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Non-Enzymatic Detection of Bacterial Genomic DNA Using the Bio-Barcode Assay

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Abstract

The detection of bacterial genomic DNA through a non-enzymatic nanomaterials based amplification method, the bio-barcode assay, is reported. The assay utilizes oligonucleotide functionalized magnetic microparticles to capture the target of interest from the sample. A critical step in the new assay involves the use of blocking oligonucleotides during heat denaturation of the double stranded DNA. These blockers bind to specific regions of the target DNA upon cooling, and prevent the duplex DNA from re-hybridizing, which allows the particle probes to bind. Following target isolation using the magnetic particles, oligonucleotide functionalized gold nanoparticles act as target recognition agents. The oligonucleotides on the nanoparticle (barcodes) act as amplification surrogates. The barcodes are then detected using the Scanometric method. The limit of detection for this assay was determined to be 2.5 femtomolar, and this is the first demonstration of a barcode type assay for the detection of double stranded, genomic DNA.

Introduction

Polymerase chain reaction (PCR)-based amplification techniques[1–3] have become standard methodologies for the detection of nucleic acids.[4, 5] With the advent of quantitative real time PCR and variants of it such as reverse transcription PCR, one can now detect nucleic acid targets in a highly quantitative manner and assess important processes like gene expression.[6–10] Though there are many benefits to PCR such as sensitivity, production of a usable product fragment, and the ability to sequence that fragment, there are times when these features of PCR are not necessary and the cumbersome nature of PCR is a disadvantage. For example, in the case of point-of-care biological detection applications, where speed is critical and the enzymatic constraints and cost of PCR are limiting,[11] an enzyme-free approach could be a major advantage.

The recently developed bio-barcode assay for the detection of protein and nucleic acid targets is potentially capable of filling this void. This assay has several forms, [12–16] and has shown promise in the high sensitivity detection of protein and oligonucleotide targets. In addition, it has the ability to simultaneously detect many different targets in one sample.[17–19] Recently it has been adapted to a microfluidic chip-based format, an important step towards automation.[20] With respect to nucleic acids, thus far, all proof-of-concept work has involved short nucleic acids in very clean environments (buffer). The complexities of the target and sample media are often limiting factors for any nucleic acid assay, especially ones that rely on enzymes for amplification. This made us wonder if the bio-barcode assay could overcome such limitations. Herein, we describe the development of a new version of the bio-barcode assay that utilizes blocking strands to inhibit target rehybridization and

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allows one to detect double stranded genomic DNA at a limit-of-detection (LOD) of 2.5 fM (7.5×10^4 copies/50μL). Proof-of-concept studies in the context of *Bacillus subtilis* DNA detection are reported.

Gold nanoparticles heavily functionalized with oligonucleotides (oligo-AuNPs), are the cornerstone of the bio-barcode assay.[21] These oligo-AuNPs have a variety of attributes with respect to probe design. They are easily functionalized,[21] highly tailorable,[22, 23] remarkably stable,[24] catalytic,[25] and cooperative binders (they exhibit unusually sharp melting transitions when hybridized to complementary DNA).[26] These sharp melting transitions can confer a considerable selectivity advantage to the oligo-AuNPs over their PCR primer counterparts.[27] Oligo-AuNPs serving as amplification agents in the bio-barcode assay, through the chemical release of their oligonucleotide "barcodes," have several potential advantages over Taq-Polymerase or other DNA replication enzymes. For example, the oligo-AuNP probes are stable for extended periods (greater than 6 months) at ambient temperature,[21] and are resistant to degredation,[28] while polymerases, like most enzymes, need to be stored at 4 °C or below. Oligo-AuNPs also function in a host of complex conditions such as sodium chloride concentrations up to 1 M,[29] different buffers such as Tris (2-Amino-2-(hydroxymethyl)-1,3-propanediol), Phosphate (Na_2HPO_4, NaH_2PO_4), Borate ($Na_2B_4O_7$), MOPS (3-(*N*-Morpholino)-propanesulfonic acid) and in the presence of metal ions or small molecules without adverse effects on their activity.[30–37]

