Abstract

GeneCapture is a company at the Hudson Alpha Institute for Biotechnology working on the development of a portable medical device to quickly identify any pathogen based on a DNA hybridization process. Existing amplification-based tests, such as the Polymerase Chain Reaction (PCR), can fail in accurately identifying these pathogens due to the rapid changeability of these pathogens. The GeneCapture DNA hybridization process uses a surface-immobilized stem-loop probe (SLP) hybridization technology with a universal fluorescent detector to identify multiple genetic expressions without the need of amplification by taking the advantage of the natural abundance of RNAs.

My research with GeneCapture is to design SLPs that can target the RNA signatures for the pathogen *Staphylococcus aureus aureus* (*S. aureus*). I have done research in creating two surface-immobilized stem-loop probes: BioD and PurK. Then using a prototype of the GeneCapture device, I have conducted experiments which uses a 2-step DNA hybridization process to test if these SLPs are viable options that can be used to detect *Staphylococcus aureus aureus*.

Introduction

The GeneCapture device is designed to rapidly and effectively screen patients who carry the Methicillin- resistant *Staphylococcus aureus aureus* (MRSA) bacterium. The final design of the device would require only one manual step of placing the patient sample into a disposable cartridge. The cartridge would include all the necessary processing technology and equipment to detect the pathogen of interest. Using a Direct RNA capture technique to detect the *S. aureus* bacteria overcomes the limitations of existing techniques that cannot adapt to the changeability of *S. aureus* strains. Providing fast treatment to *S. aureus* infected patients can prevent future out breaks of antibiotic resistance bacteria and reduce hospital spending. (GeneCapture 2012)

Methicillin-resistant *Staphylococcus aureus aureus* (MRSA)

Methicillin-resistant *Staphylococcus aureus aureus* is a type of staphylococcus bacteria that has evolved to be resistant to many antibiotics through the process of acquiring or losing genetic material. MRSA bacteria can develop when antibiotics are used too often or are incorrectly used causing the bacteria to change so that these antibiotics no longer work. (Healthwise, Incorporated 2013).

The *S. aureus* bacteria normally live on the human skin and in the nose without causing problems. However, MRSA can cause infections that occur in a hospital setting. These cases are called healthcare-associated Methicillin-resistant *Staphylococcus aureus aureus* (HA-MRSA). In most of these cases, people infected by the MRSA bacteria already have a weakened

immune system or a chronic illness thus becoming more vulnerable to infection. These infections occur in wounds, eyes, bones, blood, from surgeries, artificial joints, and intravenous tubing. More recently, MRSA has infected healthy people. Community associated Methicillin-resistant *Staphylococcus aureus aureus* (CA-MRSA) infections can occur through cuts and wounds among people who have had close contact with others who are infected. One of the reoccurring cases of MRSA outbreaks comes from athletes. Locker rooms promote skin to skin contact and bodily secretions making athletes vulnerable to MRSA infections. Most cases of CA-MRSA begin as mild skin infections such as pimples or boils. These mild infections can become fatal in a short amount of time if left untreated. Being able to quickly identify the MRSA bacteria would enable patients to obtain the necessary care quickly and prevent the spread of the bacteria to others. (Mayo Clinic Staff 2012)

Polymerase Chain Reaction

Traditionally, the Polymerase Chain Reaction (PCR) has been used to detect bacterial genetic strains by accumulating millions of DNA sequences. Detecting small amounts of genetic material can be used in many biological areas such as identifying bacterial infections, cloning, and genetic engineering. This process takes up to 4 hours and easily subjected to inaccuracy from environmental factors. (PubMed, n.d.)

The PCR process goes through four main steps: 1) denaturation, 2) annealing, 3) DNA synthesis, and 4) exponential amplification (PubMed n.d.). Denaturation separates the original double stranded DNA double helix by heating the strand to 94-96 ℃. The annealing process is where primers are added to bind to the 5' end of each DNA strand. Primers are short pieces of single-stranded DNA that are complementary to the target sequence. Primers are the starting point for the DNA polymerase to bind to on the original DNA sequence for the DNA synthesis step (PubMed, n.d.). DNA polymerase begins to synthesize new strands of DNA complementary to the target starting at the end of the primer sequence. The DNA polymerase elongates this new complementary strand by added deoxynucleotide triphosphates (dNTPs), which are single units of nucleotide bases (e.g. A, T, G and C). Once the new complementary sequences are synthesized, the finally product will be two double helix strands of the exact same DNA sequence. The PCR process repeats and amplifies the original sequence strand exponentially.

GeneCapture's device can detect small amounts of bacterial genetic material without having to use the PCR process. PCR is limited by the process's dependence on enzyme, primer usage, and sample purity. Enzyme performance depends on many factors and can be easily inhibited by the conditions of the system (Slish, n.d.). If the primer is not specific to the DNA template, cross reactivity with non-target DNA sequences can result in non-specific amplification of DNA (Slish, n.d.). If the sample is contaminated with foreign DNA, PCR can amplify the undesirable DNA strands (Slish, n.d.). PCR based diagnostic target only one to three MRSA genomic sequences, based on the similarities between MRSA strains (GeneCapture 2012). PCR can fail to

detect new MRSA bacteria which have mutated from preexisting strains because PCR targets a limited number of MRSA genes.

GeneCapture Direct RNA Capture

The goal for the device is to effectively detect MRSA in patients in one hour. The patient sample is placed in the cell lysis chamber of the device, where moving beads are used to break open the cells. The solution containing RNA will be passed over the hybridization slide where the Direct RNA Capture hybridization takes place to detect the MRSA RNA material within thirty minutes. (GeneCapture 2012)

The GeneCapture device uses a 2-step hybridization technique called "Direct RNA Capture" to detect the RNA material. Direct RNA Capture uses several stem loop probes (Figure 1) unique to the MRSA bacteria and fluorescence technology to detect multiple genetic signals simultaneously (GeneCapture 2012). The design process of the chosen stem loop probes will be further discussed in the Materials section. A visualization of the 2 step hybridization method is shown the figures below. Using stem loop probes to detect genetic material eliminates the need for enzymes and allows for specific detection of low concentrations of RNA material. First the stem loop probes are attached on a glass slide (Figure 1). As the liquid solution containing RNA passes over the stem loop probes, the stem loop probes bind to their target RNA which is complementary to the loop region of that probe (Figure 2). After this first hybridization step, the slide is cooled. As the slide is cooling, any probes that had not bound to their targets will close whereas probes that have bound to their target will remain in an opened position (Figure 3). After cooling the slides, a fluorescent dye solution is introduced to the probe and RNA solution for the second hybridization step (Figure 3). In the second hybridization step, the fluorescent dye binds to the stem portion of the probes that have remained open after the cooling step. Finally, the slide is scanned using fluorescence detecting technology. If the fluorescent dye is detected, then the fluorescent detector had bound to an opened probe bound to the MRSA RNA resulting in a MRSA positive patient sample.

