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Kinetic screening of nuclease activity using nucleic acid probes

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Dear Editor,

Please find attached the revised version #2 of our manuscript ***"Kinetic screening of nuclease activity using nucleic acid probes"*** . We have addressed all of the editors' concerns in the revised manuscript. We describe the changes and additions made in the revision in a point-by-point response to the editors' concerns. To address the concerns of the second revision, we have revised the supplementary figure 1 (button clicks), 2 additional supplementary figures (Button clicks) have been added, and substantial changes have been made to the main text and the supplementary files.

I would like to thank you for considering our revised manuscript for publication and I am looking forward to hearing from you.

Sincerely,

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TITLE:

Kinetic Screening of Nuclease Activity using Nucleic Acid Probes

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KEYWORDS:

Screening method, nucleases, biomarkers, nucleic acids, probes, nuclease activity, diagnostic tool, substrates

SUMMARY:

Altered nuclease activity has been associated with different human conditions, underlying its potential as a biomarker. The modular and easy to implement screening methodology presented in this paper allows the selection of specific nucleic acid probes for harnessing nuclease activity as a biomarker of disease.

ABSTRACT:

Nucleases are a class of enzymes that break down nucleic acids by catalyzing the hydrolysis of the phosphodiester bonds that link the ribose sugars. Nucleases display a variety of vital physiological roles in prokaryotic and eukaryotic organisms, ranging from maintaining genome stability to providing protection against pathogens. Altered nuclease activity has been associated with several pathological conditions including bacterial infections and cancer. To this end, nuclease activity has shown high potential to be exploited as a specific biomarker. However, a robust and reproducible screening method that can efficiently harness this activity remains highly desirable.

Herein, we introduce a method that enables the screening of nuclease activity using nucleic acid

probes as substrates, with the scope of differentiating between pathological and healthy conditions. This system offers the possibility of designing new libraries of probes, with increasing specificity, in an iterative manner. Thus, multiple rounds of screening are necessary to refine the probes' design with enhanced features, at each round, taking advantage of the availability of chemically modified nucleic acids. The considerable potential of the proposed technology lies in its flexibility, high reproducibility, and versatility for the screening of nuclease activity associated with disease conditions. It is expected that this technology will allow the development of promising diagnostic tools with great potential in the clinic.

INTRODUCTION:

Nucleases are a class of enzymes capable of cleaving the phosphodiester bonds that form the backbone structure of nucleic acid molecules. The vast diversity of nucleases makes their classification rather difficult. However, there are some common criteria used to describe nucleases, such as substrate preference (deoxyribonucleic acid (DNA) or ribonucleic acid (RNA)), cleavage site (endonucleases or exonucleases), or metal ion dependency, among others[1]. Nucleases are highly conserved catalytic enzymes that have fundamental roles in both, prokaryotic and eukaryotic organisms and have been used, and continue to be used, as gene editing tools[2]. They are also fundamental actors in DNA maintenance and replication, helping to keep genome stability and participating in proof-reading processes[3]. In bacteria, for example, nucleases have been identified as important virulence factors, able to promote bacterial survival by reducing the efficacy of the host´s immune system[4-8]. In mammals, nucleases have been suggested to be implicated in apoptosis[9], mitochondrial biogenesis and maintenance[10] and mediation of antibacterial and antiviral innate immune responses[11]. More importantly, nuclease activity alterations, whether enhancement or lack of, have been implicated in a wide array of human diseases. These diseases range from a wide variety of cancers[12,13] to cardiac hypertrophy[10] or autoimmune diseases[14]. Nucleases have become interesting candidates as biomarkers for a heterogeneous group of human conditions. In fact, nucleases have already shown their potential as successful diagnostic tools for the detection of infections caused by specific bacterial agents, such as *Staphylococcus aureus* or *Escherichia coli*[15,16]. In many cancer types, expression of staphylococcal nuclease domain-containing protein 1 (SND1) ribonuclease is indicative of poor prognosis[17]. In pancreatic cancer patients, elevated ribonuclease I (RNase I) serum levels have been reported[18] and proposed to be associated with cancerous cell phenotypes[19]. In ischemic heart conditions, such as myocardial infarction or unstable angina pectoris, deoxyribonuclease I (DNase I) serum levels have been shown to be a valid diagnostic marker[20,21].

It has been hypothesized that the global blueprint of nuclease activity may be different in healthy and disease states. In fact, recent reports have used differences in nuclease activity to distinguish between healthy and cancerous phenotypes[22] or to identify pathogenic bacterial infections in a species-specific manner[15,23]. These findings have opened a new avenue for the use of nucleases as biomarkers of disease. Therefore, there exists a necessity for the development of a comprehensive screening method able to systematically identify disease associated differences in nuclease activity, which may be of key importance in the development of new diagnostic tools.