The bio-barcode assay combines target specific oligo-AuNPs with a second homogenous capture agent (a magnetic microparticle functionalized with a different target specific oligonucleotide, oligo-MMP). The oligo-MMP is used to capture and isolate the target of interest from the sample solution, prior to the addition of the oligo-AuNPs. The MMP-target-AuNP complex allows for rapid isolation and subsequent washing prior to oligonucleotide barcode release. The barcodes can be easily detected via the Scanometric method, which exhibits an LOD for short purified oligonucleotides of 100 aM.[25, 38]

Materials and Methods

Culture Media and Bacterial Strains

Routine growth and maintenance of *Bacillus subtilis 168* (American Type Culture Collection # 23857) was done in Luria-Bertani (LB) media (Fisher Scientific) and on solidified plates using LB agar (Fisher Scientific). All cultures were maintained at 30 °C on plates or in liquid form with shaking at 160 rpm and 30 °C. All growth media were sterilized by autoclave treatment prior to use.

Genomic DNA Isolation

B. subtilis cells were grown in 50 mL of liquid media in 125 mL flasks overnight and harvested after 10 hours of growth. The cells were split into two 25 mL aliquots and spun down at 8,000 rpm for 10 minutes. The supernatant was then removed, and the aliquots were resuspended in 5 mL of 50 mM Tris, 50 mM EDTA at pH 8.0 and frozen for no less than 1 hour at −20 °C. Frozen cells in a 50 mL conical tube were placed on ice, and 500 μL of 10 mg/mL lysozyme (Fisher Scientific) dissolved in 250 mM Tris, pH 8.2 were added to the tube. The cells-lysozyme mixture was allowed to slowly warm to room temperature over a two-hour period. Next, 1 mL of a 1 mg/mL solution of Proteinase K (Fisher Scientific) in 50 mM Tris, 0.4 M ETDA, 0.5 M SDS, pH 7.5 was incubated with the cells at 50 °C for 1 hour. Afterward, RNase A (1 μL, Ambion Inc./Applied Biosystems) was added to degrade all RNA contamination. Following RNA degradation, the genomic DNA was removed from the other cellular debris by phenol-chloroform extraction and ethanol precipitation. The integrity and size of the genomic DNA was confirmed by gel electrophoresis using a 1% agarose gel

with ethidium bromide (Bio-Rad, ReadyAgarose™ Gels) with 1 × TBE (Tris, Boric Acid, EDTA) buffer at 120 volts for 1 hour. (Figure S1, lane 5/6) The size of the genomic DNA isolated was compared with commercially available genomic DNA isolated by ATCC for *Bacillus subtilis 168.* (Figure S1, Lane 2).

Probe Design

Probes were designed from the alpha subunit of the tryptophan synthase gene (bp 2371552-2370749) from *Bacillus subtilis 168*. All probes were tested against the NCBI BLAST search engine, with the magnetic and gold probe sequences being unique to *B. subtilis*. Two of the three blocking sequences (center and 5′) had a homology to one other organism, while the 3′ blocker was specific only for *B. subtilis.* Probes were designed to fall within a region of the genome that could be cut easily with the restriction enzyme HpyCH4V (New England Biolabs). This was done to allow for de-circularization of the genomic DNA and prevention of super coiling during the heat denaturation step of the assay. Probe specificity was confirmed by routine Southern Blot analysis (data not shown). Oligonucleotide sequences are given in Table 1.

Oligonucleotides

All specialty oligonucleotides were purchased from Integrated DNA Technologies (Coralville, IA) and were purified by HPLC. Standard desalting was used to purify PCR primers and blocking strands. Prior to use, the oligonucleotides were stored at − 80 °C in a dried state. Working solutions of the oligonucleotides were stored at − 20 °C.