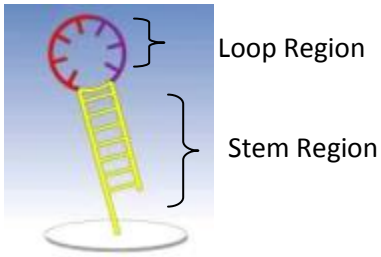

Figure 1: Stem loop probe attached to glass slide. (Dowell 2012)

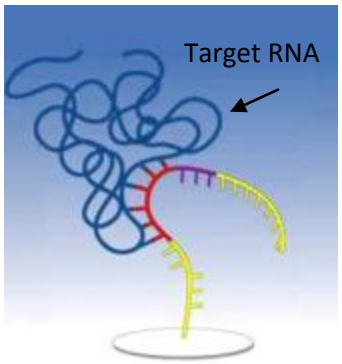

Figure 2: Target RNA bound to open probe. (Dowell 2012)

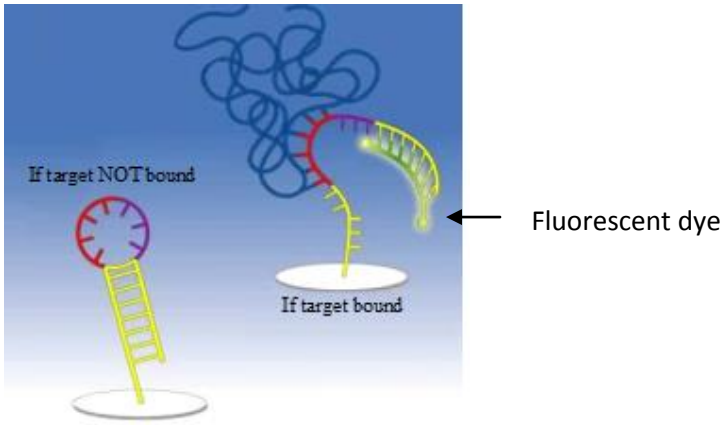

Figure 3: Probes not attached to a Target RNA returns to a closed position, while opened probes will have a fluorescent dye attached thus giving a positive MRSA result. (Dowell 2012)

Hypothesis

My research focused on designing and verifying stem loop probes that could be used in the GeneCapture device to detect the MRSA bacteria. The MRSA RNA strands that the probes I designed will target were based on literature research and finding expression changes in RNA levels after exposure to the antibiotic, Ciprofloxacin. The probes must then be designed to be complementary to the MRSA RNA and to contain the standard stem region. After designing the probes, the probes are verified to bind to their target RNA sequence by running hybridization experiments with the probes in the GeneCapture prototype.

The two probes that I designed were structured based on the MRSA genes BioD and PurK. The probes BioD and PurK have been chosen because of their high level of change in expression once exposed to the Ciprofloxacin. After designing the probes into the stem loop shape, the

probes are expected to attach to their RNA targets due to their complementary RNA loop region and to have structurally stable thermodynamic properties.

Probe Design Procedure

The stem loop probe structure includes a stem region and a loop region. The stem region is the same for each probe, whereas the loop region of the probe will be unique to the *S. aureus* RNA sequence that is to bind specifically to the desired MRSA gene. The stem and loop region are determined separately, and are later combined to form a unique genetic probe sequence. This probe sequence is then cross-referenced to genetic sequence data base sources, such as the NCBI BLAST tool, to verify that the sequence of the probe is unique only to *S. aureus*. Finally, the genetic sequence of the probe is sent to Integrated DNA Technologies (IDT), Inc. to build these probe structures.

The loops of these probes had to be built based on sequence information that are unique to the *S. aureus* bacteria from the NCBI genome database. The genes that the probes will target were chosen based on their expression change after exposing the bacteria to Ciprofloxacin (Cirz 2007). The two genes focused in this paper are the PurK gene and the BioD gene.

PurK, N5-carboxyaminoimidazole Ribonucleotide synthetase, belongs to the ATP-grasp superfamily of carbon-nitrogen bonding ligase enzymes involved in the biosynthesis of purine. Purines are synthesized as nucleotides, which are the building blocks for the production of genetic material (Thoden 1999). PurK catalyzes the conversion of 5-aminoimidazole ribonucleotides (AIR), ATP, and bicarbonate to N5-carboxyaminoimidazole ribonucleotide (N5-CAIR), ADP, and Pi by adding a carboxyl group onto the amine functional group (Thoden 1999).

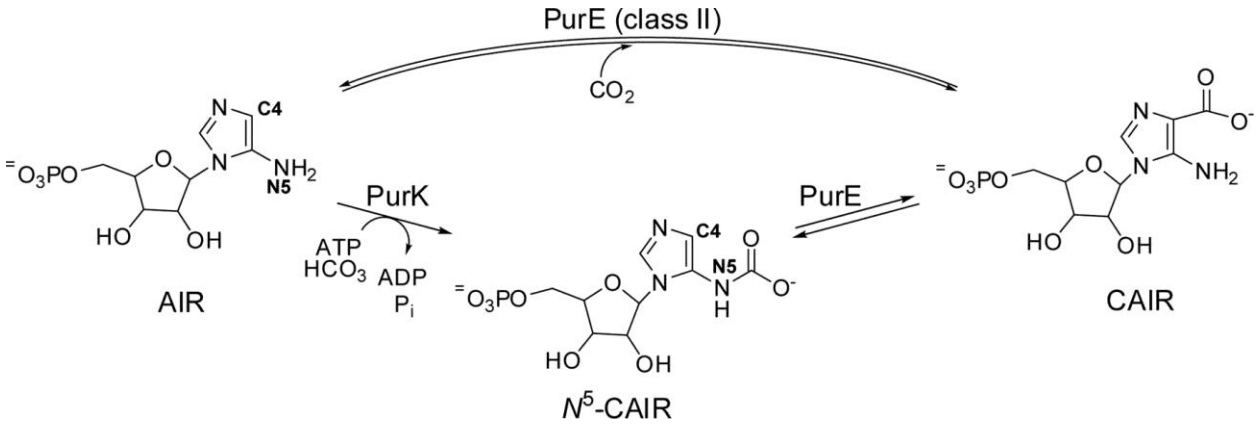

Figure 4: PurK catalyzing the conversion of AIR to N5-CAIR.

