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Gold Nanowire Based Electrical DNA Detection Using Rolling Circle Amplification

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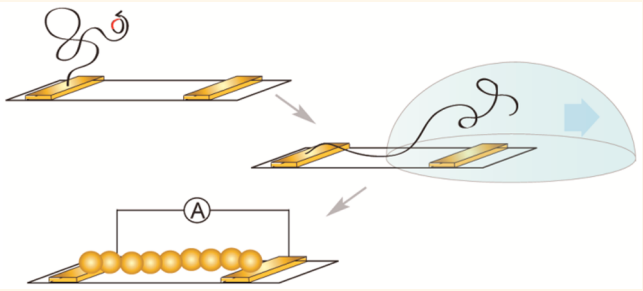
ABSTRACT We present an electrical sensor that uses rolling circle amplification (RCA) of DNA to stretch across the gap between two electrodes, interact with metal nanoparticle seeds to generate an electrically conductive nanowire, and produce electrical signals upon detection of specific target DNA sequences. RCA is a highly specific molecular detection mechanism based on DNA probe circularization. With this technique, long single-stranded DNA with simple repetitive sequences are produced. Here we show that stretched RCA products can be metalized using silver or gold solutions to form metal wires. Upon metallization, the resistance drops from TΩ to kΩ for silver and to Ω for gold. Metallization is seeded by gold nanoparticles aligned along the single-stranded DNA product through hybridization of functionalized oligonucleotides. We show that combining RCA with electrical DNA detection produces results in readout with very high signal-to-noise ratio, an essential feature for sensitive and specific detection assays. Finally, we demonstrate detection of 10 ng of *Escherichia coli* genomic DNA using the sensor concept.



KEYWORDS: gold nanoparticles · rolling circle amplification · nanoelectronics · gold conjugation · metal enhancement

There is a need for sensitive, inexpensive, and fast biosensors for point of care (POC) diagnostics. Sensitivity is important when target molecules are only present in very low numbers, which is typically the case for early diagnosis and for nucleic acid analytes. Present POC devices can measure metabolites and protein biomarkers down to the picomolar range. This is not sensitive enough for detecting the DNA or RNA of infectious pathogens or for free circulating tumor DNA in cancer diagnostics. Most electrical sensors have the advantage of meeting the size, cost, low volume, and power requirements of decentralized testing.[1]

In recent years, there has been a huge interest in constructing one-dimensional structures using DNA to produce metal-enhanced conductive wires. DNA is a versatile molecule which has unique molecular self-assembled properties in the form of the four bases, adenine, thymine, guanine, and cytosine. It also exhibits good chemical and mechanical properties like stability and rigidity. The poor electrical properties of DNA[2] can be overcome by building metal nanowires[3−5] where the double-stranded (ds) DNA acts as template for the seed-mediated growth of a silver,[4] gold,[6,7] or palladium[8,9] wire.

We propose to use rolling circle amplification (RCA) of DNA to generate gold nanoparticle (AuNP)-seeded electrically conducting metal wires that can be electronically sensed. This approach has the potential to be developed into a sensor for POC diagnostics. RCA uses DNA circularization in highly selective recognition reactions, coupled to virtually background-free signal amplification that generates long tandem-repeated single-stranded DNA molecules.[10,11] RCA can be combined with padlock probes[12] for nucleic acid detection and proximity ligation[13,14] for protein detection, which both generate DNA circles upon specific recognition of a target molecule. A padlock probe is a single-stranded synthetic DNA sequence, and upon

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circularization, the padlock probe can be amplified in an isothermal rolling circle amplification reaction using phi29 polymerase. Specificity in a padlock probe reaction is achieved through the requirement of two oligonucleotide hybridization events and a DNA ligation reaction.[12,15] For ligation to occur, the padlock probe is thus required to hybridize in juxtaposed position and the two-terminal nucleotides of the padlock probe to be engaged in correct base-pairing when brought together in a head-to-tail fashion.[16] The rate of RCA is about 90 kb DNA per hour in a highly processive DNA polymerization reaction, resulting in a 45 μm long single-stranded product containing 1000 repeats of the padlock probe after 1 h, making this a very suitable technique for constructing a one-dimensional structure of long single-stranded (ss) DNA. This technique allows us to design the repeated sequences, making it possible to include a sequence to where we can hybridize a detection probe containing a AuNP.