Quantitative PCR

The copy number of genomic DNA per mL (isolated from *B. subtilis* cells) was determined using quantitative real-time PCR (qPCR). A LightCycler 2.0 instrument and LightCycler Software Version 4.0 (Roche Applied Sciences) were used to run the qPCR reactions and quantify the data respectively. Primers were designed to amplify a 1066 base pair fragment of the genomic DNA from *B. subtilis* (Table 1). A LightCycler FastStart DNA Master SYBR Green kit (Roche Applied Sciences) was used to generate the PCR reactions. Each PCR reaction was carried out in a 20 μL capillary (Roche Scientific) by placing the capillary tube in a cooling rack (4° C), combining and mixing the reagents in an eppendorf tube at 4°C, spinning the reactants into the capillary tubes, and thermally cycling. Since the real-time reporter used (SYBR Green) is an intercalating fluorophore where total fluorescence depends on product length, a standard curve for the qPCR had be constructed before hand. To do this, end-point PCR was run using Qiagen's Taq PCR Master Mix following the manufacturer's protocol. During each amplification, primer concentrations of 0.5 μM were used with approximately 0.1 μg of template. The reactions were done in 100 μL PCR tubes in strips of 8 (Fisher Scientific) on a GeneAmp® PCR System (Applied BioSystems). The thermal profile consisted of an initial 8-minute denaturation step at 95 °C, followed by 45 cycles of denaturation at 95 °C for 55 seconds, annealing at 52 °C for 45 seconds, and extension at 72 °C for 120 seconds. Following the 45 cycles, the product sequence was completed with an extension step at 72 °C for an additional 10 minutes. The PCR product size was confirmed by gel electrophoresis using a 1% agarose gel with ethidium bromide (Bio-Rad, ReadyAgarose™ Gels) with 1 × TBE (Tris, Boric Acid, EDTA) buffer at 120 volts for 1 hour (Figure S1, Lane 3/4). The remaining PCR product, not run on the gel, was purified using a MinElute PCR purification kit (Qiagen Inc.) following the manufacturer's instructions. The PCR product was quantified using UV-visible spectroscopy, and then was used to generate a standard curve for qPCR. (Figure S2)

Magnetic Particle Functionalization with DNA

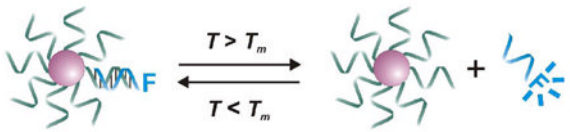
Oligonucleotide-functionalized magnetic microparticles (Oligo-MMPs) were prepared according to literature procedures.[17, 39] Briefly, 2.8 μm amine-functionalized magnetic microparticles (Dynal Corp/Invitrogen) were coupled to thiolated oligonucleotide strands using the hetero-bi-functional crosslinker sulfo-SMPB (Pierce Biotechnology). Unreacted amine sites were passivated with sulfo-NHS acetate (Pierce Biotechnology). Oligo-MMPs were stored at 4 °C in 10 mM phosphate buffered saline (0.15M NaCl, PBS) with 0.001% sodium azide as a preservative. Oligo-MMPs were washed three times with PBS prior to use in the assay.

Gold Nanoparticle Functionalization with DNA

Oligo-AuNP probes were prepared according to literature procedures.[39] Briefly, 4 nanomoles of freshly reduced thiolated DNA was added to 1 mL of 13 ± 2 nm gold nanoparticles and shaken gently overnight. The system was buffered to a phosphate concentration of 10 mM (pH 7.0) including 0.01 percent sodium dodecyl sulfate (SDS). Over the course of one day, the sodium chloride concentration was brought to 0.15 M in a stepwise manner. Particles were then spun (13,000 rpm) and rinsed four times (10 mM phosphate, 0.15 M NaCl, 0.01% SDS, pH 7.4) to remove any unbound DNA. Probes were stored in excess DNA until needed, at which time they were purified as described above.