Herein, we introduce and describe a new in vitro screening approach (**Figure 1**) to identify sensitive and specific probes capable of discriminating between nuclease activity in healthy and unhealthy, or activity specific to a type of cell or bacteria. Taking advantage of the modularity of nucleic acids, we designed an initial library of quenched fluorescent oligonucleotide probes consisting of a comprehensive set of different sequences and chemistries, both being important parameters for the screening methodology. These oligonucleotide probes are flanked by a fluorophore (fluorescein amidite, FAM) and a quencher (tide quencher 2, TQ2) at the 5´ and 3´ ends respectively (**Table 1**). By using this fluorescent resonance energy transfer (FRET) based fluorometric assay to measure the kinetics of enzymatic degradation, we were able to identify candidate probes with the potential to discriminate differential patterns of nuclease activity associated with healthy or disease states. We designed an iterative process, in which new libraries are created based on the best candidate probes, that allows the identification of specific candidate probes in subsequent screening steps. Moreover, this approach takes advantage of the catalytic nature of nucleases to increase sensitivity. This is achieved by making use of the activatable nature of the reporter probes and the ability of nucleases to continually process substrate molecules, both representing key advantages over alternative antibody or small molecule-based screening methods.

This approach offers a highly modular, flexible and easy to implement screening tool for the identification of specific nucleic acid probes capable of discriminating between healthy and disease states, and an excellent platform for the development of new diagnostic tools that can be adapted for future clinical applications. As such, this approach was used to identify the nuclease activity derived from *Salmonella* for the specific identification of this bacteria. In the following protocol, we report on the methods for screening bacterial nuclease activity using kinetic analysis.

PROTOCOL:

1. Oligonucleotide library design and preparation

1.1. Library design

1.1.1. Based on the following criteria design a library of oligonucleotide probes between 8 and 12-mer long to avoid secondary structure formation, with equal length for all probes.

1.1.1.1. Include at least one DNA and one RNA random sequence containing a combination of adenine (A), guanine (G), cytosine (C) and thymine (T)/uracil (U) (**DNA and RNA probes in Table 1**).

NOTE: The DNA and RNA sequences described in **Table 1** have been useful as the initial substrates for classifying an unknown type of nuclease activity, either DNase or RNase. These two sequences are recommended as the starting point for any screening, as they have shown a wide capability to detect nuclease activity profiles in bacteria and tissue samples. If nuclease activity is not observed with these DNA and RNA sequences, the design of additional oligonucleotides is

required.

1.1.1.2. Include polynucleotide sequences consisting of a single type of nucleotide to determine sequence dependence for a given nuclease activity (DNA-Poly A, DNA-Poly T, DNA-Poly C, DNA-Poly G, RNA-Poly A, RNA-Poly U, RNA-Poly C and RNA-Poly G in **Table 1**).

1.1.1.3. Using the same sequence of nucleotide residues as the initial DNA and RNA sequences, include sequences that contain nucleoside analogs harboring chemical modifications at the 2'-position of the ribose sugar, such as 2'-Fluoro and 2'-O-Methyl, as a stringent step for increasing the selectivity of the nucleases.

1.1.1.3.1. Include fully modified sequences consisting entirely of the 2'-Fluoro nucleoside analogs or the 2'-O-Methyl nucleoside analogs (All 2'-F and All 2'-OMe, **Table 1**)

1.1.1.3.2. Include chimeric sequences consisting of unmodified natural purines and 2'-Fluoro pyrimidine nucleoside analogs (RNA Pur-2'F, **Table 1**).

1.1.1.3.3. Include chimeric sequences consisting of unmodified natural purines and 2'-O-Methyl pyrimidine nucleoside analogs (RNA Pur-2'OMe, **Table 1**).

1.1.1.3.4. Include chimeric sequences consisting of unmodified natural pyrimidines and 2'-Fluoro purine nucleoside analogs (RNA Pyr-2'F, **Table 1**).

1.1.1.3.5. Include chimeric sequences consisting of unmodified natural pyrimidines and 2'-O-Methyl purine nucleoside analogs (RNA Pyr-2'OMe, **Table 1**).

1.2. Oligonucleotide probe preparation and storage

NOTE: Oligonucleotides are synthesized by the phosphoramidite method. After synthesis, the oligonucleotides are purified using high performance liquid chromatography (HPLC) and the mass is measured by mass spectrometry.