In this paper, we generate and stretch RCA products over a gap of 5 μm between two electrodes to produce conductive metal wires. Upon formation of conductive wires, an electrical signal is generated which is measured as a change in resistance. We demonstrate the use of this technique to detect 100 pM of synthetic DNA target and 66 fM of genomic DNA from *Escherichia coli*.

RESULTS AND DISCUSSION

Gold electrodes are fabricated using optical lithography, thermal evaporation, and a lift-off process (Supporting Information Figure S1). To initiate RCA, the electrodes are covered with primers by producing a self-assembled monolayer (SAM) of oligonucleotides on the gold surface (Figure 1). (Supporting Information Table S1 provides the oligonucleotide sequences.) For proof of concept, a padlock probe is used to directly target the surface primer. In a combined hybridization and ligation step, the padlock probe is hybridized in a head-to-tail fashion and ligated to the surface primer using the enzyme T4 DNA ligase, whereafter the padlock probe is amplified by RCA (Figure 1). RCA products tend to coil into dense DNA blobs with a diameter of 1 μm both in solution and on solid phase.[11,17,18] To bridge the electrode gap, the RCA products have to be stretched by applying force. For this, we use a very similar method to the receding meniscus principle, also called molecular combing, to stretch the RCA products.[19−22] The surface containing the RCA products is washed with a low concentration of the anionic surfactant sodium dodecyl sulfate (SDS) in a weak TNT buffer. The RCA products are stretched as a result of drying the surface using an air flow, thus creating an air/liquid meniscus. Moving the meniscus aligns the RCA products in the direction of the flow, and the DNA is left strongly adhered to the surface. To facilitate metallization, we align 10 nm sized AuNPs along the stretched RCA product. This is achieved by hybridization of short oligonucleotide detection probes with conjugated AuNPs to the repeated sequence along the RCA products (Figure 1).

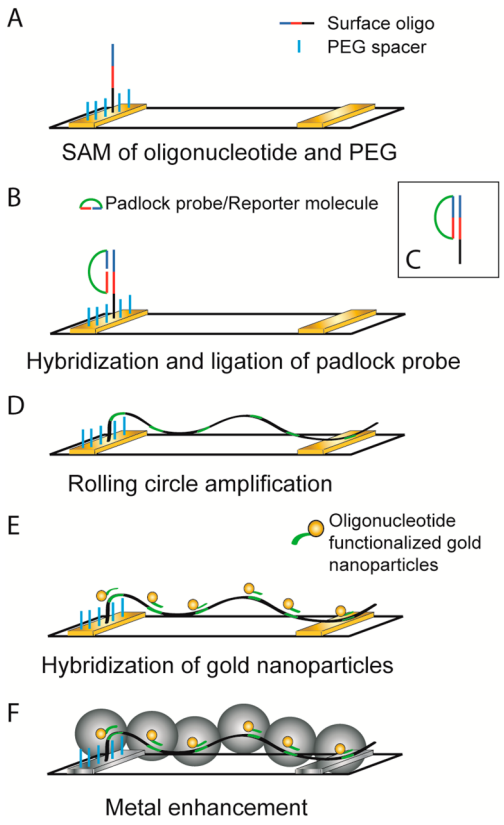


Figure 1. Schematic illustration of formation of metalized wires from stretched rolling circle amplification products to generate an electrical signal. For generating metalized wires, a padlock probe, whose ends have been designed to hybridize head-to-tail on a target sequence, is hybridized and ligated to a self-assembled monolayer (SAM) of oligonucleotides containing the target sequence and poly(ethylene glycol) (PEG) alkane backfiller on a gold electrode (A−C). (D) This circle is then used for RCA, creating a long single-stranded product on the electrode. (E) Oligonucleotide-functionalized gold nanoparticles are then hybridized to the product. (F) With the aid of these particles, the single-stranded DNA threads are metalized using either a silver or gold salt solution to form metal wires.

