Electronic Supplementary Information (ESI)

Materials and Methods

Reagents

All electrochemical reagents were purchased from Sigma Aldrich and used as received, unless otherwise stated. DNA oligos were purchased from Integrated DNA Technologies (USA). Sequences are given in Table S1. Strepavadin-HRP was purchased from BD Biosciences (USA, Cat # 554066) and used at 1:500 dilution in 1xPBS buffer.

Table S1 Sequences of DNA oligos used in 5' to 3' orientation. Modifications are as indicated. DNA base being interrogated is highlighted in bold font and underlined.

Oligos	5'-Sequence-3'
Capture	HS-C3-CCAGTTACAGATCGTCATGTTC
Black Probe	CCAGTTACAGATCGTCATGTTCAACGCCCCTCGACGCACCTAACC**G**
Red Probe	Phos-CCCTACCCGCCTACCTAACCGA-C6-biotin
Blue Probe	TCGGTTAGGTAGGCGGGTAGGG**C**
Grey Probe	Phos-GGTTAGGTGCGTCGAGGGGCGTT
Matched Target	GGGATTCGGTTAGGTAGGCGGGTAGGG**C**GGTTAGGTGCGTCGAGGGGC GTTTTTA
Unmatched Target	GGGATTCGGTTAGGTAGGCGGGTAGGG**T**GGTTAGGTGCGTCGAGGGGC GTTTTTA

LCR protocol

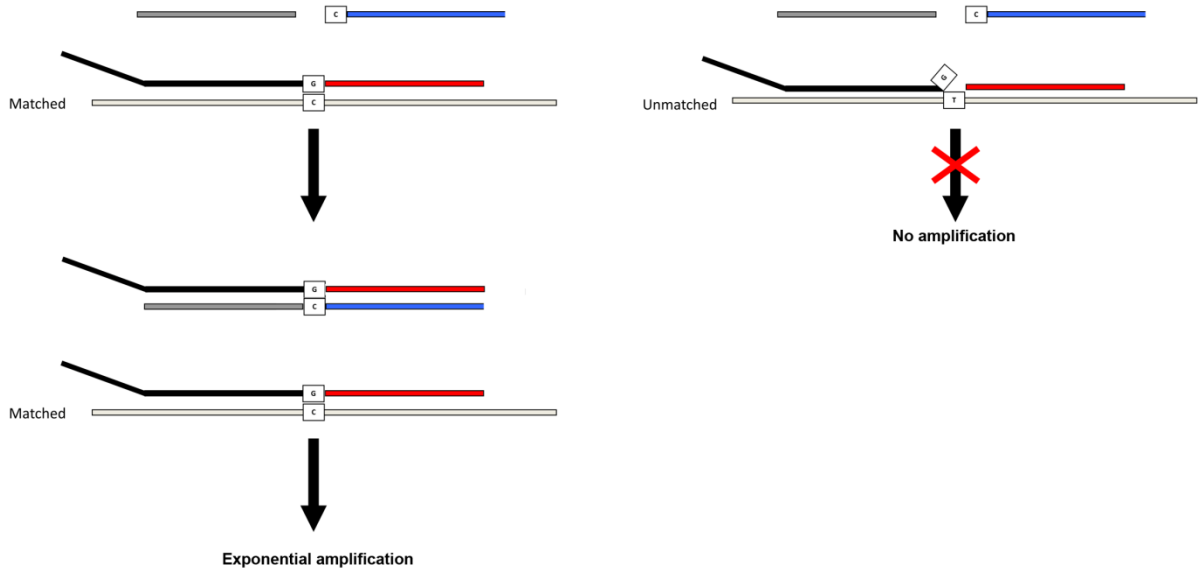

Fig. S1 Ligase chain reaction. Perfectly matched sequences allowed for the black and red probes to anneal and ligate whereas no ligation can occur in the presence of an unmatched sequence. Ligated black/red DNA can then serve as a template for the blue and grey

probes, and ligated blue/grey serve as templates for subsequent black and red probe reaction, resulting in exponential amplification.