1.2.1. Spin down the lyophilized oligonucleotide probes using a centrifuge and dilute them in Tris-EDTA (TE) buffer to prevent the nuclease degradation. Determine the dilution volume according to the yield of each probe to render a stock solution with a concentration of 500 pmol/µL.

1.2.2. Store lyophilized oligonucleotides at 4 °C or room temperature for a short term. For long term storage (months to years), store lyophilized oligonucleotides at -20 °C. Upon resuspension of lyophilized oligonucleotides in TE, store the stock solutions at -20 °C or, preferably, at -80 °C.

2. Bacterial culture

2.1 Take one porous glass bead from the cryogenic storage vial under sterile conditions.

2.2 To isolate individual bacterial colonies (*Salmonella* and *E. coli*), use the quadrant method[24] by streaking/rolling the bead directly onto a Petri dish containing Tryptic Soy Agar (TSA) medium supplemented with defibrinated sheep blood.

2.3 Incubate the culture at 37 °C for 24 h.

3. Supernatant preparation

3.1 Transfer a single colony from solid media to 50 mL Tryptic Soy Broth (TSB) and incubate at 37 °C for 24 h at 200 rpm.

3.2 Dilute the culture (1:500) in TSB (sub-culture) and incubate at 37 °C for 24 h at 200 rpm.

NOTE: It is expected that after 24 h of incubation the bacterial cultures are in the stationary phase reaching values higher than 10^9 CFU/mL.

3.3 After the incubation period, transfer the cultures to capped sterile tubes and centrifuge at 4,500 *x g* for 30 min.

3.4 Collect the supernatant and use it immediately. Alternatively, store the supernatants at 4 °C or -20 °C.

4. Nuclease Activity Assay

4.1. Preparation of working solutions

4.1.1. Prepare a 20 µL working solution for each probe in 1.5 mL nuclease free microcentrifuge tubes by diluting (1:10 ratio) the stock solution (500 pmol/ µL) for a final working concentration of 50 pmol/ µL. For that purpose, mix 18 µL of Phosphate Buffer Saline (PBS) containing $MgCl_2$ and $CaCl_2$ with 2 µL of probe stock solution.

NOTE: Be aware that during the preparation of the working solution, the substrate oligonucleotides are more vulnerable to nuclease degradation, therefore, it is recommended to prepare the working solutions just before setting up the reaction.

4.1.2. Avoid direct light contact during the preparation and keep in the dark (wrapped in foil), when dealing with fluorophores.

4.2. Reaction set up

4.2.1. Pre-warm the fluorometer to 37 °C.

4.2.2. Prepare a set (one tube/probe) of 1.5 mL nuclease free microcentrifuge tubes for each sample (TSB, *E. coli* and *Salmonella*) and label accordingly.

4.2.3. Carefully add 96 µL of TSB sterile culture media, *Salmonella* supernatant or *E. coli* supernatant (from the step 3.4.). Subsequently, add 4 µL/tube of probe working solution accordingly. Perform this step at room temperature.

NOTE: 10 probes were used for the first round of the screening and 6 probes for the second round.

4.2.4. Mix thoroughly by pipetting up and down to obtain a homogenous solution. Avoid introducing air bubbles into the samples while mixing.

4.2.5. Load 95 µL of each solution (probe + supernatant or culture media) into a separate well of a black bottom, non-treated 96 well plate. Minimize the formation of bubbles in the wells upon loading by dispensing carefully with the tip close to the wall of the well.

4.2.6. Cover the plate with its lid. Visually inspect the lid and check for pen markings or dust particle accumulation that may introduce measurement artifacts. Replace the lid for a new one if that is the case.

4.3. Measurement set up

4.3.1. Open the acquisition software (e.g., Gen5 3.05 or any other available compatible software) by clicking the software shortcut icon (**Figure S1A**).

4.3.2. Select **Read Now** from the task manager window and choose **New…** to create the kinetic measurement protocol (**Figure S1B**).

4.3.3. Click on **Set Temperature** in the dialog window titled **Procedure** and select 37 ˚C. Confirm and save the settings by pressing **OK** (**Figure S1C**).

4.3.4. Click on **Start Kinetics** in the dialog window titled **Procedure**. Then in the pop-up dialog window, titled **Kinetic Step**, select 2 h in the **Run Time** input box and 2 min in the **Interval** input box. Confirm and save the settings by pressing **OK** (**Figure S1D**).

4.3.5. Click on **Read** in the dialog window titled **Procedure**. Then in the pop-up dialog window, titled **Read Method**, select **Fluorescence Intensity** as a detection method, **Endpoint/Kinetic** as a read type and **Filters** as optics type. Confirm and save the settings by pressing **OK** (**Figure S1E**).