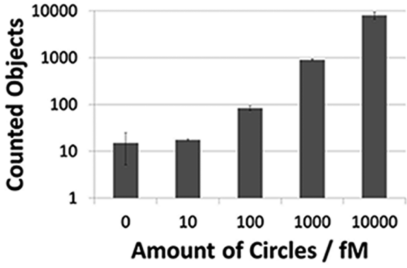


Figure 2. Digital quantification of rolling circle products, with a limit of detection of 100 fM using a synthetic target.

RCA is an established technique for amplifying DNA or RNA.[23,24] Before transferring the system to the gold electrodes, we confirmed that rolling circle products (RCPs) form successfully in solution by digital quantification[18] as seen in Figure 2. Digital quantification which utilizes fluorescence microscopy allows precise

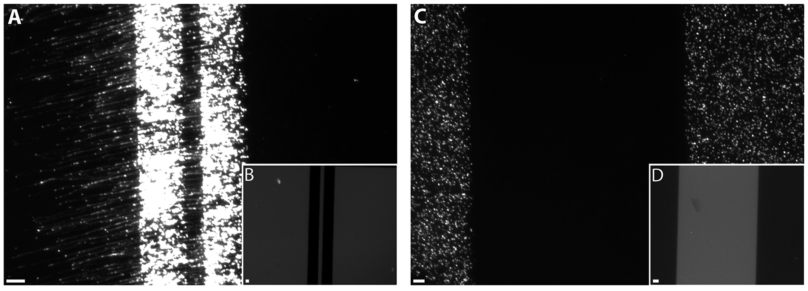


Figure 3. Effect on stretching of RCA products when including a backfiller in the SAM is investigated. The stretched RCA products are labeled with fluorescence-tagged detection probes and observed using fluorescence microscopy. (A) Stretched RCA products are achieved when methylated PEG alkane backfiller, MPEG, is used, and (B) no background of product is seen when only MPEG is used to form the SAM. This is observed using an electrode gap of 5 μm. (C) Collapsed RCA products, seen as bright dots on the electrodes that have a gap of 50 μm, are achieved when PEG_4 backfiller is used, and (D) also here no background is observed when only PEG_4 is used to form the SAM. Scale bar is 5 μm.

quantification of RCPs over a dynamic range of 7 orders of magnitude. The limit of detection was 100 fM using a synthetic target using this technique.

We then demonstrate formation of a self-assembled monolayer of a 44 bases long oligonucleotide (surface primer) on the gold electrodes. The SAM is evaluated by the amount and behavior of the RCPs formed on the electrodes using 100 pM of synthetic DNA target. For the SAM evaluation, the electrode gaps of 5 and 50 μm are used, but for measuring the generated electrical signal, only the 5 μm electrode gap is used. Producing a SAM of oligonucleotides on gold particles or on a gold surface is a widely known and used technique where the oligonucleotides form a strong bond with the gold atoms through a thiol-to-metal interaction. The spacing of the conjugated oligonucleotides and the formation of the SAM are important for hybridization of a complementary oligonucleotide.[25] By introducing a poly ethylene glycol (PEG) backfiller, the alignment of ssDNA can be improved,[26,27] the possibility for nonspecific attachment is reduced, and the ssDNA is extended into the solution, thus making it more accessible for hybridization. We also observed that a higher proportion of the self-assembled oligonucleotides is hybridized and ligated to the padlock probe, which can then be amplified during RCA. PEG backfillers are used in preference to alkanethiol backfillers because PEGs prevent nonspecific adsorption of both DNA and proteins,[28] unlike alkanethiols which only prevent nonspecific adsorption of DNA. We also found that forming the SAM using coadsorption[28] produced consistently more rolling circle products compared to a two-step sequential adsorption process.[26] This agrees well with previously published findings by Steel *et al.*, where they showed that for longer ssDNA probes (>24 bases) a coadsorption process is more suitable.[25] The bond between the surface primer and the gold surface is sensitive to high amounts of dithiothreitol (DTT), especially if high temperatures are used.[29] For this reason, DTT was used in low concentrations and the temperature was kept at 37 °C throughout all hybridization and enzymatic reaction steps.