BioD, Desthiobiotin synthetase, belongs to the cycloligase family, which forms carbon-nitrogen bonds. The enzyme catalyzes the chemical reaction

ATP + 7,8-diaminononanoate + CO_2 = ADP + phosphate + dethiobiotin

, which inserts a carbon dioxide molecule in between the N7 and N9 nitrogen atoms of 7,8-Duanubioekargibuc acid (DAPA) (BioD 2014). This reaction is necessary for the biosynthesis of biotin. Bacteria can synthesis biotin which is a cofactor for several enzymes required for all organisms for the production and metabolism of fatty acids and amino acids (Dtb Synthetase, n.d.) for cell growth.

The PurK gene was chosen as a gene because after thirty minutes the gene's expression decreased by 6.8 fold and after 120 minutes continued to decrease by 12.7 fold. BioB was chosen because after thirty minutes the gene's expression was increased by 8.5 folds and after 120 minutes continued to increase by 7.4 folds. Even though only PurK and BioB genes are analyzed in this paper, a variety of other genes from the Ciprofloxacin paper were chosen to be templates for the stem loop probes. Having many unique probes would allow for more confidence that the kit can correctly detect the presence of *S. aureus* in a patient sample.

The stem is made up of two artificial complementary strands (Figure 5). The length and sequence of the two strands is unique and will not bind to any other bacterial RNA. The sequence and length of the stem were created to be strong enough to remain closed if no complementary binding occurs in the loop region, but still be able to open when specific binding has occurred in the loop region of the probe. The specific length of stem was also chosen so that once the probes are printed onto glass slides and the probes are high enough off the slide to have a solution-like environment. These criteria are the basis in standardizing the construction of the stems of the design probes.

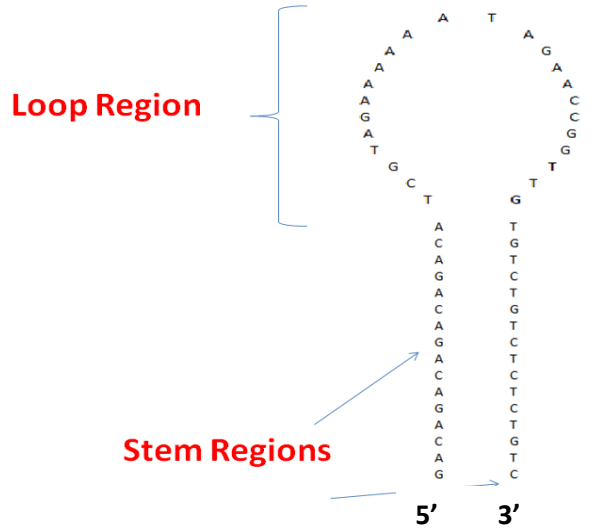

Figure 5: Example of the structure of a stem-loop probe.

After choosing a gene to target, the NCBI gene sequence data base was used to find all the genetic sequences for that specific gene. In the case of PurK, the NCBI website provided 20 sequences of the PurK gene and the BioD gene had 24 sequences. These sequences are then compared using a sequence data base program called Adapt. Adapt first compares small segments of the gene to find which sequences are conserved between all the selected *S. aureus* strains. Then the sequences are compared to find which of these segments are unique to *S. aureus* and will not cross react to other pathogens. The more unique the gene structure is to *S. aureus*, the more accurate the kit will be in detecting *S. aureus*.

Next, each loop region of the probes is placed between the two sequences of the stems. This newly generated potential probe must now be computer generated into a 3-D structure. Then the 3-D structure must be verified to be structurally stable. The structurally stability of the probes is based on the parameters of the melting temperature, the change in free energy (ΔG), and the shape of the 3-D structure. These parameters were optimized for the conditions in which these probes will be used for by GeneCapture. The mFold website is used to generate the 3-D structure of the genetic sequence. Once the structure is generated, we verify to see if the structure has a hair pin loop shape and that the melting temperature and change in free energy (ΔG) is within the GeneCapture's chosen parameters.

If the sequences of the probe meet the folding thermodynamics set by GeneCapture, the final genetic sequence of the probes are then compared against the most current NCBI web database of pathogen sequences and human RNA. The completed probe must not have any cross reactivity against host genes or other pathogens. The BLAST program has a database containing all organisms, which the program uses to compare the desired genetic sequence to that of the sequences in its database. The BLAST program on the NCBI website is used to cross reference the probe genetic sequence to other organisms' genetic sequence. The output of the system displays how closely the sequence of the probe matches with a sequence of another organism. This is the final verification step for designing the stem loop probes. As long as the probes have good thermodynamic folding stability and are specific in binding to *S. aureus* chosen genes, then these designs will be sent to IDT inc. to build these chosen genetic structures.

Microarray Construction

A microarray is a collection of genetic material attached to a solid surface arranged in a particular order (Learn.Genetics, 2014). They are used to measure the expression levels of many different types of genes simultaneously (Learn.Genetics, 2014). Once the probes have been received from IDT inc., the probes are printed as a microarray onto the glass slide. For these experiments, the Perkin Elmer Piezarray microarrays were created "in house" using a microarray printer (DNA Microarray, n.d.).