4.3.6. In the pop-up dialog window titled **Read Step (Kinetic)**, select **Green** from the **Filter Set**. Confirm and save the settings by pressing **OK** (**Figure S2A**).

4.3.7. In the dialog window titled **Procedure**, select **use lid** and click on **validate** to ensure that the created protocol is valid by receiving a pop-up dialog window (**Figure S2B**).

4.3.8. Select **protocol** in the menu bar and choose **procedure** (**Figure S2C**).

4.3.9. In the dialog window titled **Procedure**, define the wells to be measured (**Figure S2D**).

4.3.10. Enter the name of the experiment in the file name input box (**Figure S2E**).

4.3.11. Load the plate with its lid into the plate reader. Ensure the plate is in the right orientation.

4.3.12. Start the acquisition by clicking **read new** button in the toolbar (**Figure S2F**).

4.4. Data Analysis

4.4.1. Click on one of the measured wells in the dialog window titled **Plate 1** (**Figure S3A**).

4.4.2. Click **select wells** and include all the measured wells in the **well selection dialog** window. Confirm and save the settings by pressing **OK**. (**Figure S3B**).

4.4.3. Select **data** in the dialog window titled **Plate 1** to visualize the tabulated results (**Figure S3C**).

4.4.4. Export the data to a spread sheet by selecting **quick export** from the context menu (**Figure S3C**).

4.4.5. In the spread sheet, label the data columns accordingly for each sample and probe.

4.4.6. Generate kinetic graphs, using **line with markers** style, by plotting relative fluorescence units (RFU) versus time (x-axis: timeline of the reaction and y-axis: RFUs).

NOTE: The description of generating a graph is applicable using spreadsheet software (e.g., Excel). However, any software can be used to generate graphs from the raw data obtained, by plotting RFU versus time.

4.4.7. Calculate the rate of the enzymatic reaction for each measured interval according to the following formula[25]: rate=$\frac{If-Ii}{Tf-Ti}$, where *If* is RFU at the maximal interval time point and *Ii* is RFU at the minimal interval time point, and *Tf* and *Ti* are the maximal and minimal interval time points, respectively.

4.4.8. Select the rate with the highest value (R_{max}) for each curve. If there is more than one R_{max} per sample, select the one that occurs at the earliest measurement time point.

4.4.9. Calculate the ratio between R_{max} and the average value of the time interval at which R_{max} occurs rate coefficient = $\frac{R\max}{(Tf+Ti)/2}$

4.4.10. Calculate the fold difference (FD) between the rate coefficient of *Salmonella and E. coli* for each probe.

4.4.11. Consider a fold difference value higher than 3 as significant.

4.4.12. Consider the probes with an FD>3 as candidate probes for the next screening round.

5. Screening

5.1. First Round of Screening

5.1.1. Perform the nuclease activity assay (as described in section 4) using DNA, RNA and polynucleotide probes.

5.1.2. Evaluate the preference for DNA chemistry or RNA chemistry by comparing the number and performance (FD value) of DNA and RNA candidate probes.

5.1.3. Select type of nucleic acid chemistry rendering the greatest number of candidate probes (FD>3) and the best performing probes (FD value), as illustrated in **Figure 1**.

5.2. Second Round of Screening

5.2.1. Perform the nuclease activity assay (as described in section 4) using fully modified probes and chimeric probes based on the nucleic acid chemistry selected in the first screening round.

5.2.2. Evaluate the preference towards sequences containing 2'-Fluoro and 2'-O-Methyl nucleoside analogs by comparing the results obtained for 2'-Fluoro and 2'-O-Methyl modified candidate probes.

5.2.3. Select the candidate type of nucleoside analog modification rendering the greatest number of candidate probes (FD>3) and the best performing probes (FD value), as illustrated in **Figure 1**.

REPRESENTATIVE RESULTS:

Figure 1 shows the work flow of this methodology, which is divided into two screening rounds. In the first round of screening, we used 5 DNA probes (DNA, DNA Poly A, DNA Poly T, DNA Poly C and DNA Poly G) and also 5 RNA probes (RNA, RNA Poly A, RNA Poly U RNA Poly C and RNA Poly G). The raw data of this screening round can be found in **Supplementary Table 1.** In the second

round, the chemically modified probes were synthesized by replacing the RNA sequence with chemically modified nucleosides (All 2'-Fluoro and All 2'-OMethyl) or by the combination of RNA and purines or pyrimidines chemically modified (RNA Pyr-2'F, RNA Pyr-2'OMe, RNA Pur-2'F and RNA Pur-2'OMe). The raw data of this screening round can be found in **Supplementary Table 2.** A detailed description of the sequences can be found in **Table 1.** The results obtained from the first screening round are shown in **Figure 2**, where *Salmonella* culture supernatants report a clear preference for RNA probes over the DNA probes. In contrast, *E. coli* and culture media controls show very limited capability to degrade RNA probes. In addition, we have calculated the fold difference (FD) using the rate coefficients between *Salmonella* and *E.coli* (**Supplementary Table 3** and **Supplementary Table 4**) in order to identify the best performing probes. The calculations were performed as described in the protocol section.