After demonstrating formation of a SAM of surface primer and that RCPs can be formed successfully on the electrodes, we set out to stretch these by molecular combing to bridge the electrode gap. It has previously been reported that the extent of stretching is influenced by surface characteristics and the use of surfactants.[20] In order to optimize the stretching of RCA products, we investigated the influence of two PEG backfillers with different function groups giving rise to either hydrophobic or hydrophilic surface properties. We compare MPEG (*O*-(2-mercaptoethyl)-*O'*-methylhexa(ethylene glycol)) and PEG_4 ((11-mercaptoundecyl)tetra(ethylene glycol)) to evaluate the effect of backfillers on both stretching of the rolling circle products and the amount of products formed (Supplementary Note 2). The PEG backfillers are tested in three primer-to-backfiller ratios (1:10, 1:50, and 1:100), and the results are analyzed by fluorescence microscopy imaging. For both backfillers, a ratio of 1:10 between primer and backfiller produces the best results (Figure S2). Due to the different properties of the backfillers, they have significantly different impact on the formation of stretched rolling circle products (Figure 3). When PEG_4 is used, the RCA products tend to collapse on the surface and form coils (Figure 3C). This is also the case when no backfiller is used. For MPEG, thread-like products are formed (Figure 3A). This may be due to the hydrophobic properties of the surface caused by the methyl tail group aligning SDS along the surface with the hydrophobic part toward the backfiller and the negatively charged group exposed to the RCA products. This repels the negatively charged DNA from the surface, making it stretch as the dry/wet interface moves along the surface.

DNA itself is not very conductive and in many instances can be regarded as an insulator. By metalizing DNA, it is possible to obtain conductive wires. To facilitate metallization, we align 10 nm sized AuNPs along the stretched RCA product. This is achieved by hybridization of short oligonucleotide detection probes (20 bases long) with conjugated AuNPs to the repeated sequence along the RCPs (Figure 1). AuNPs will then be bound at approximately 20 nm spacing, due to the length of the padlock probe, if all repeats are saturated with detection probes. Occasionally, larger

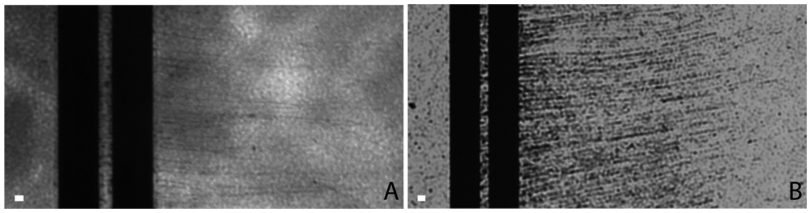


Figure 4. RCA products detected with AuNP and electrical conductivity enhanced with (A) silver for 20 min and (B) with gold for 20 min. Gold enhancement produces more defined threads with less background. Scale bar is 5 μm.

TABLE 1. Measurements of Resistance after 10, 15, and 20 min of Enhancement Using Either Silver or Gold[a]

metal	resistance after 10 min enhancement (Ω)	resistance after 15 min enhancement (Ω)	resistance after 20 min enhancement (Ω)
Ag			
positive	111×10^9	1×10^{12}	856×10^3
negative	143×10^9	50×10^{12}	2.5×10^{12}
Au			
positive	227×10^9	346×10^9	28.0
negative	67.5×10^9	31.5×10^9	112×10^9

[a] Increase in resistance between measurements is most likely due to oxidation of the metal.