Melting Analysis

In order to determine the maximum assay stringency, or the upper temperature limit to heat denature and eliminate non-specific hybridization events in solution, we determined the melting temperatures (T_m) for each of the probes (oligo-AuNP and oligo-MMP probe sequences) with their respective targets in a 1:1 ratio (Equation 1). In a typical melting experiment, AuNPs were functionalized with a 5′-thiol-modified probe sequence (Table 1) and allowed to hybridize to one equivalent of a 5′ Fluorescein (FITC)-modified complementary DNA sequence specific to the targeted regions within the tryptophan synthase gene (*trp 1*; AuNP target, *trp 3*; MMP target).



Equation 1

All experiments were allowed to equilibrate for over 24 h in 10 mM phosphate buffer, 0.15 M NaCl, 0.1% SDS, pH 7.4 (assay buffer) to ensure that equilibrium has been reached. Binding of the nanoparticle probes to a complementary target sequence modified with a molecular fluorophore resulted in quenching and decreased fluorescence intensity. Subsequent heating resulted in dissociation of the probe/target complex and a recovery of fluorescence intensity, providing a way to spectroscopically monitor the melting transition (Figure 1). Fluorescence measurements were performed on a Molecular Devices Gemini EM Microplate spectrofluorometer with temperature control. Comparison of the *trp 1* (Au NP) probe-target (T_m = 64.1 ± 0.5 °C) and *trp 3* (MMP) probe-target (T_m = 70.1 ± 1.1 °C) complexes after dissociation revealed a difference of 6 °C in the T_m. The lowest T_m value was used to determine the thermal stringency for the assay. A temperature 14 °C below that lowest melting temperature (40 °C)was chosen to ensure that no probe-target complexes would dehybridize under stringent conditions.

Preparation of Oligonucleotide Arrays

N-hydroxy succinimide (NHS) activated Codelink glass microscope slides (Amersham Biosciences) were used to support microarrays of amine-terminated oligonucleotides complementary to the Au particle-bound barcode sequences and were prepared according to the manufacturer's protocol. The "capture" oligonucleotides were printed in triplicate using a GME 418 robotic pin-and-ring microarrayer (Affymetrix). The chips were allowed to couple overnight at 70% humidity, and were then passivated in 0.2 % SDS at 50 °C for 30 minutes to hydrolyze all unreacted NHS groups.