The DNA microarrays fabricated by GeneCapture are covalently coated with Dendron modified surfaces, which improves the hybridization process. Dendrons are cone-shaped structures that

create a solution like environment for immobilized probes by lifting the probes up above the glass surface. Dendrons can control the spacing between each immobilized probes which reduces non specific binding and enhance selectivity of the probes. (Soon, 2005)

The probes are mixed into a buffer solution containing Mg Cl2 NaHCo Dimethyl sulfoxide DMSO. DMSO is a spotting (or microarray printing) aqueous solution for many organic molecules. The DMSO solution "wets" the hydrophobic dendron cones and then through a series of chemical mechanisms the probes bind to the DMSO structure which is connected to the glass slide. A series of experiments have been done to determine the appropriate of spotting buffer needed to avoid the probes from occupying too many of the dendron cones. Overcrowding the cones can cause the probes to bind nonspecifically. The microarray has a 24 X 24 spotting pattern with pairs of up to 12 probes.

Experiment procedure

The objective of these hybridization experiments is to verify if the stem loop probes that were created will attach to the unique mRNA strands indicative of the SOS-mediated response of *S. aureus* due to the exposure from the antibiotic, Ciprofloxacin. The experiments require using a prototype of the GeneCapture kit. The prototype of the kit does not contain an electrical motor which allowed GeneCapture to have control the flow of the solutions across the glass slide.

The buffers in these experiments contain standard sodium phosphate with EDTA but vary in the amount of ethanol. Each buffer has a specific task in the experiment. The main component of the buffers is standard Sodium phosphate with EDTA (SSPE) and Sodium dodecyl sulfate (SDS). SSPE is a stabilizing salt solution and SDS is a detergent that wets the slide. The Norm buffer contains 2 X of standard sodium phosphate with EDTA. EDTA takes out Ca^{2+} ions on the slides. Ca^{2+} in the solution is promotes instability in the RNA structure by catalyzing the autocleave use of the RNA phosphate backbone. The Norm buffer is used as way to wash away unwanted debris off the probes. The targets are placed in a different buffer solution containing 2X SSPE with 0.1% SDS and 10% ethanol. Ethanol increases the activity of the target by driving the targets out of the SDS solution by making the targets less thermodynamically stable. This increase instability of the targets increases the rate at which the targets want to bind complementary on the probe. Once bound on the probe, the target and probe binding is thermodynamically favored.

All the components of the GeneCapture kit are assembled by hand (Figure 6). This includes closing and opening the appropriate valves, placing caps on chamber well holding the different buffers, and the precise placement of the glass slide onto the back of the kit allowing for the solution to uniform flow over probes. Next, the heating element is turned on to equilibrate. The 1^{st} temperature setting is set at a range of 37.0 ℃ to 47.0℃ so that mismatched targets (RNA strands from patient samples) will not bind to the probes, while correct targets will bind

complementary to the loop region of the probes. Once that temperature has been reached, the heating element is placed on the back of the slide securing and heating the slide. While the heating element is heating the slide to the appropriate temperature, Norm buffer is poured into the first chamber well using a syringe. 1.6 mL of target solution is pulled into a three milliliter (mL) syringe and placed onto the central port.

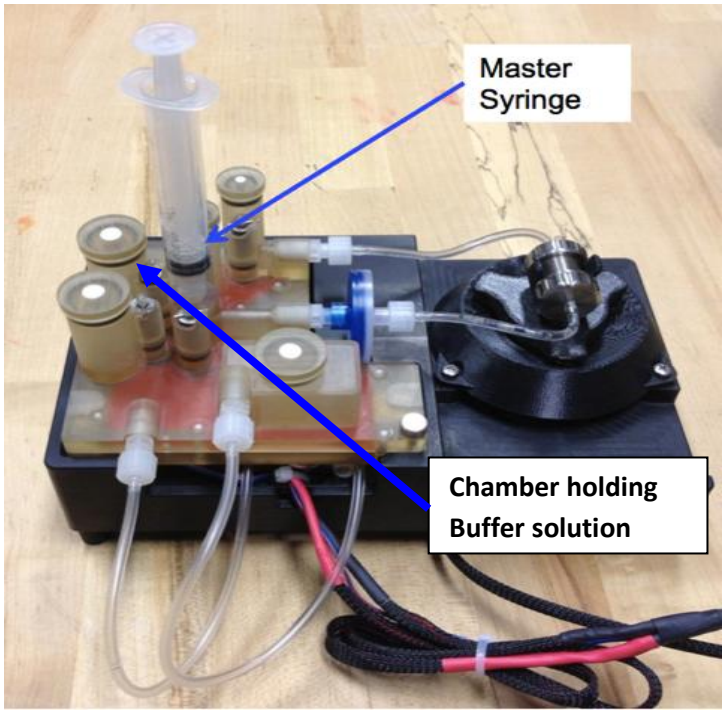

Figure 6: Prototype of GeneCapture medical device.

The first hybridization of the target onto the probe begins by flowing the target solution through the opening between the central port opening and the slide opening. The solution is drawn back and forth using the syringe to promote mixing of the target solution across the glass slide. The large scale mixing concept for hybridizing targets onto probes is unique because traditionally the slides are placed in a tube of target solution and set stationary for a period of time. The traditional diffusion limited hybridization method is slow and incomplete so many attempts have been made to mix tiny volumes during hybridization. GeneCapture mixes the target solution on the glass slide because mixing causes uniform flow so that targets increases its contact with probes on the surface of the slide giving a higher probability of target and probe binding. After mixing the target solution over the probes for ten or twenty minutes, the syringe pushes all of the target solution off the slide, and then the syringe is taken off the central port and filled with air. The syringe is placed back onto the port and air is pressed over the slide to remove any remaining solution.

The next step is to wash off any free or nonspecific bonded targets off the probe slides. The valve connecting the port and the slide is closed, and the valve connecting the first chamber well

and the port is then opened. The norm buffer in the first chamber well is drawn up by the same syringe. Then, the valve between the first chamber well and the port is closed, and the valve between the port and the slide is opened. The syringe is pressed down pushing the 1.5 mL of norm buffer onto the slide. The syringe presses a 0.5 mL of buffer up and down flowing the norm buffer across the slide and washing off any free or nonspecifically bonded targets After one minute of mixing, a cooling fan attached near the slide is turned on to cool the slide to 23.0 ℃. Mixing and cooling the slide allows for any of the probes who did not bind to any targets to close and return to its closed stem loop shape. Flowing the norm buffer is continued across the slide until the temperature of the slide is equilibrated to 23.0℃. Once the fan has stopped and the temperature of the slide is 23.0 ℃, the syringe is taken off the port, filled with air, placed back onto the port, and pushes the air across the system to push off any leftover wash solution.