These results suggest the presence of an RNase type of activity derived from *Salmonella* that could be used to select specific RNA-based probes. Based on the identification of RNA as the preferred nucleic acid type for *Salmonella* nucleases, we have designed a new library using chemically modified nucleotides to be used in the second round of screening aimed at increasing the specificity of the probes. **Figure 3** shows the kinetic profiles of the probes containing chemically modified nucleotides. Interestingly, the RNA Pyr-2'OMe and RNA Pur-2'OMe have shown the best performing kinetic behavior when compared with the controls. These results suggest that *Salmonella* has an important RNase activity that can be used for selecting probes capable of specifically recognizing this bacteria. As such we observed that 2'-OMe chemically modified nucleosides are more suitable for the type of RNAses secreted by *Salmonella*. With this in mind, the protocol described in this contribution offers the possibility of exploring the use of nuclease activity as a biomarker.

FIGURE AND TABLE LEGENDS:

Figure 1: Bacteria cultures and workflow of the screening process. Preparation of bacteria cultures and supernatants (left). Description of the workflow for the two screening rounds. First screening: The preference for DNA or RNA is evaluated using 10 probes. Second screening: Based on the nucleic acid preference, additional probes containing chemically modified nucleotides are evaluated to identify the best performing substrates for a given nuclease activity.

Figure 2: First kinetic screening round. Kinetic profiles of *Salmonella*, *E. coli* and culture media (TSB) using DNA and RNA probes. Nuclease activity is represented by relative fluorescence units (RFU). The graphs are representative for at least 3 individually performed experiments. The different samples are labeled as indicated in the graph. Fold difference (FD) values were calculated using the rate coefficients of *Salmonella* and *E. coli* for each probe.

Figure 3: Second kinetic screening round. Kinetic profiles of *Salmonella*, *E. coli* and culture media (TSB) using chemically modified probes. Nuclease activity is represented by relative fluorescence units (RFU). The graphs are representative for at least 3 individually performed experiments. The different samples are labeled as indicated in the graph. Fold difference (FD) values were calculated using the rate coefficients of *Salmonella* and *E.coli* for each probe.

Table 1: Nucleic acid probe sequences. List of all the nucleic acid probes used in this study.

Supplementary Figure 1: Measurement set up. Button clicks and dialog windows describing the stepwise process performed in the acquisition software to set up the different measurement parameters. (**A**) desktop icon. (**B**) Task manager dialog window. (**C**) Procedure and Temperature Set up dialog windows. (**D**) Procedure and Kinetic Step dialog windows. (**E**) Procedure and Read Method dialog windows.

Supplementary Figure 2: Measurement set up. Button clicks and dialog windows describing the stepwise process performed in the acquisition software to set up the different measurement parameters. (**A**) Procedure and Read Step (Kinetic) Dialog windows. (**B**) Procedure dialog window (**C**) "Protocol" menu bar. (**D**) Well selection dialog window. (**E**) File name input box. (**F**) **Run New** icon used to start the acquisition within the software.

Supplementary Figure 3: Data analysis. Button clicks and dialog windows describing the stepwise process performed in the acquisition software to export acquired data into a spread sheet for further analysis. (**A**) Plate Matrix dialog window. (**B**) Plate and Well Selection dialog windows. (**C**) Plate dialog window and **Quick Export** context menu.

Supplementary Figure 4: Third screening round - sequence preference optimization: Description of the different steps involved in an additional screening round aimed at assessing sequence variations.

Supplementary Figure 5: Fourth screening round - Specificity evaluation round: Description of the different steps involved in an additional screening round aimed at increasing specificity.

Supplementary Figure 6: Fifth screening round – Reaction parameter optimization: Description of the different steps involved in an additional screening round aimed at reducing non-target cross reactivity by modulating nuclease activity.

Supplementary Table 1: Raw data from the first screening round. For each probe (labeled in red, on top), the acquisition time and the raw fluorescence values were reported for 3 samples tested in this study (TSB, *E. coli* and *Salmonella*), along with the calculated rate value for each interval. The calculations were carried out as described in the methods section.

