*p*-value < 0.05, Table S1). These quick response times of only a few minutes compare favorably to other sensing modalities.<sup>33–36</sup> We can therefore conclude that the presence of amplified DNA has increased the rate of signal suppression. The value of the rate constant of signal suppression is not important for this system and varies inevitably between different probes; what matters is that the rate is greater in the presence of target amplicons than in the RPA blank solution.

For longer time periods, the electrode fouling and probetarget hybridization can no longer be significantly distinguished from each other. Therefore, a time threshold of 15 min should be set to detect the signal suppression of the RPA target product. After 15 min, the nonspecific fouling of the RPA reagent may cause false-positive results. These findings highlight the importance of time as a variable when making measurements in complex media. It is encouraging that shorter times allow for better detection as point-of-care diagnostic devices require rapid detection times. The ability to make measurements directly in the RPA product matrix without purification is, to the best of our knowledge, reported here for the first time. Without the need for purification steps, the overall time to have a result in any POC device is shortened, and all the equipment required for purification is removed. The combination of RPA and E-DNA sensing is, therefore, highly suited to incorporate into POC diagnostic devices. We are currently integrating this method into paper- and textile-based point-of-care NAT diagnostics.

# CONCLUSIONS

We have combined RPA, an isothermal amplification technique performed at 38 °C in under 30 min, with a stemloop probe for the electrochemical detection of amplified DNA. This method shows three distinct advantages: (i) It does not require any purification after the amplification step, which eliminates the need for purification equipment in POC devices. (ii) It is highly sensitive. The technical limit of detection of the sensor is 0.5  $\mu$ M; however, RPA is able to amplify as few as 10 copies/ $\mu$ L *S. epidermidis* genomic DNA, which can, therefore, be quoted as the limit of detection for the whole system, which is much lower than previous reports. (iii) It is quick because the binding kinetics of the DNA target to the DNA probe is faster than those of the nonspecific fouling in the complex RPA product media. This allows detection of the target DNA amplicons in less than 15 min, and the total time for amplification and detection was 70–75 min. These combined features open up possibilities to integrate RPA into the nextgeneration POC nucleic acid tests.

# EXPERIMENTAL SECTION

Materials. We purchased the TwistAmp Basic RPA kit and Oligonucleotide primers from TwistDX Limited (Cambridge, U.K.) and Eurofins Genomics Europe Shared Services GmbH (Germany), respectively. The stem-loop oligonucleotide probe was customized by Integrated DNA Technologies, Inc. (IDT, Iowa, USA). S. epidermidis ATCC 12228 was purchased from ATCC LGC Standards (USA). Lambda exonuclease enzyme, phosphate-buffered saline (PBS) tablets pH 7.4, nuclease-free water, and 10× Tris-borate-EDTA (TBE) buffer were purchased from Fisher Scientific (Sweden). Gold wire of 100  $\mu$ m diameter (99.99% trace metals basis), silver wire of 100  $\mu$ m diameter (99.99% trace metals basis), Tris (2-carboxyethyl) phosphine hydrochloride (TCEP), 6-mercapto-1-hexanol (6hydroxy-1-hexanethiol, 97%), and sulfuric acid (99.999%) were purchased from Sigma-Aldrich (Sweden). The QIAamp DNA kit (QIAGEN, Germany) was used for the extraction and purification of genomic DNA.

**RPA Primers and Stem-Loop Oligonucleotide Probe Design.** To amplify the 210 bp target amplicon for the SE-0105 gene of *S. epidermidis* ATCC 12228, we designed TwistAmp Basic kit primers through Primer3 Output and IDT primer design tools. The DNA sequences of the primers are shown in Table 1. We designed a stem-loop (S-L) probe of an oligonucleotide modified with methylene blue (MB) and disulfide (C3S-S) modifications at the 5' and 3' ends, respectively. The synthesis and modifications of the probe were done by Integrated DNA Technologies (IDT) and verified through electrospray ionization mass spectroscopy (ESI-MS, see Figure S3). To convert the double-stranded RPA target amplicon to ssDNA through the lambda exonuclease enzyme, the forward RPA primer was modified with a phosphate group at the 5' end, as shown in Table 1.