Genomic DNA Bio-Barcode Assay

Bacillus subtilis cells were isolated from culture by centrifugation, and the genomic DNA was isolated as described above using lysozyme and proteinase K. The genomic DNA was then cut using the restriction endonuclease HpyCH4V (New England Biolabs). A restriction digestion step was needed to prevent DNA super-coiling during the heating and subsequent detection. A dilution series of genomic DNA was made for testing with the bio-barcode assay. Additionally, an aliquot of the genomic DNA was quantified using qPCR as described above. Assays were assembled in nuclease free eppendorf tubes (Ambion Inc) containing 5 μL of genomic DNA sample, 1 μL of each blocking oligonucleotide (200 μM) and 32 μL of assay buffer. The assays were mixed thoroughly and placed at 95 °C for 10 minutes to denature the genomic DNA fragments. After 10 minutes, the temperature was lowered to 72 °C, and 10 μL of oligo-MMPs (20 mg/mL) were added to each tube. The reactions were mixed well, and placed at 40 °C for 2 hours while mixing in an end over end manner to ensure that the oligo-MMPs did not settle. The oligo-MMPs with the target bound were then washed 3 times with 100 μL of assay buffer to remove all unbound nucleic acids and remaining components from the restriction digest. This step is especially important as DTT, which is used in the restriction buffer, can react with the oligo-AuNP probes in the next step. To the washed oligo-MMPs-target complexes 40 μL of assay buffer and 10 μL of 500 pM freshly cleaned oligo-AuNPs were added. The reactions were vortexed, and placed at 40 °C with end-over-end mixing for one hour. The reactions were then washed five times using 1000 μL of assay buffer to remove all unbound oligo-AuNPs. The supernatant was removed after the 5^{th} wash and the complexes were resuspended in 50 μL of 0.5 M DTT in assay buffer. The tubes were then placed at 50 °C for 15 minutes, followed by 45 minutes 25 °C to liberate the thiolated oligonucleotide barcodes from the surface of the gold nanoparticle through ligand exchange.[14] Following barcode release, the oligo-MMPs were isolated using a magnet and the remaining supernatant was transferred to a new nuclease free eppendorf tube. Next, 15 μL of sample was pipetted into a slide chamber well (Nanosphere Inc) assembled on a chip prepared as described above. The barcode samples on the chip were heated to 60 °C and then allowed to hybridize to their complement for 1 hour at 37 °C while shaking at 120 rpm. The chips were then washed three times in assay buffer and reassembled with clean slide chambers. To each well 15 μL of universal probe solution (500 pM universal AuNP, 10% formamide in assay buffer) was added. The probes were allowed to hybridize for 45 minutes at 37 °C while shaking at 120 rpm. The gaskets were then removed and the slides were washed twice in 0.5 M $NaNO_3$, 0.2 % Tween 20, 0.1 % SDS, washed three times in 0.5 M $NaNO_3$ and finally quickly dipped in cold (4 °C) 0.1 M $NaNO_3$. The slides were spun dry, and equal parts silver stain solution A and B (Nanosphere Inc.) were placed on top of the slide so that the entire surface was covered. The silver enhancement was carried out for 3 minutes before being terminated by washing with Nanopure® water (18 MΩ, Barnstead). The slide was dried and imaged using a high resolution Verigene ID (Nanosphere Inc.), and the spot intensity was analyzed using GenePix Software (Molecular Devices). A representative image of the data obtained from an assay (as displayed in false color using GenePix Software) can be seen in Figure 3A.

Safety Considerations

To the best of our knowledge, this assay presents no serious hazards, though caution should be taken to avoid skin and eye contact with the silver enhancement solution. In addition, when the assays are used in conjunction with unknown biological samples or known bacterial samples, all proper government safety protocols should be followed.

Results and Discussion

A typical assay was performed by digesting circular genomic DNA isolated from *Bacillus subtilis* cells with 1 unit of HpyCH4V to yield smaller linear DNA fragments, Scheme 1. In eppendorf tubes, 5 μL of target DNA at various concentrations, 1 μL of each blocking oligonucleotide (200 μM) and 32 μL of buffer, were mixed, and the target strands were denatured at 95 °C for 10 minutes. Following denaturation, the samples were cooled to 72 °C, and 10 μL of oligo-MMPs (20mg/mL) were added to the reaction vessel and placed on a rotating shaker at 40 °C for 2 hours to facilitate target capture. Following target capture, the samples were thoroughly washed to remove all contaminants, and the oligo-AuNP probes (500pM) were added to the assay. After hybridization for an hour at 40 °C, the sandwich complexes were washed extensively, and the barcodes were chemically (dithiothreitol, DTT) released for scanometric detection.[25] The slides after silver amplification were imaged with a Verigene ID system (Nanosphere Inc.), which records the scattered light from the silver amplified spots and provides quantitative information regarding the concentration of barcode oligonucleotides.