Supplementary Table 2: Raw data from the second screening round. For each probe (labeled in red, on top), the acquisition time and the raw fluorescence values were reported for 3 samples tested in this study (TSB, *E. coli* and *Salmonella*), along with the calculated rate value for each interval. The calculations were carried out as described in the methods section.

Supplementary Table 3: Calculations based on the first screening round. For each probe (labeled in red, on top), the following calculations were performed for 3 samples in this study (TSB, *E. coli* and *Salmonella*): maximum rate values, minimal and maximal interval time point, rate coefficient,

fold difference values between *Salmonella* and *E. coli* over TSB and fold difference values between *Salmonella* and *E. coli* (highlighted in yellow). The calculations were carried out as described in the methods section and the calculation formulas and the step by step calculations are shown in the spreadsheet.

Supplementary Table 4: Calculations based on the second screening round. For each probe (labeled in red, on top), the following calculations were performed for 3 samples in this study (TSB, *E. coli* and *Salmonella*): maximum rate values, minimal and maximal interval time point, rate coefficient, fold difference values between *Salmonella* and *E. coli* over TSB and fold difference values between *Salmonella* and *E. coli* (highlighted in yellow). The calculations were carried out as described in the methods section and the calculation formulas and the step by step calculations are shown in the spreadsheet.

DISCUSSION:

Alterations of nuclease activity have been associated with a wide variety of disease phenotypes, including different types of cancer and bacterial infections. These alterations are proposed to be the causative agent of a condition[14], while in other cases they are the consequence of a detrimental physiological event[20] or pathogenic agent[16,26]. Not surprisingly, attempts to use nucleases and nuclease activity as a diagnostic biomarker have been described[15,22,23,26], with considerable promise. Accordingly, the establishment of a robust and reproducible screening approach for the systematic evaluation of nuclease activity in disease and for the identification of specific and sensitive probes seems pertinent. To address this necessity, we have developed a robust, modular and easy to implement screening platform based on a fluorescent assay, that allows the discovery of novel disease biomarkers and the identification of sensitive and specific nucleic acid probes in parallel, by using the catalytic action of nucleases.

Powerful screening tools such as small molecule high throughput screening (HTS)[27], systematic evolution of ligands by exponential enrichment (SELEX)[28] or phage display[29] have been previously reported, which allows the identification of high affinity recognition molecules (e.g., small molecules, aptamers or binding-peptides). The screening approach presented here allows the selection of probes that can identify known and unknown nuclease activity. This approach is compatible with in vitro, ex vivo and in vivo screening models. However, the reactive nature of nucleases confers an obvious advantage over the aforementioned approaches, in that the probe-nuclease interaction is not static, but it´s a dynamic process. This means that the nucleases (target) become an intrinsic signal amplification module for the reporter probes since several reporter probes can interact with a single nuclease.

As in any other screening method, the generation and management of the initial library is essential. The nature of nucleic acid probes provides great flexibility in the design and creation of a library and allows the introduction of varying degrees of complexity depending on the screening application. Library complexity can be introduced at different levels including sequence motifs, nucleotide chemistry, phosphate backbone chemistry, and oligonucleotide length. Modulating the complexity of the library allows screening probes, not only for their capacity to successfully identify nuclease activity associated with disease but also for properties compatible

with subsequent in vivo applications. Furthermore, a nucleic acid base library offers several advantages over its antibody or peptide counterparts. On the one hand, antibody production is well known to be cumbersome, requiring animals or complex eukaryotic culture systems, which increase the cost and introduce batch variability[30,31]. Moreover, the high molecular weight and immunogenicity limit their application[28,32]. On the other hand, the generation of biological peptide libraries usually requires either viral or bacterial expression systems[29,30], increasing the complexity of the screening process. Chemical peptide libraries avoid this problem, at the expense of using convoluted bead-based systems or multiple rounds of expensive peptide synthesis[33]. All these problems are circumvented by using a nucleic acid library. Once the initial library has been established, the screening methods are quick and straightforward. The method described herein serves as a platform for additional screening rounds, such as, sequence optimization (**Supplementary Figure 4**), specificity evaluation (**Supplementary Figure 5**) or optimization of modulatory elements of nuclease activity, such as metal cofactors (typically divalent cations) and chelators, which are very useful to increase the specificity of the selected probes (**Supplementary Figure 6**).