**RPA Amplification and Gel Electrophoresis.** We carried out the RPA amplification in 50  $\mu$ L reaction volumes by preparing a "master mix" solution containing 2.1  $\mu$ L of forward primer (8  $\mu$ M), 2.1  $\mu$ L of reverse primer (8  $\mu$ M), 29.5  $\mu$ L of rehydration buffer, and 8.2  $\mu$ L of nuclease-free water followed by rehydrating a freeze-dried RPA enzyme pellet (lyophilized polymerase and recombinase enzymes) using the prepared master mix. We then added either 5  $\mu$ L of 10 copies/ $\mu$ L genomic S. epidermidis DNA template suspension (extracted and purified with QIAamp DNA kit, QIAGEN, Germany) or nuclease-free water to make up a total of 50  $\mu$ L of RPA target and RPA blank solutions, respectively. The RPA reaction was initiated by adding 2.5  $\mu$ L of MgAc (240 mM) to the lid of the reaction tubes: capping the tubes, spinning magnesium acetate into the solution, inverting the tubes vigorously 8-10 times to mix, and spinning down the solution once again. Next, we placed the tubes in a thermal cycler (UNO96, VWR) at a constant 38 °C for 30 min. After 4 min from the start of the reaction, we took out the tubes and inverted them vigorously 8-10 times to mix, spun down (MiniStar microcentrifuge,

VWR), and returned them to the thermal cycler for the total reaction time. The RPA product solutions were stored at 4 °C until use. We confirmed the efficiency of the RPA reaction through agarose gel electrophoresis. We stained 5  $\mu$ L of the unpurified RPA target (10 copies/ $\mu$ L genomic *S. epidermidis* DNA template) and the RPA blank solutions with 1  $\mu$ L of DNA staining dye (Thermo Scientific #SM0373) and loaded 5  $\mu$ L of the solutions in a prepared 3% agarose gel in TBE (0.5×) buffer.

**Digesting RPA Products to Single-Stranded DNA.** We mixed RPA target and RPA blank solutions each with 1  $\mu$ L of lambda exonuclease enzyme (1 U) and 4  $\mu$ L of lambda exonuclease buffer, provided in the enzyme kit for 50  $\mu$ L of reaction mixture, incubated them at 37 °C for 25 min, and stopped the reaction by raising the temperature to 80 °C for 10 min. The solutions were then stored at -20 °C.

Fabrication of the E-DNA Sensor. The labeling protocol was adopted from the Xiao et al. reported protocol.<sup>37</sup> Briefly, we electropolished gold microwires (100  $\mu$ m diameter) by cycling the electrodes in 0.5 M sulfuric acid solution from -0.4to +1.35 V (vs Ag/AgCl) at a scan rate of 100 mV/s until the cyclic voltammogram stabilized.<sup>38</sup> 3  $\mu$ L of 10 mM TCEP was added into each of 1  $\mu$ L of 200  $\mu$ M solutions of thiolated MBmodified oligonucleotide probe and incubated in the dark for 1 h. After incubation, we added 1  $\mu$ L of TCEP (10 mM) to the probe solution and incubated it for another 30 min until the solution turned transparent. Afterward, we brought the final volume of the reduced DNA probe solution up to 200  $\mu$ L by diluting with PBS buffer (1×, pH 7.4). The electrochemically cleaned gold microwires were then directly transferred to 200  $\mu L$  of reduced probe solution and incubated at room temperature for 2 h in the dark followed by rinsing with MQ-water and drying with nitrogen gas. We transferred the electrodes to the freshly prepared 2 mM 6-mercapto-1-hexanol solution and incubated them for 4 h at room temperature in the dark to passivate the unlabeled electrode sites. Afterward, we rinsed the 6-mercapto-1-hexanol by soaking the electrodes in four MQ-water vials subsequently for the whole 2 min and stored the labeled gold electrodes in PBS buffer  $(1 \times, pH 7.4)$ up to 1 month at 4 °C in the dark.