gaps are seen along RCA threads (results not shown) as a result of some sites being occupied by a detection probe lacking AuNPs or inaccessible to the detection site due to incomplete stretching. Similar findings have been reported elsewhere.[30−32] A string of AuNPs 20 nm apart gives a resistance of TΩ or greater, which agrees with similar studies[2] where dsDNA was measured in an electrode gap of 30 nm. Adding either a Ag(I) or a Au(III) solution to the AuNPs forms approximately 150−300 nm wide metal wires (Figures 4 and 5) which dramatically reduces the resistance from TΩ to kΩ for silver and Ω for gold (Table 1 and Supplementary Notes 2 and 3). This gives a very high signal-to-noise ratio where a negative control, lacking stretched rolling circle products, has a resistance of GΩ or greater. Table 1 illustrates the change in resistance when allowing the electrical conductivity enhancement process to continue for 10−20 min, where a drop in resistance was observed after 20 min of enhancement.

Silver enhancement is a heterogeneous reaction resulting in formation of differently sized silver clusters. The high resistance observed when using silver enhancement can be due to these large clusters becoming separated from each other.[5] Unlike silver enhancement, gold enhancement is a more homogeneous reaction that does not form clusters,[33] and thus more defined threads with less background are achieved (Figure 5). The gold enhancement solution itself does not, or to a very small extent, produce background which a silver enhancement solution can. However, some background is observed due to the stickiness of DNA. During the hybridization of the AuNP-conjugated detection oligonucleotides to the stretched RCPs, some of these tend to stick to the glass surface. These are enhanced as well during the metallization, but because of the randomness of these and the small amount that is

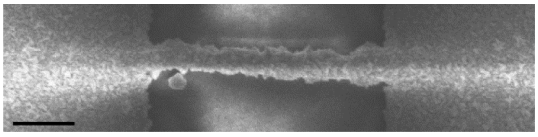


Figure 5. SEM image illustrates gold enhancement, which results in formation of a homogeneous gold wire along the rolling circle product. The gold wire is about 300 nm wide. Scale bar is 1 μm.

formed, they do not align to generate an electrical signal which is seen in the negative controls (Table 1).

We also demonstrate that the technique can be used to detect *E. coli* genomic DNA. A sample containing purified DNA from *E. coli* is fragmented using BstUI and denatured at a high temperature for a short period of time. Thereafter, the sample is subject to molecular probing and amplification as outlined in Figure 6. For molecular probing, an NAD^+-dependent ligase, Tth ligase, is used due to the higher specificity of these ligases compared to ATP-dependent ligases. A padlock probe and a capture probe are hybridized to the target whereupon the padlock probe is ligated. The biotinylated capture probe is then allowed to bind to streptavidin-coated Dynabeads, and unbound excess padlock probes are washed away. The circles are then amplified by RCA and digested by MlyI to form ssDNA reporter molecules with one tag sequence at each end. The reporter molecules can be circularized upon hybridization and ligation to a target sequence and amplified in a second RCA cycle.[34] We confirmed that RCPs form by hybridizing and ligating the reporter molecules to a primer in solution, which is called a circle-to-circle amplification (C2CA),[35] for further amplification and digital quantification utilizing fluorescence microscopy. In solution, we see a linear relationship between the amount of *E. coli* DNA and counted blobs where the LOD