The kinetic fluorometric assay used in this study can be optimized for both, bacterial and cellular cultures, with the screening being performed in friendly microtiter plates. In the case of cellular assays, this protocol is compatible with both, suspension and monolayer cultures, which, post-optimization, can be used according to the necessities of the in vitro model being studied. We have identified several critical steps that require optimization prior to screening. These include the cell number or bacterial density to be assayed, probe concentration, fluorometer detector gain settings or the implementation of in-built background correction methods. One limitation of this technology is the need of specific nuclease activity present in the sample. Without this feature, the screening approach for the selection of candidate probes is not feasible. Another limitation is the self-quenching ability of guanines when they are in close proximity to the fluorophore. This characteristic needs to be considered when designing the library.

The enzymatic nature of the reaction being measured makes it necessary to consider plate loading times. Minimizing loading times will reduce background differences between different experimental conditions. Several options exist to overcome this problem, such as slowing the enzymatic reaction during loading by using ice-cold reagents, or using automated loading systems, though the latter increases costs considerably, but also the throughput. Moreover, kinetic measurements are a great improvement over static measurements, providing a complete and more realistic picture of the dynamics of nuclease´s catalytic reaction, especially in terms of interactions between probes and nucleases.

In summary, our protocol offers a versatile, robust and reproducible screening method for the identification of sensitive and specific nucleic acid probes for the detection of nuclease activity associated with the disease, which overcomes major hurdles of alternative screening methods. We anticipate that this screening technology will allow the development of novel diagnostic tools in a multitude of conditions, with easy translatability to the clinic.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