The second hybridization involves binding the 13 nucleotide (nt) detector onto probes that have bonded specifically to their targets. A new syringe containing a 1.5 mL of detector is placed onto the central port. Next, the syringe presses down the 13 nt detector through the opening and onto the slide. The plunger of the syringe is pressed up and down rapidly flowing the 13 nt detector solution across the slide. This back and forth flow of detector is done for only 20 seconds. After 20 seconds, the detector is washed off by removing the syringe, filling the syringe with air, and pressing air across the slide.

Immediately after the detector is washed off, a final wash with norm buffer is necessary to remove any unwanted detector. A new syringe filled with norm buffer is placed onto the port and is pressed down until a 1.5 mL of norm buffer is onto the slide. The temperature is then set to 28.0℃. Once the temperature has been equilibrated for two minutes, a 0.5 mL of norm buffer is flowed back and forth across the slide. After mixing for one minute, temperature is set to 23.0 ℃, and the norm buffer is mixed until the slide has cooled to 23.0℃ from the air fan. Once the slide is cooled, the remaining one mL wash is pushed over the slide. The syringe is removed, filled with air, and air is pressed over the slide removing remaining norm buffer. Finally, the slide is ready for the final wash step. The slide is removed from the kit and dunked into a tube of 0.2X standard sodium citrate (SSC), removing any debris. The slide is placed in the SSC solution for only one second. The SSC is dried off the slide using a nitrogen gun.

After drying the slide completely, the slide is labeled and stored into a dark slide holding container. It is necessary to keep the slides dark to limit the exposure of the detector to any light. The slide is scanned using the GenePix 4000B microarray scanner. From the fluorescent detection of the scanned slide images, an analysis can be made of how well the probes bonded to their targets.

Fluorescent Detector

The fluorescent detector is designed to be complementary to the linear open arm region of the probe after it has bound to the target. The detector is a single strand of 13 nucleotides that match

the end of the probe and has a fluorescent dye attached to the 5' end. The fluorescent dye used is Alexa 647, developed by Molecular Probes, Inc. (ThermoFisher 2014). The Alexa 647 dye is a far-red-fluorescent dye detected using 635 nm laser lines. Alexa647 can detect low abundance of biological structures due to its high efficiency of the fluorescence process (fluorescence quantum yield) and high photostability (ThermoFisher 2014).

Fluorescent Scanner

The GenePix 40000B microarray scanner uses a laser excitation method which uses 635 nm and 532 nm lasers that are directed onto the slide after passing through a series of filters and mirrors (Molecular Devices, 2013). These wavelengths correspond to the optimal wavelengths used to excite common fluorescent dyes (Molecular Devices, 2013). The lasers scan the microarray slide slowly in the y-direction, and rapidly scan in the x-direction. A pair of high-sensitivity, low- noise photo multiplier tubes (PMTs) are used to detect the emitted fluorescent light. PMTs convert photons that reach its active surface into electrons. The amount of current produced by the flow of electrons is directly proportional to the amount of photons emitted from the fluorescence. If there is a high concentration of fluorescence, then the more intense the signal is. To the user, the machine produces an image of the slide and the fluorescence is shown as a bright light on the image. The larger the signal produced, then the brighter the image is (Axon Instruments, 2011).

Conditions of Experiment

The objective of these hybridization experiments is to verify if the stem loop probes that were created will attach to the unique mRNA strands indicative of the SOS-mediated response of *S. aureus*. To verify that the stem loop probes worked, the concentration of targets in the solution was varied to determine the smallest concentration of target in solution that could be detected by the probes with small amounts of background noise. Targets in the solution are segments of DNA which will bind complementary to the probes. RNA is not used because RNA oligonucletides are not readily bought from companies. However, DNA should have enough binding interactions to use for analysis in this stage of this experiment. Tables 1 and 2 list the concentration of target added in each of the BioD and PurK experiments respectively.

The solution for each hybridization experiments contained the desired targets and the hybridization buffer described in the Materials section. The buffer in the BioD experiments used hybridization buffer. However, the buffer used in the PurK experiments also contained "dirty" components. The dirty components are cell lysates, which are broken up pieces of proteins, nucleotides, etc. which was used in this experiment to mimic the background seen in an actual patient sample. Tables 1 and 2 summarize how each experiment for each probe was varied by changing the concentration of targets in the solution.

Each experiment contains a positive control probe and a negative control probe. The positive control probe has an artificial DNA sequence, which targets only the positive control target (Pos1T). The negative control probe was created to not bind to any targets. Having the positive control and negative control indicates the performance of each experiment.

The experiments were done on a 24 spots by 24 spots microarray (Figure 7). Each microarray contained up to 12 different types of probes, thus each probe occupied 48 different spots. For analysis, the 24 by 24 microarray was divided into 8 smaller blocks with each probe appearing on 6 spots within each of the smaller blocks. Dividing the spots into smaller blocks allows for a convenient method for averaging the data. Further averaging of the data is done by printing the spots in pairs. Each pair of probes is printed far from each other within each block to create a controlled environment setting. Dividing the microarray into smaller blocks is a simple way to exclude part of the array if there was a problem with printing or hybridization during the analysis.

The scanner reads the fluorescence across the array at a wavelength of 635 nm and calculates the mean intensity of fluorescence per area of the spot to quantify the data. The intensity of each probe is first averaged between each pair of probes, then averaged within each block, and finally averaged across the entire microarray. Any binding that has occurred that is not between the probe and its complementary binding target is called non-specific binding, which is also known as background noise. To take account the amount of non-specific binding in the analysis, the average intensity of each probe detected by the scanner is divided by the average intensity of the

negative control, thus the signal-to-noise ratio (SNR) of the intensity of each probe as compared to the negative control probe is calculated as shown in Eq. 1.

$$SNR = \frac{Average\ intensity\ of\ each\ Probe}{Average\ intensity\ of\ negative\ control\ probe} \qquad Eq.1$$

This ratio tells us how much complementary target to probe binding has occurred compared to the non-specific binding. The change in SNR allows for comparison between each experiment because the actual intensities of the probes and negative control vary with each experiment.