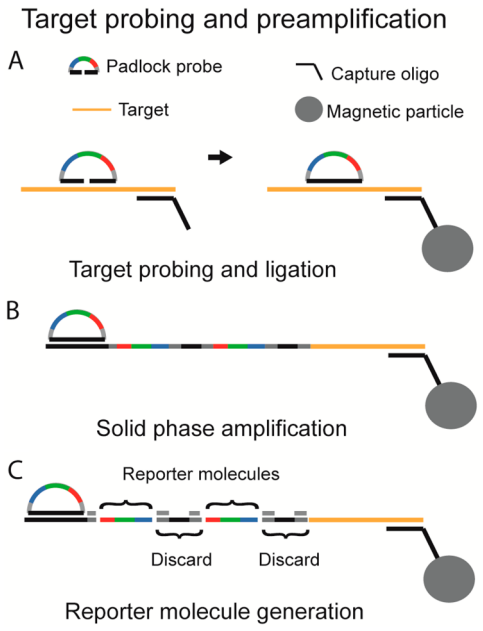
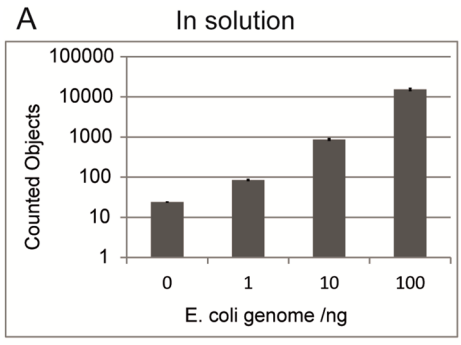


Figure 6. For target probing (A), a padlock probe hybridizes to a target sequence and forms a circle in the presence of ligase. This is then captured to streptavidin-coated Dynabeads by a biotinylated capture probe, and unbound excess padlock probes are washed away. (B) Circles are amplified by RCA, creating a long single-stranded product with tandem repeats of the padlock probe. (C) Restriction oligonucleotides are hybridized to the RCPs to direct digestion by MlyI to form reporter molecules with specific tag sequences at the 3′ and 5′ ends. The reporter molecules can then be circularized in solution for digital quantification or on gold electrodes for generation of metalized wires (Figure 1).



On gold electrodes

B

E. Coli	Resistance /Ω
0 ng	$1x10^{12}$
1 ng	$8x10^{11}$
10 ng	93
100 ng	73

Electrical analysis

Figure 7. For E. coli detection, target probing and a preamplification step was used as illustrated in Figure 1. (A) Digital quantification illustrates a linear relationship between target amount and counted objects where a LOD is seen at 1 ng, which equals 6.6 fM. (B) Electrical measurements illustrate a change in resistance from 10 ng, *i.e.*, 66 fM of E. coli.

is 1 ng, which corresponds to 6.6 fM (Figure 7A). The reporter molecules are also hybridized and ligated to the surface primer on the electrodes for a second RCA where stretched RCPs are generated and metalized using a Au(III) solution. With this technique, a change in resistance is seen from 10 ng corresponding to 66 fM of *E. coli* DNA where the measured resistance decreases by nearly 10 orders of magnitude to 93 Ω. A negative control without *E. coli* DNA, which has had the same treatment as the positive sample, shows a resistance of TΩ. By plotting the resistance against the concentration of target DNA, an on/off relationship is observed, which is expected considering the type of electrical measurements used.

A high signal-to-noise ratio is a valuable asset for a good sensor. We show that our concept offers a signal-to-noise ratio that is nearly 10 orders of magnitude. In this study, we have not come even close to realizing the full potential of this favorable signal-to-noise ratio, for example, in terms of quantification, but future work will aim to utilize this strength to develop a biosensor with high sensitivity, specificity, and wide quantitative dynamic range with the possibility of multiplexing. Previous work by Hardenbol *et al.* has demonstrated that padlock probes can be multiplexed to interrogate large sets of single-nucleotide DNA variants.[36]

CONCLUSION

To summarize, we used rolling circle amplification to produce long single-stranded DNA with simple repetitive sequences to bridge an electrode gap of 5 μm. RCA products are stretched by molecular combing, and 10 nm AuNPs are aligned along the DNA strand by hybridization of a complementary sequence attached to the particles. The conductivity of the AuNP string is enhanced using silver or gold solutions to form 150−300 nm wide silver or gold wires. This reduces the resistance from TΩ, when only 10 nm AuNPs are present, to kΩ for silver wires and Ω for gold wires. We demonstrate that this electrical readout is compatible with the padlock probe and RCA molecular probing mechanism by detecting genomic DNA from *E. coli*. By combining RCA with metallization seeded by AuNPs, an electrical detection technique is produced with a very high signal-to-noise ratio.