Electrochemical Measurements. We carried out the electrochemical DNA sensing (E-DNA sensing) using threeelectrode geometry consisting of an S-L probe labeled 100  $\mu$ m diameter gold wire as a working electrode, silver wire (100  $\mu$ m diameter) as a pseudo-reference electrode, and a 100  $\mu$ m diameter gold electrode (double in length relative to the working electrode length) as a counter electrode.  $1 \times PBS$  was used as the electrolyte. The synthetic target, synthetic control, unpurified RPA blank, or unpurified DNA target products were separately added into the PBS solution, and the electrodes were immersed in the mixture to the same depth (5 mm) to characterize the probe performance. We conducted electrochemical characterization (VSP, Cromocol potentiostat, Bio-Logic Scientific instrument, Scandinavia) by performing cyclic voltammetry (CV) between -0.2 and +0.2 V versus the silver pseudo-reference electrode at a scan rate of 50 mV/s as well as square wave voltammetry (SWV) in the same setup between -0.05 and +0.15 V at a frequency of 50 Hz, amplitude of 20 mV, and a step potential of 10 mV.

To obtain a baseline signal, we incubated the E-DNA sensor in 500  $\mu$ L of target-free PBS buffer (1×, pH 7.4), carried out CV and SWV in 5 min intervals until a stable peak current was observed (typically after 10–20 min), and used that scan as a

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baseline for further comparison. Afterward, we added 1  $\mu$ L of either unpurified RPA target or unpurified RPA blank solutions treated with the lambda exonuclease enzyme to the same 500  $\mu$ L of PBS media (1×, pH 7.4, thereby performing a 500-fold dilution of the RPA products) and measured the electrochemical responses by SWV every 5 min over 20 min. Each measurement was repeated three times with three individual E-DNA sensors for RPA target and RPA blank solutions. We also investigated the E-DNA sensor performance by incrementally increasing the synthetic target concentration in 500  $\mu$ L of PBS buffer (1×, pH 7.4) for 10 min of hybridization time. This was repeated on three individual E-DNA sensors in triplicate SWV measurements on each electrode, and the calibration plot was plotted based on the obtained results of the mean and standard deviations from three SWV measurements on each of the three electrodes for each concentration.

We calculated the signal suppression of the E-DNA sensors by subtracting the peak  $\Delta I$  for RPA blank and RPA target solutions from the peak  $\Delta I$  for PBS and dividing the obtained value by the baseline current peak (the peak  $\Delta I$  for PBS), eq 2

signal suppression (SS) = 
$$(\Delta Ib - \Delta Iss)/\Delta Ib)$$
 (2)

where  $\Delta I b$  is the baseline current and  $\Delta I s$  is the suppressed current after hybridization of the ssDNA target. The limit of detection (LOD) of the E-DNA sensor is based on the  $3\sigma$ (standard deviation) of the average signal suppression of three blanks divided by the slope of the calibration plot.<sup>39</sup>

## ASSOCIATED CONTENT

## Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.0c00341.

Figure S1. Square wave voltammograms (SWV) of three separate E-DNA sensors showing their responses in the presence of PBS (1×) and after incubation with 0.2–8  $\mu$ M synthetic target DNA, Figure S2. Signal suppression of the three individual E-DNA sensors incubated in PBS (1×) for 20 min in 4  $\mu$ M synthetic ssDNA negative control (NC) and 4  $\mu$ M synthetic ssDNA target for 10 min of incubation, and Figure S3. Electrospray ionization mass spectroscopy (ESI-MS) spectrum of the stem-loop probe from Integrated DNA Technologies. Table S1. ANOVA analysis of the sensor responses (PDF)

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#### **Author Contributions**

This manuscript was written through contributions of all authors. All authors have given approval to the final version of the manuscript.

# Notes

The authors declare no competing financial interest.

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