Types of Conditions for BioD	Repeats of Experiment	Solution	Amount of Targets
1	**2**	**Hybridization Buffer**	**No targets**
2	**2**	**Hybridization Buffer**	**1nM desired Targets**

Table 1: Experimental Conditions for BioB.

Types of Condition for PurK	Repeats of Experiment	Solution	Amount of Targets
1	**6**	**Dirty Hybridization Buffer**	**No Targets**
2	**2**	**Dirty Hybridization Buffer**	**1 nM desired Targets**
3	**4**	**Dirty Hybridization Buffer**	**250 pM desired Targets**
4	**6**	**Dirty Hybridization Buffer**	**100 pM desired Targets**
5	**3**	**Dirty Hybridization Buffer**	**50 pM desired Targets**
6	**3**	**Dirty Hybridization Buffer**	**10 pM desired Targets**

Table 2: Experimental Conditions for PurK.

Microarray Figures

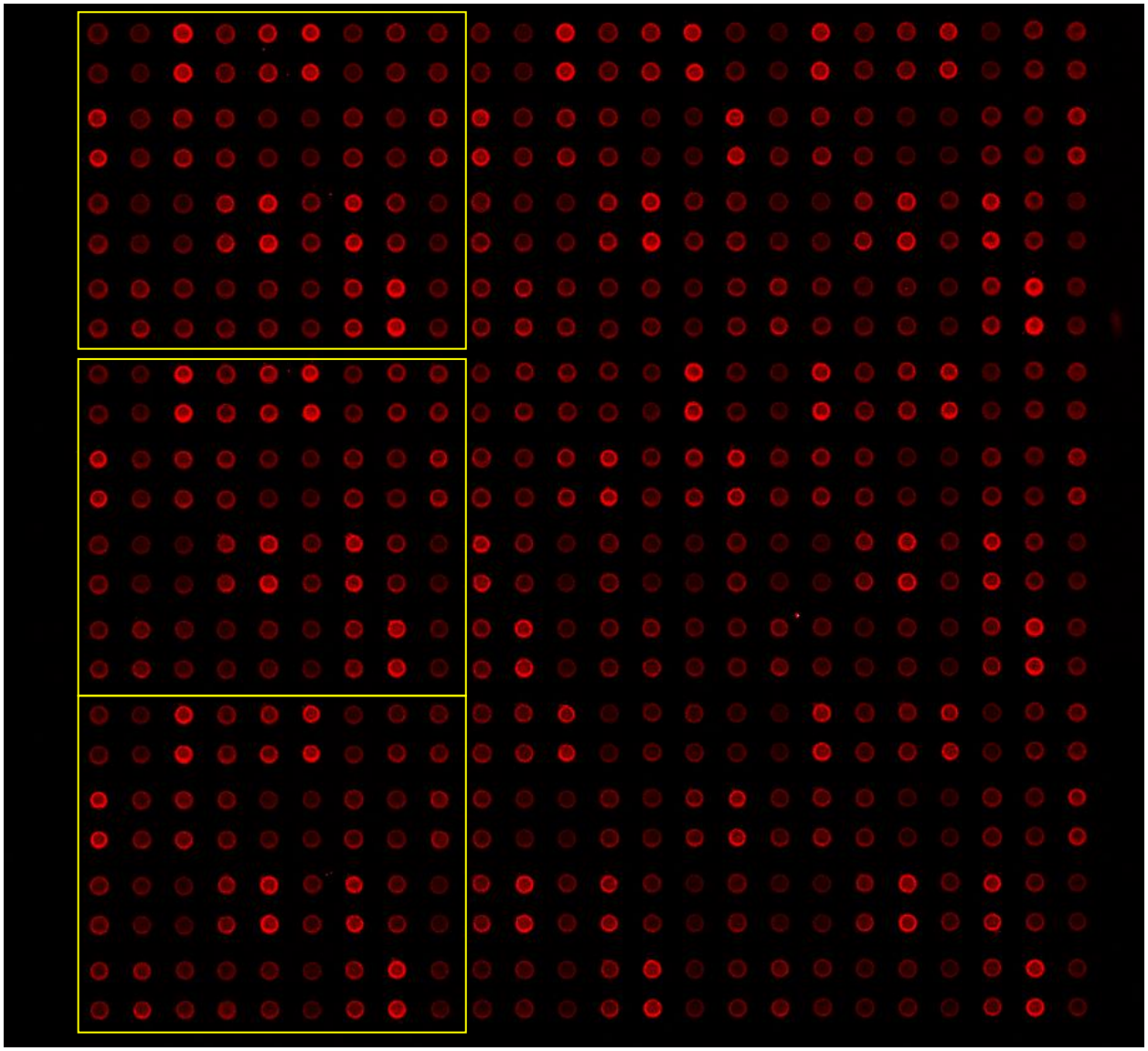

Figure 7: Microarray of hybridization experiments using only Buffer.