METHOD

Electrode Fabrication. One hundred nanometer thick gold electrodes are produced using contact photolithography and a lift-off process. A thin 10 nm titanium layer is used for gold adhesion to the glass substrate.

Reagents. All oligonucleotides are purchased from Biomers or IDT. Gold nanoparticles are purchased from BB International,

silver enhancement kit from Invitrogen and gold enhancement kit from Nanoprobes. All enzymes are purchased from Fermentas except for BstUI which is purchased from New England Biolabs and Tth ligase that is purchased from Genecraft. *O*-(2-Mercaptoethyl)-*O′*-methylhexa(ethylene glycol) and (11-mercaptoundecyl)tetra(ethylene glycol) are purchased from Sigma-Aldrich.

Preparation of Alkanethiol Oligonucleotide Modified Gold Electrodes. Prior to preparation of alkanethiol oligonucleotide modified gold electrodes, the gold surface is cleaned with warm Piranha solution for 30 min (3:7 30% hydrogen peroxide and concentrated sulfuric acid), whereafter the electrodes are rinsed thoroughly with Milli-Q water and dried. (**Warning! Extreme care needs to be exercised when preparing and handling Piranha solution due to the very exothermic reaction.**)

The thiol modified oligonucleotide is reduced by incubating with 0.1 M DTT in 0.18 M phosphate buffer pH 8 for 1 h. DTT is removed using a NAP-5 column, leaving an unprotected thiol group on the oligonucleotide.

To produce the self-assembled monolayer of oligonucleotide with and without backfiller on the gold electrode, 1 μM oligonucleotide with 0−100 μM of *O*-(2-mercaptoethyl)-*O′*-methylhexa(ethylene glycol) or (11-mercaptoundecyl)tetra-(ethylene glycol) is incubated overnight at 4 °C in 0.05% SDS, 1 M NaCl, and 10 mM phosphate buffer pH 8. The electrode is then washed with 0.1× SSC (saline−sodium citrate, 1.5 mM sodium citrate and 15 mM NaCl) and dried.

Rolling Circle Amplification on Electrodes. One hundred nanomolar padlock probes are phosphorylated in PNK buffer (50 mM Tris-HCl (pH 7.6 at 25 °C), 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine) with 1 mM ATP and 0.1 units of T4 polynucleotide kinase. For hybridization and ligation of the phosphorylated padlock probe, 100 pM of the padlock probe is incubated in 1× ligation buffer (10 mM Tris acetate, pH 7.5, 10 mM magnesium acetate, 50 mM potassium acetate) with 1 mM ATP, 0.2 μg/μL BSA, and 0.05 U/μL T4 DNA ligase. Ligation is performed for 2 h at 37 °C, whereafter the electrodes are rinsed with 1× TNT buffer (0.15 M NaCl, 10m M Tris-HCl (pH 8.0), and 0.05% Tween-20) and 0.1× SSC. Rolling circle amplification is performed for 2 h at 37 °C using 1× phi29 buffer (50 mM Tris-HCl pH 7.5, 10 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$), 0.25 mM dNTP, 0.2 μg/μL BSA, and 0.2 U/μL phi29 polymerase. The electrodes are then washed with 0.1× TNT and 0.1% SDS and dried by airflow to produce threadlike structures.

Metallization of ssDNA. To produce metalized RCA wires, 10 nm oligonucleotide-functionalized gold particles are used. Functionalized gold particles (50 nM) are hybridized to the RCA products at 37 °C for 1 h using 0.5 M NaCl, 20 mM Tris-HCl, 20 mM EDTA, and 0.01% Tween. Unbound gold particles are removed by washing with 0.1× TNT and 0.1% SDS. The electrodes are dried by airflow. The gold particles are then used as nucleation sites for further metal enhancement using silver (LI silver enhancement kit) or gold (gold enhance from Nanoprobes) solutions. The gold particles are enhanced in sequential steps, each 5 min long, where the resistance is measured after each step. The enhancement procedure is stopped upon a large drop in resistance.