To detect genomic DNA using the bio-barcode assay, separation of the duplex targets into their single strand forms is critical for probe binding. However, the conditions required to thermally denature DNA are very harsh (95 °C), and the oligo-MMPs (iron oxide nanoparticles embedded in a polymer scaffold) deteriorate under such conditions. Chemical denaturants are not an option, as they prevent the oligo-MMPs from hybridizing to the target as well. To overcome the challenge of denaturing DNA duplexes and keeping them apart long enough to allow the oligo-MMPs to hybridize required the implementation of blocking oligonucleotides, a modification of a strategy that has been used in scanning probe detection techniques.[40] These blocking oligonucleotides (blockers) consisted of three different 35 base pair sequences, designed to flank the particle probe binding sites. In the assay, the blockers were used in great excess (~$1{:}10^6$, target: blocker) to prevent strand rehybridization (Figure 2A).[40] When the duplex DNA is heated to 95 °C with an excess of blockers, the duplex thermally denatures, and as the solution cools, the kinetics of blocker binding should be faster than that of native strand re-hybridization.[41] This should result in open regions of the duplex. To test this hypothesis we ran the bio-barcode assay under various conditions to determine the effectiveness of the blockers (Figure 2B). The left most sample labeled “no target” was run with digested λ-phage DNA as a negative control and 4 μM of each of the three blocking oligonucleotides. The sample in the center was run with 250 fM target only, and the third sample (far right) was run with 250 fM target and 4 μM of each of the three blockers. The impact of the blockers is significant. The signals obtained for the λ-phage DNA and the target without blocking strands fall within each other’s standard deviations, while the target sample that contained the blockers shows a six-fold increase in signal. These experiments demonstrated that the blocking oligonucleotides are critical to the success of the bio-barcode assay’s ability to detect genomic DNA.

In order to evaluate the sensitivity and dynamic range of the assay in the presence of blockers, a digestion mixture was diluted into a series of solutions. The bio-barcode assay is capable of detecting bacterial genomic DNA down to the low femtomolar concentration range, with a limit of detection of 2.5 fM (final concentration in the assay, 7.5×10^4 copies

under the stated conditions, Figure 3). The row of spots labeled 250 fM clearly exhibits the most intense red color, indicating the strongest signal (Figure 3A). The spots at 25 fM target concentration show an orange/yellow color indicating a moderate to high signal intensity. The 2.5 fM spots show a yellow/green intensity, which is distinct from the blue color seen at the 0 fM (no target) row at the top of the slide. Additionally, the quantified data (5 independent experiments) presented in Figure 3B show that the signal at 2.5 fM is greater than three standard deviations above the control signal. The normalized assay is log linear through the femtomolar concentration range, and becomes non-linear above 1 pM due to saturation of the scattering signal as read by the Verigene ID. This saturation issue can be easily solved by diluting the barcodes prior to their detection by the scanometric method, thereby allowing one to span the femtomolar and higher concentration range with this method.[25]

Conclusion

We have demonstrated the ability of the bio-barcode assay to detect bacterial genomic DNA with an LOD of 2.5 fM. The integration of blocking oligonucleotides proved to be a critical addition to the assay, which ultimately allowed for the detection of genomic duplex DNA isolated from *B. subtilis* cells. This work paves the way for the transition of the bio -barcode assay from a laboratory technique to one that can be deployed in the field for the rapid and accurate detection of biological terrorism agents. *Bacillus subtilis* was chosen as a model system since it is a close family member of the lethal bacterium *Bacillus anthracis*, which in its spore form is the biological weapon Anthrax.[42] In the future, the bio-barcode assay may be coupled with automated field-deployable sample collection technologies to potentially produce a system for continuous biological surveillance.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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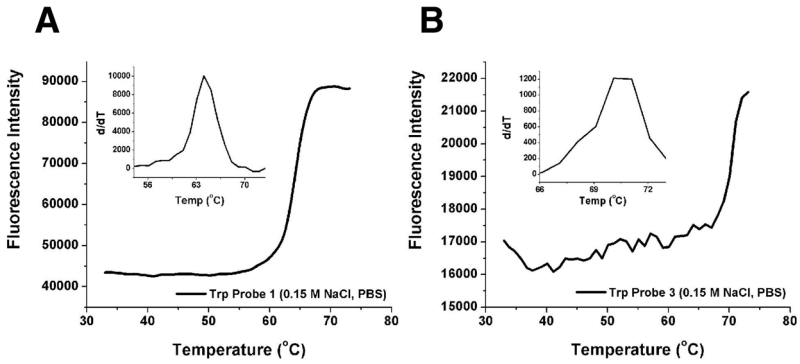