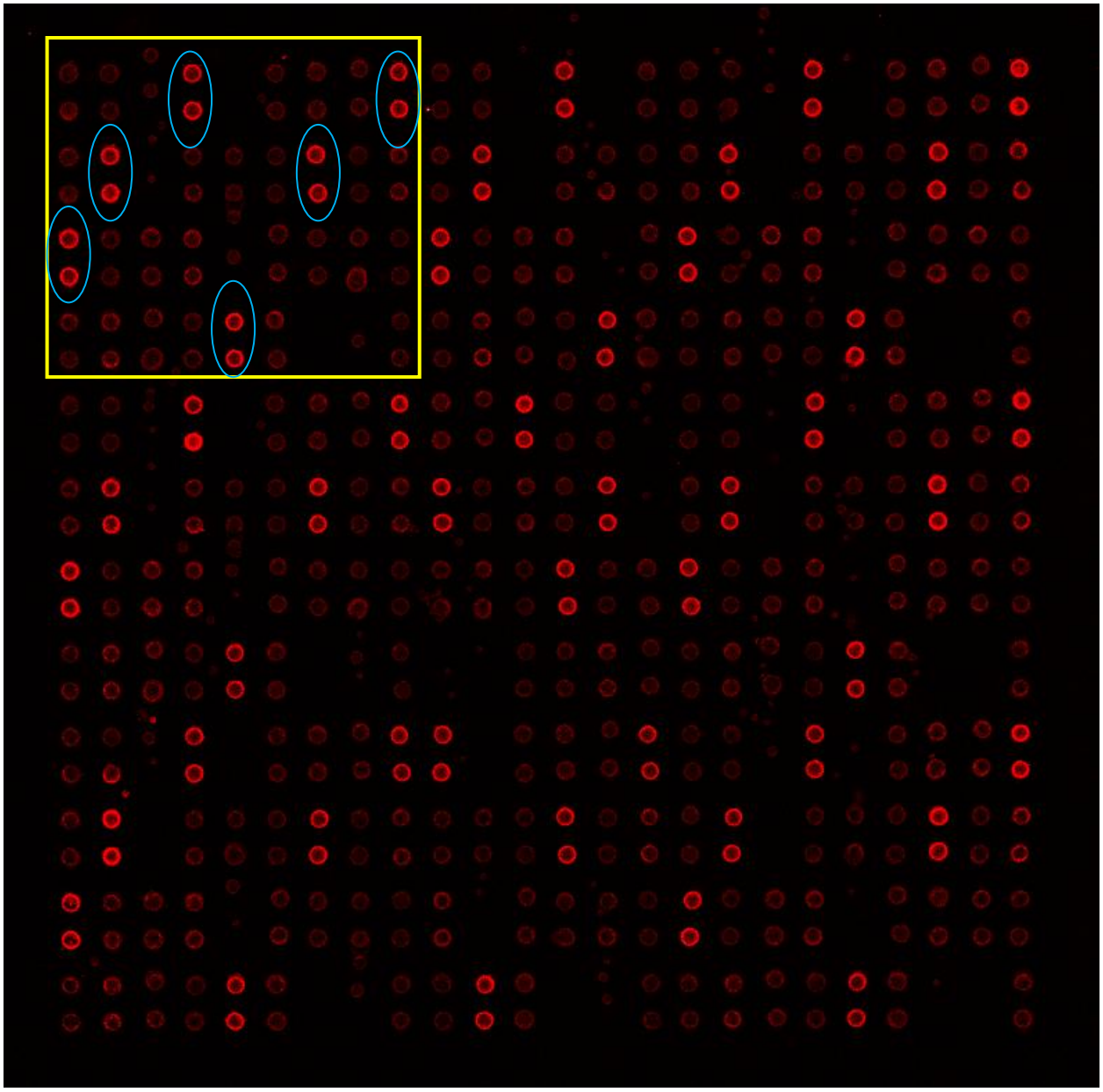

Figure 8: Microarray of hybridization experiments using Dirty Buffer with 500 pM Pos1T.

Analysis of Microarray

The microarrays for two experiments are shown in Figures 7and 8. Each microarray contains 12 probes, but for this paper, only the probes BioD and PurK are analyzed. In Figure 8, the blue circles encompassing two spots are an example of a pair of the same probes printed together. Each pair of spots should have the same mean intensity value, but it can be seen in the uneven brightness between the spots that the mean intensity values have some variation, which is why an average needs to be taken for more confident values. The yellow boxes in both figures show how the microarray was separated into smaller sections for analysis. The mean intensities of each

pair of spots were averaged together, then that averaged value is averaged with all the other pair of spots of the same probe within the yellow blocks, and finally that averaged value is averaged between all of the 8 blocks.

As previously stated, separating the microarray into smaller blocks allows for confidence in significant values. In Figure 8, there are spots missing from the microarray due to an error in the microarray printing machine. Creating the smaller blocks allows us to still analyze the data because averages between each pair of probes can still be taken.

Figure 7 shows the intensity of the fluorescence of each spot on the microarray after the hybridization experiments using only hybridization buffer with no targets added. This figure shows that some non-specific binding has occurred because there was some fluorescent dye detected across the microarray as shown in the brightness of the spots. The uneven intensity of each spot indicates that each probe has a different non-specific binding pattern. However, further studies show that the amount of non-specific binding for each probe is consistent between each experiment.

Figure 8 shows the hybridization experiments after using dirty buffer with only the positive control target. The figure shows that the spots with the positive control probes are brighter than the surrounding spots, which is expected because only the positive control target was added.

Signal to Noise Ratio Data Analysis

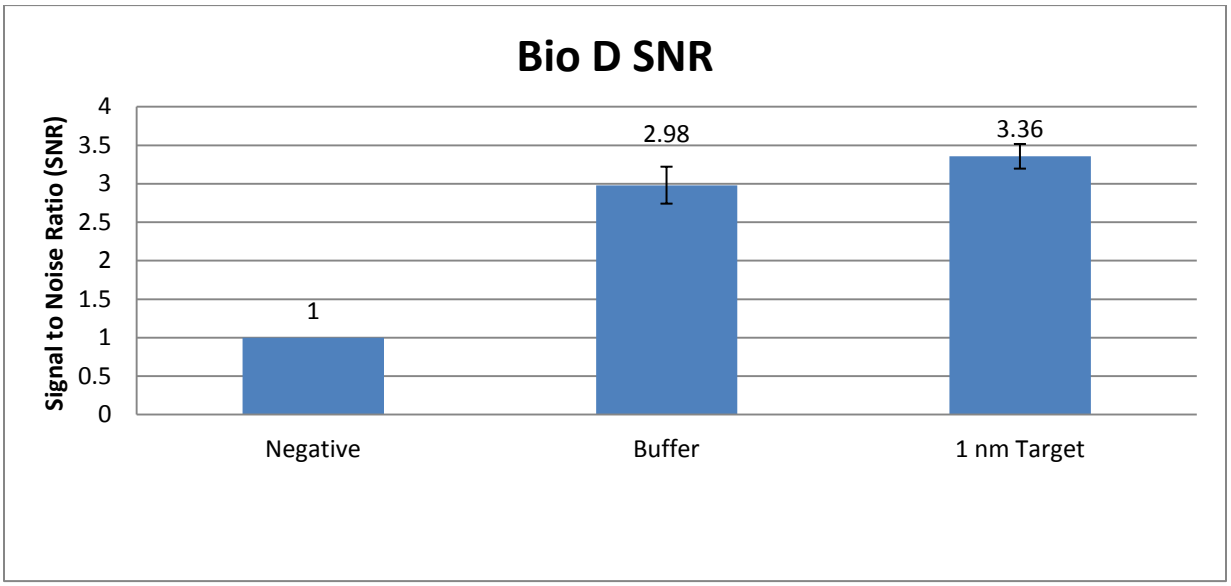

Figure 9: Signal to Noise ratio of BioD in different solutions.