Fig. S1 shows the LCR mechanism of DNA amplification. Taq ligase (NEB) was used for LCR as recommended. 100 nM of each DNA probe was used in a 25 µL LCR reaction. 50 ng of salmon sperm DNA was used as a blocking agent and to simulate background DNA situations in a cellular system. Following thermocycling (1 cycle of 95°C for 3 min, 15 cycles of 95°C for 30 s followed by 62.5°C for 2 min), products were electrophoresed through agarose and stained with ethidium bromide, and visualized on a UV transilluminator. For fluorescence intensity quantification, gels were scanned on a Typhoon 9400 (GE Health) and densitometry was performed using ImageJ.

In this proof of concept study, the 0.04 pM template was chosen as lower concentration because at this concentration, 75 bp long 'knife' products could be readily visualized by ethidium bromide fluorescence, and hence any detectable voltammetric signal could be validated. In addition, 0.04 pM also approximates the typical copy number in 50 ng of cellular DNA. The upper limit of the template concentration used in this study was 40 pM because LCR became non-specific if overloaded with excess template.

Functionalization of gold electrodes

Gold marcrodisk (diameter = 3 mm) working electrodes were purchased from CH Instrument (Austin, USA). Prior to electrochemical experiment, the electrodes were cleaned physically with 0.1 micron alumina, sonicated in acetone for 20 min, and chemically with piranha solution (H_2SO_4:H_2O_2; 3:1) for 30 seconds to remove any organic impurities (*Note: Piranha is a highly toxic and hazardous chemical. Wear proper PPE while dealing with such chemicals. Also dispose them by flushing lots of water down the sink*) and finally electrochemically in 0.5 M H_2SO_4 until characteristic gold electrode profiles were achieved. The effective working area of the electrodes were determined under linear sweep voltammetric conditions for the one-electron reduction of $K_3[Fe(CN)_6]$ [1.0 mM in water (0.5 M KCl)] and used of the Randles-Sevcik relationship.[1]

$$i_p = 0.4463nF(nF/RT)^{1/2}AD^{1/2}\upsilon^{1/2}C \quad ..... \quad ..... \quad ..... \quad (1)$$

where i_p is the peak current (A), n (=1) is the number of electrons transferred, A is the effective area of the electrode(cm^2), D is the diffusion coefficient of $[Fe(CN)_6]^{3-}$ (taken to be $7.60 \times 10^{-6} cm^2 s^{-1}$), C is the concentration (mol cm^{-3}), υ is the scan rate (Vs^{-1}), and other symbols have their usual meanings.

Self assembly of monolayer (SAM) was then made using 4 µM of thiolated DNA and left to incubate for 12-16 h in 10xPBS. After which electrodes were washed in 10xPBS back filled with 0.5 mM 6-mercarpto-1-hexanol in 95:5 10xPBS/glycerol solution for 1 h at ambient conditions. Functionalized electrodes were then washed in 10xPBS and use immediately for hybridization.

Electrochemical experiments

Following LCR, products were mixed with 8.3 µL of 40xPBS to give a final concentration of 10xPBS. LCR products were then hybridized to previously functionalized electrodes for 1h at 40°C. After which, electrodes were washed once in 10xPBS and twice in 1xPBS. For methylene blue (MB) detection, electrodes were then incubated in 1.0 mM MB for 30 min at room temperature and washed three times with 5xPBS (50 mM phosphate buffer, 685 mM sodium chloride, and 13.5 mM potassium chloride, pH 7.4). For HRP detection, electrodes were incubated with HRP solution for 15 min at ambient temperature and washed three times with 5xPBS.

Electrochemical detection was performed using standard three-electrode system with a Ag/AgCl reference, Pt counter electrode, and DNA-modified gold electrode as working electrode. Initially, voltammetric responses were collected in both N_2-saturated and ambient 5xPBS running buffer with the use of cyclic voltammetry. Since both conditions yielded similar relative responses, ambient conditions were used for subsequent all voltammetric measurements. For HRP experiments, running buffer was supplemented with 1.0 mM H_2O_2 to monitor the electrocatalytic reduction current. The relative response was defined as

$$\textit{Relative response} = (R_x / R_{40\ \text{pM}\ \mathbf{M}}) \quad \ldots\ldots \quad \ldots\ldots \quad \ldots\ldots \quad (2)$$

where R was the current density (ampere/cm^2); x was 40 pM **M**, 4 pM **M**, 0.4 pM **M**, 0.04 pM **M**, 40 pM **UM** or **NoT**.