Figure 1. Probe Melting Analysis

A) Melting curve for the duplex formed between an oligo-AuNP probe and its fluorophore labeled complement (sequences given in Table 1) B) Melting curve for the duplex formed between the oligo-MMP probe and its fluorophore labeled complement (sequences given in Table 1). The fluorescence of the complementary strands is quenched when they are bound to the AuNP and is recovered when the duplexes melt with the fluorophore strand being released into solution.

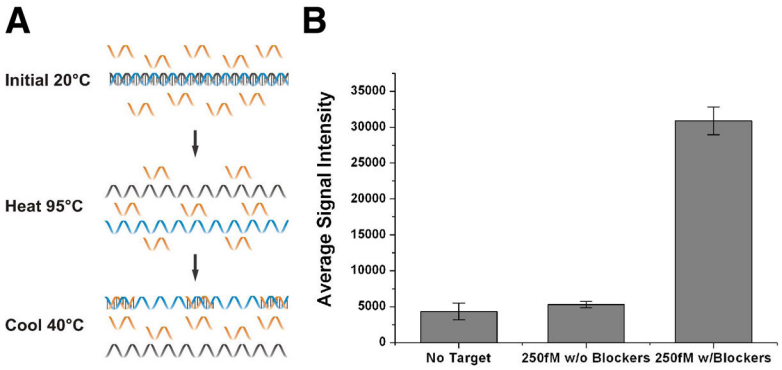


Figure 2. Blocking Oligonucleotide Functionality

A) Scheme showing how the blocking oligonucleotides are designed to prevent genomic DNA strand rehybridization. **B)** This graph shows the importance of the blocking oligonucleotides to the function of the bio-barcode assay. It is clearly seen that without blockers the signal obtained in the assay is the same as that with no target, while in the presence of blockers a large signal is obtained indicating that the genomic DNA is available for hybridization to probes.

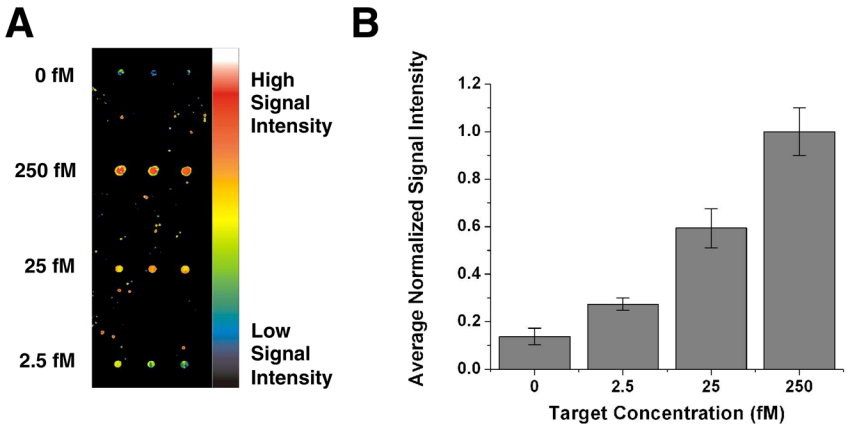
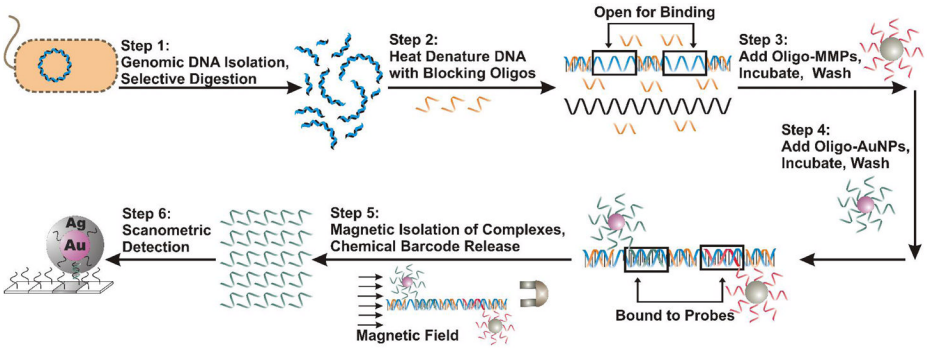