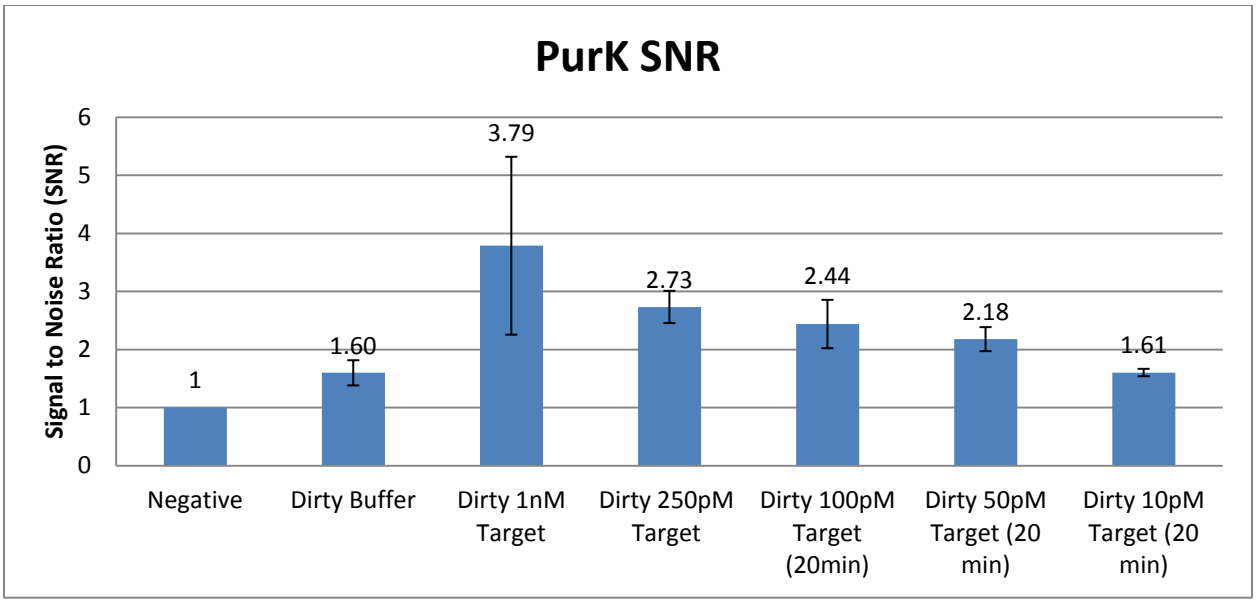

Figure 10: Signal to Noise ratio of PurK in different solutions.

The SNR of BioD for the conditions with only buffer and no targets averaged to be 2.98 shown in Figure 9. Having no target in the solution and a SNR much greater than the SNR of the negative probe, which is 1.00, indicates that the BioD probe had opened up without having a target bound to the loop region allowing for non-specific binding. This indicates that the probe may be too weak to remain in an initial closed position. The SNR increases to 3.36 when 1 nm of target was added to the solution. The BioD probes can have some complementary binding to their targets because the SNR increased when its target is added. However, the SNR increase is small and not significant. BioD is not an ideal probe to use because the BioD has an SNR much greater than 1.00 when no target was present in the solution thus further investigation of this probe was discontinued.

When Pur K is in the dirty hybridization buffer solution with no targets, SNR is averaged to be 1.60. Some of the PurK probes have opened up without targets, but compared to BioD, which has a SNR of 2.98, PurK should be further investigated because the SNR value is closer to 1.00 than other probes under these conditions.

In the dirty buffer with varying concentrations of target added (1nM, 250pM, 100pM, 50pM, and 10pM), the SNR values of PurK consistently remained much higher than 1.60 value indicating that the PurK probes open when its target binds to the probes' loop region. The largest concentration of target added was 1nM and the smallest concentration added was 10 pM. We expect to see a lower SNR when less targets are added to the buffer. In Figure 10, the SNRs of PurK does decrease as the concentration of target is lowered as shown. This indicates the amount of PurK probes that bind is relative to the concentration of target added. This is

significant because the objective is to develop probes that can bind to its target even if there is a small concentration available in the sample. Comparing the SNR of PurK at 10 pM and no targets added, we see that the 10pM SNR value (SNR=1.61) is too close to the SNR value with no target (SNR=1.60) indicating that 10pM does not a give a confident signal of complementary target to probe binding. Lowering the concentration of target allows us to find that the lowest amount of target required for PurK detection cannot be lower than 10pM.

From the data gathered from the PurK experiments, making the first hybridization step run for 20 minutes allow results in a higher signal than at 10 minutes. This is expected because the longer the hybridization step, the more time available for the probes to bind to their target.

Further investigation of the PurK probe is done because with no target, the SNR values are consistently close to 1.00 indicating that most of the probes remain closed and that probes are strong enough to remain closed at these initial conditions. When its target is introduced to the system, the increase in SNR values indicate that the probes can bind complementary to its target. The PurK probe is a potential probe used to detect the *S. aureus* in the GeneCapture pathogen detection device.

Conclusion

The BioD results show that BioD is not a probe that should be further investigated. The experiments show that the BioD has too much non-specific binding, and cannot be used to obtain accurate signal in detecting *S. aureus* or any other pathogens in the GeneCapture device. The high SNR value with only 50 pM of target in the PurK experiments indicates that the PurK probe has a high complementary target to probe binding. Lowering the concentration of PurK target allowed us to find that the minimum amount of target needed for good PurK target to probe binding is at 10 pM. Finding this minimum concentration gives us confidence that the GeneCapture device can detect the *S. aureus* bacteria because normal cellular concentration levels of this target RNA is only 50 pM. Varying conditions such as temperature and buffer should continue to find the best conditions at which probes have the lowest non-specific binding and the highest target signal.

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