Nuclease treatment

With the MB intercalation approach, the voltammetric responses could be attributed to the three species of DNA on the electrodes: ssDNA free capture probes, unligated "short knife" dsDNA and ligated "long knife" dsDNA. To remove free ssDNA capture probes, mung bean nuclease (NEB, USA) was used to treat the DNA-modified electrodes as recommended by the manufacturer. After hybridization, electrodes were treated with mung bean nuclease for

30 min at 30°C. To inactivate the nuclease, electrodes were washed three times with 0.01% SDS in 1xPBS solution and prepared as indicated earlier for MB detection.

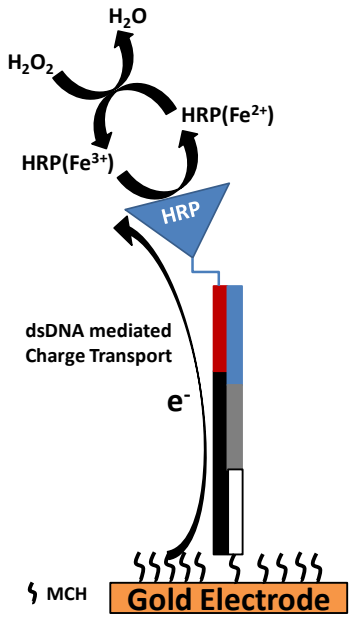

Fig. S2 Schematic representation of electrocatalytic reduction of H_2O_2 by HRP (Fe2+) at the DNA-modified electrodes. $HRP(Fe^{2+})$ is the product of one electron reduction of $HRP(Fe^{3+})$. Electron flow from the electrode surface to LCR product (red/blue + black/grey + white)-bound $HRP(Fe^{3+})$. Electrochemically generated $HRP(Fe^{2+})$ can easily reduce H_2O_2 and regenerate $HRP(Fe^{3+})$ that can continue on in the catalytic cycle. As depicted in the Scheme, $HRP(Fe^{3+})$ attachment is only possible at the top of the dsDNA, requiring charge transport through the DNA film. This process will continue until all H_2O_2 in the solution has been reduced or electrode is no longer at a potential to reduce $HRP(Fe^{3+})$.

EC methods can detect various amounts of LCR products

Figure S3 shows the cyclic voltammetric profiles when LCR products are detected electrochemically by MB (Fig. S3A), MB response after nuclease digestion (Fig. S3B) and by HRP labelling (Fig. S3C). With MB detection, two distinct peaks were observed; at -300 mV and -250 mV. The observed response could be attributed to the 3 species of DNA on the electrodes; ssDNA free capture probes, unligated "short knife" dsDNA and ligated "long knife" dsDNA. Since we were only concerned with detecting difference between ligated and unligated LCR products, removal of the ssDNA free capture probes could help de-convolute the observed MB response. A possible solution was to treat the electrodes after hybridization with a ssDNA specific endonuclease such as the mung bean nuclease. After nuclease digestion of ssDNA, the -250 mV peak was largely abolished, suggesting that the -250 mV

peak corresponded to that of ssDNA. However, the response profile was still similar to that prior to ssDNA removal (Fig. 4), suggesting that MB response from of unligated LCR probes (short dsDNA) was the dominating EC response at low concentrations.