Figure 3. Genomic DNA Detection Results

A) This image is a representative slide from a single assay; showing that 2.5 fM is distinguishable from the 0 fM (no target) sample. The grey scale image from the Verigene ID is converted to color using GenePix 6.0 software (Molecular Devices). **B)** The data shown above is an average of 5 independent runs of the genomic DNA bio-barcode assay.



Scheme 1. Genomic Bio-Barcode Assay

The first step is to isolate the genomic DNA from the bacterial cells and cut it with a restriction enzyme. This cut prevents the DNA from super coiling during heating and gives smaller target pieces. The next step is to introduce blocking oligonucleotides designed to flank the probe binding sites and prevent strand rehybridization after thermal denaturation. The blocking oligonucleotides are used in excess to out compete the native strand during hybridization. The target region is now “propped” open and accessible for probe binding. Magnetic microparticles (oligo-MMPs) are used to capture the targets from the sample and then washed. An excess of oligonucleotide modified gold nanoparticle probes (oligo-AuNPs) is added to the assay solutions which results in the sandwiching of the target with the oligo-MMP. Unbound oligo-AuNPs are removed by washing. The barcodes are chemically released for scanometric detection and quantification.

Table 1

Oligonucleotide Sequences

Name	Sequence
Forward Primer	5′-AGA CTC TAA TGC AGT CAC CAA CGC-3′
Reverse Primer	5′-TGC TCC CAA TAT AAC GTA TGC TGC-3′
Magnetic Probe	5′-HS- $(CH_2)_6$ -iSp18-CCG CAA TGA GTT CAA TTC ATC CGT GTA CCC-3′
Gold Probe	5′-AAG CCA TGA GGT GAC GTA TAT TTC TTT AGT-iSp9-AGC TAC GAA TAA-$(CH_2)_3$-SH-3′
Scanometric	5′-HS- $(CH_2)_6$ - AAA AAA AAA ATT ATT CGT AGC T–3′
Chip Capture	5′-ACT AAA GAA ATA TAC GTC ACC TCA TGG CTT-$(iSp18)_2$-NH_2-3′
Center Blocking	5′-TTG AAC AAG CCG AGG GGT TCG TCT ACT GTG TAT CT-3′
5′ Blocking	5′-ATT GAC GGT CTG CTT GTT CCG GAT CTG CCA TTA GA-3′
3′ Blocking	5′-TGT TCC GGT TGC TGT AGG GTT CGG TAT ATC AAA CC-3′
Melting Au NP	5′-HS-$(CH_2)_6$-A_{10}-AC TAA AGA AAT ATA CGT CAC CTC ATG GCT T-3′
Melting MMP	5′-HS-$(CH_2)_6$-A_{10}- GG GTA CAC GGA TGA ATT GAA CTC ATT GCG G-3′
AuNP Target (*trp1*)	5′-FITC-AA GCC ATG AGG TGA CGT ATA TTT CTT TAG T-3′
MMP Target (*trp 3*)	5′-FITC-CC GCA ATG AGT TCA ATT CAT CCG TGT ACC C-3′

iSpX = Polyethylene Glycol (X units of ethylene oxide)