Electrical Measurements. Electrical measurements are carried out using an Agilent B1500A semiconductor device analyzer with Easy expert software. The current between the electrodes is measured by varying the voltage from 0 to 100 mV and then back to 0 V. The resistance is then calculated using the reciprocal of the slope obtained when plotting current *versus* voltage.

Rolling Circle Amplification for Digital Quantification Using a Synthetic Target. One micromolar padlock probes are phosphorylated in PNK buffer (50 mM Tris-HCl (pH 7.6 at 25 °C), 10 mM $MgCl_2$, 5 mM DTT, 0.1 mM spermidine) with 1 mM ATP and 0.1 units of T4 polynucleotide kinase. For hybridization and ligation, 20 nM of the padlock probe is incubated in 1× phi29 buffer with 60 nM surface primer, 1 mM ATP, and 0.02 U/μL T4 DNA ligase. Ligation is performed for 15 min at 37 °C. The circles are diluted to 100 pM, 10 pM, 1 pM, and 100 fM in sterile filtered MQ water to be used for RCA. RCA is performed for 1 h at 37 °C using 1× phi29 buffer (50 mM Tris-HCl pH 7.5, 10 mM $(NH_4)_2SO_4$, 10 mM $MgCl_2$), 0.125 mM dNTP, 0.2 μg/μL BSA, and 0.08 U/μL phi29 polymerase. The RCPs are then labeled with 5 nM Cy3 modified oligonucleotide in a hybridization buffer (1 M NaCl, 20 mM EDTA, 20 mM Tris-HCl pH 8, and 0.1% Tween) for 2 min at 70 °C and 15 min at 55 °C.

E. coli Preparation. Two O/N broth culture of *E. coli* DA5438 (approximately 10^9 CFU/mL), approximately 3−4 colonies, are incubated overnight with agitation at 37 °C in 10 mL of TSB. The DNA is extracted using MagnaPure 96 DNA and viral NA SV kit using a pathogen universal protocol.

Preamplification of E. coli. A 20 ng/μL *E. coli* solution is fragmented in SmartCut buffer from New England Biolabs using 10 units of BstUI at 60 °C for 10 min. The fragments are then prepared in three different amounts (100, 10, and 1 ng) and denatured at 95 °C for 5 min before hybridized and ligated to 100 nM phosphorylated padlock probe in Tth ligase buffer (20 mM Tris-HCl pH 7.6, 25 mM KCl, 10 mM $MgCl_2$, 10m M DTT, 1 mM NAD, and 0.1% Triton X-100), 0.2 μg/μL BSA, and 250 mU/μL Tth ligase at 55 °C or 5 min. The denatured *E. coli* is at the same time hybridized to a biotinylated capture oligonucleotide, which is after hybridization and ligation conjugated to 5 μL of MyOne T1 streptavidin magnetic beads for 5 min at room temperature. Before use, the beads are washed three times in wash buffer containing Tween (10 mM Tris-HCl pH 7.6, 5 mM EDTA, 0.1 M NaCl, and 0.1% Tween). After conjugation, the beads are washed once using the wash buffer before resuspending in an RCA mix (0.2 μg/μL BSA, 0,125 mM dNTPs, 1× phi29 buffer, and 100 mU/μL phi29 polymerase). RCA is performed for 60 min at 37 °C before digestion at 37 °C using FastDigest MlyI and two restriction oligonucleotides in FastDigest buffer and 0.2 μg/μL of BSA. Digestion is stopped after 15 min by heat inactivation at 80 °C for 5 min. The monomers are then either hybridized and ligated to the surface primer in solution and amplified for digital quantification or hybridized and ligated to the surface primer on the electrodes for generation of stretched metalized RCPs and electrical measurements.

Conflict of Interest: The authors declare no competing financial interest.

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Supporting Information Available: Detailed methods and supplemental figures. This material is available free of charge *via* the Internet at http://pubs.acs.org.

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