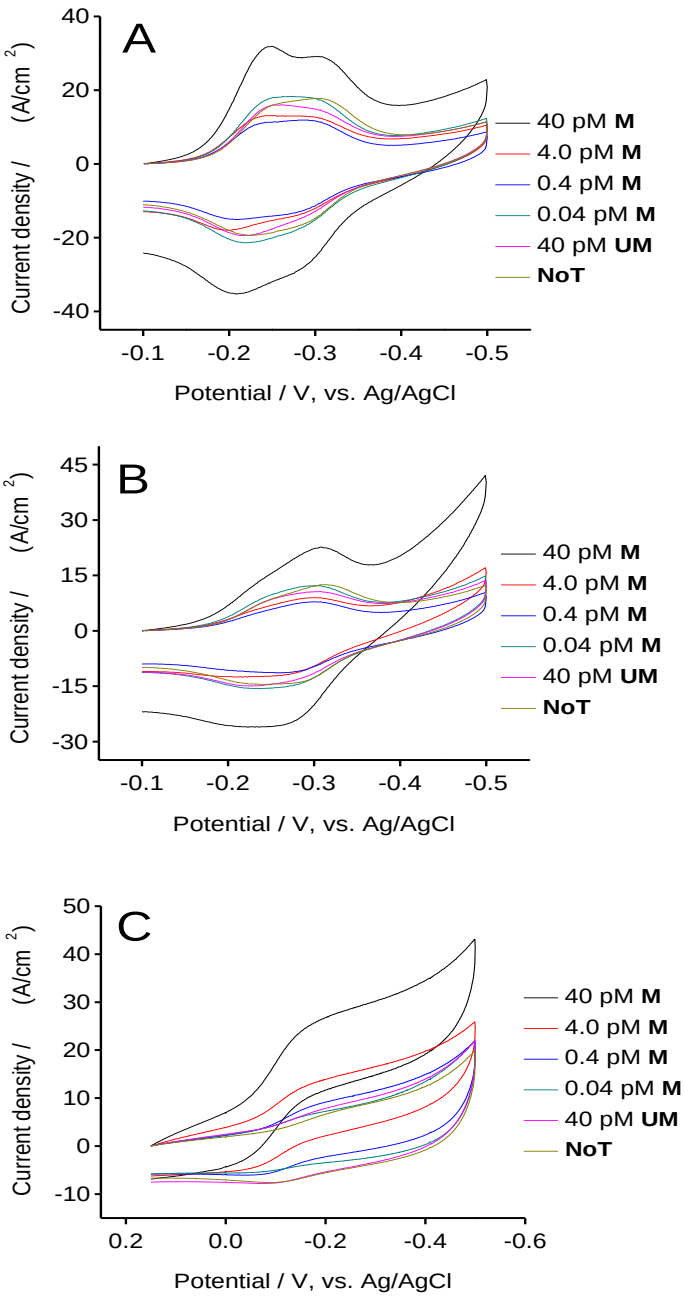

Fig. S3 Cyclic volatmmograms in 5xPBS buffer at a scan rate of 100 mVs^{-1} for reduction of MB at MB/DNA-modified electrodes before (A) and after (B) digestion. (C) Electrocatalytic reduction of H_2O_2 in 5xPBS buffer at a scan rate of 100 mVs^{-1} at HRP/DNA -modified electrodes.

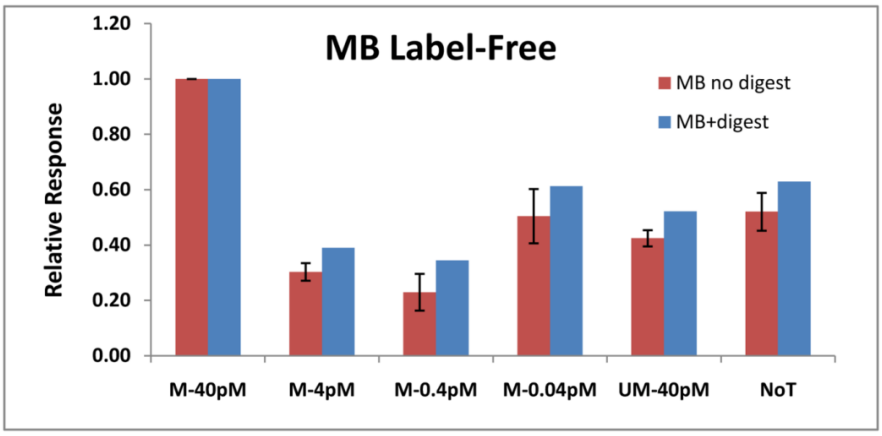

Fig. S4 Similar relative response profiles from MB before and after nuclease treatments. Red: MB response profiles before nuclease treatment. Blue: MB response profile after nuclease treatment. Errors bars are standard deviation from 3 independent experiments.

References

S1. A. J. Bard and L. R. Faulkner, *Electrochemical methods : Fundamentals and Applications*, John Wiley & Sons: New York, 2001.