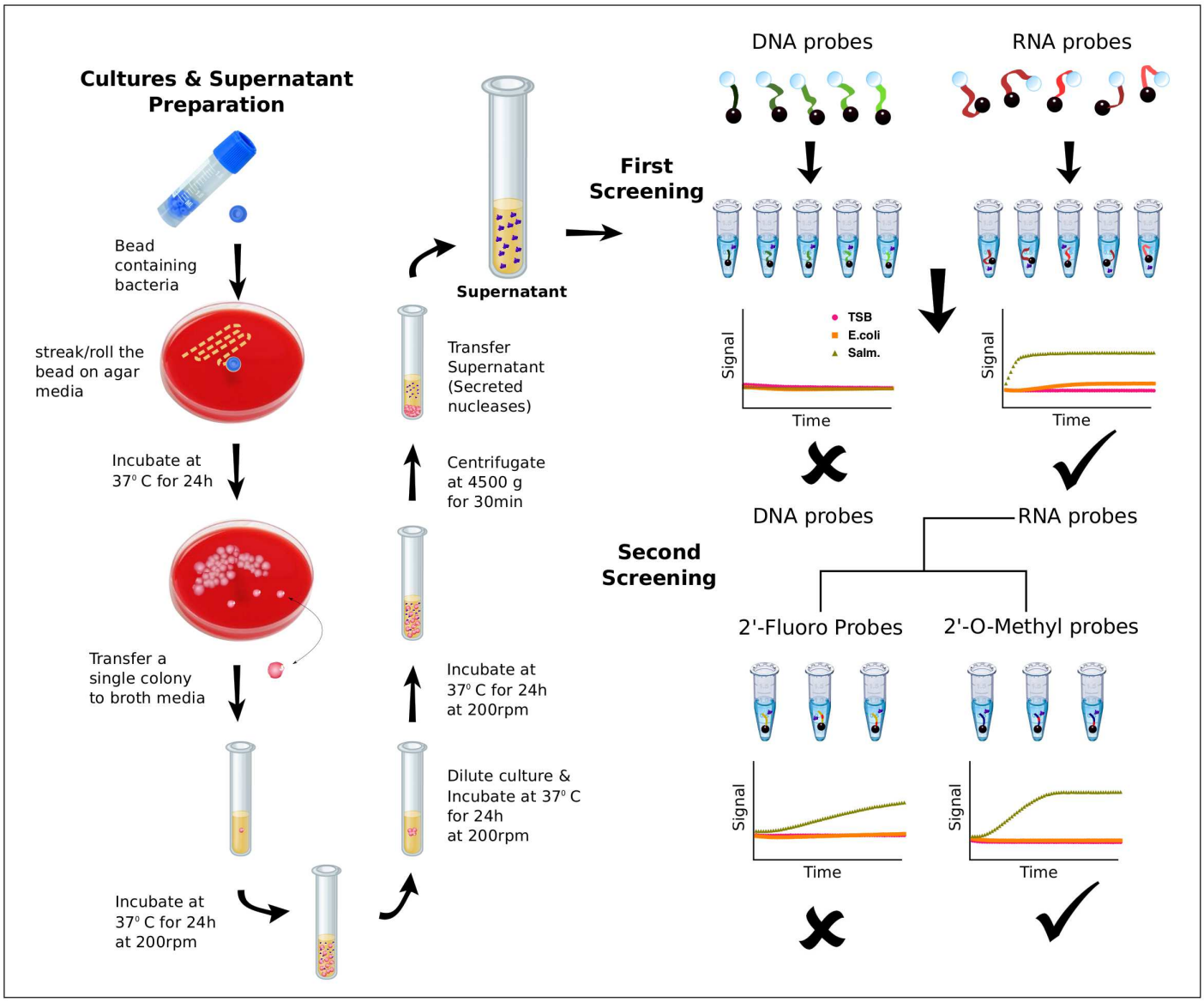

Figure 2

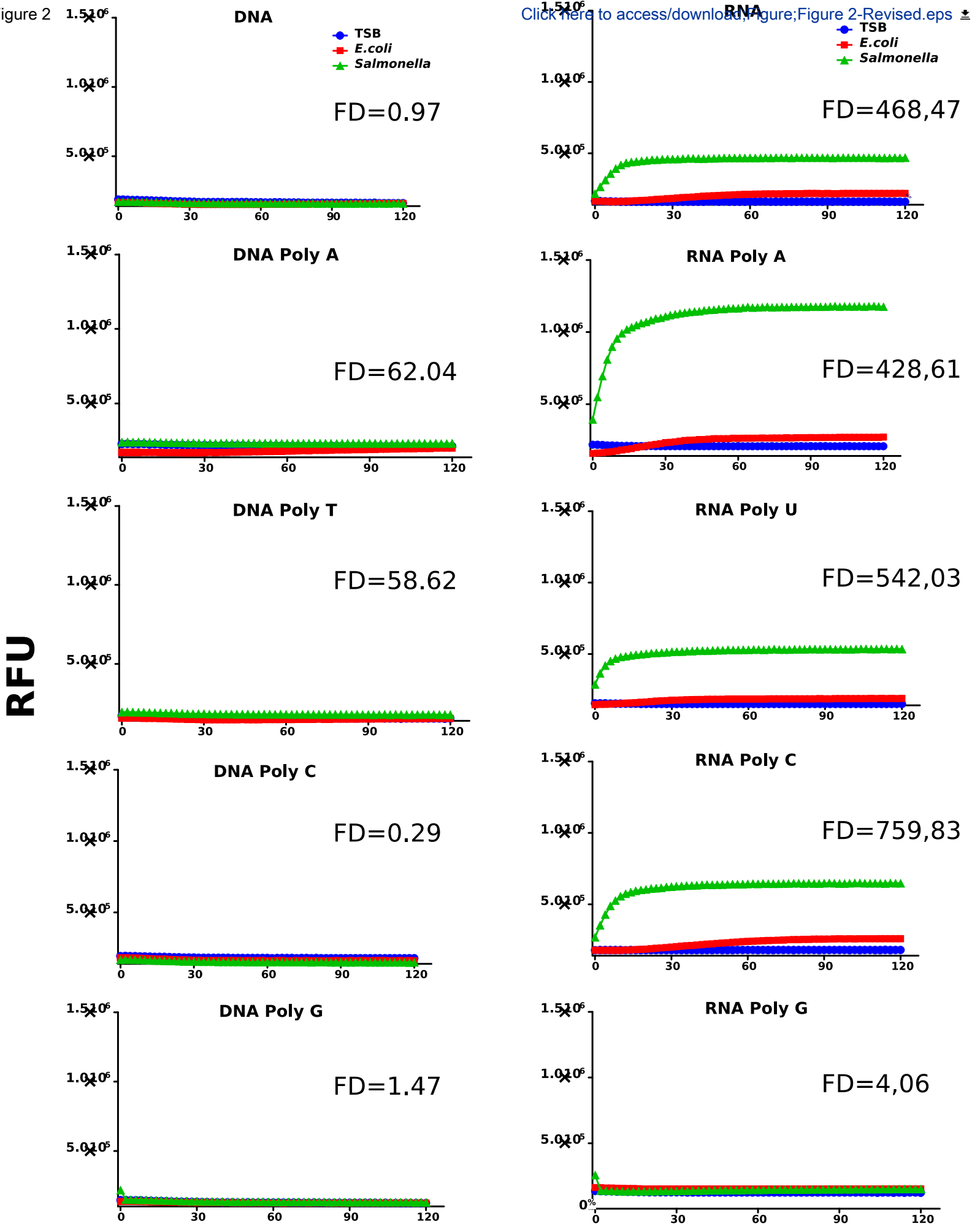

Figure 3

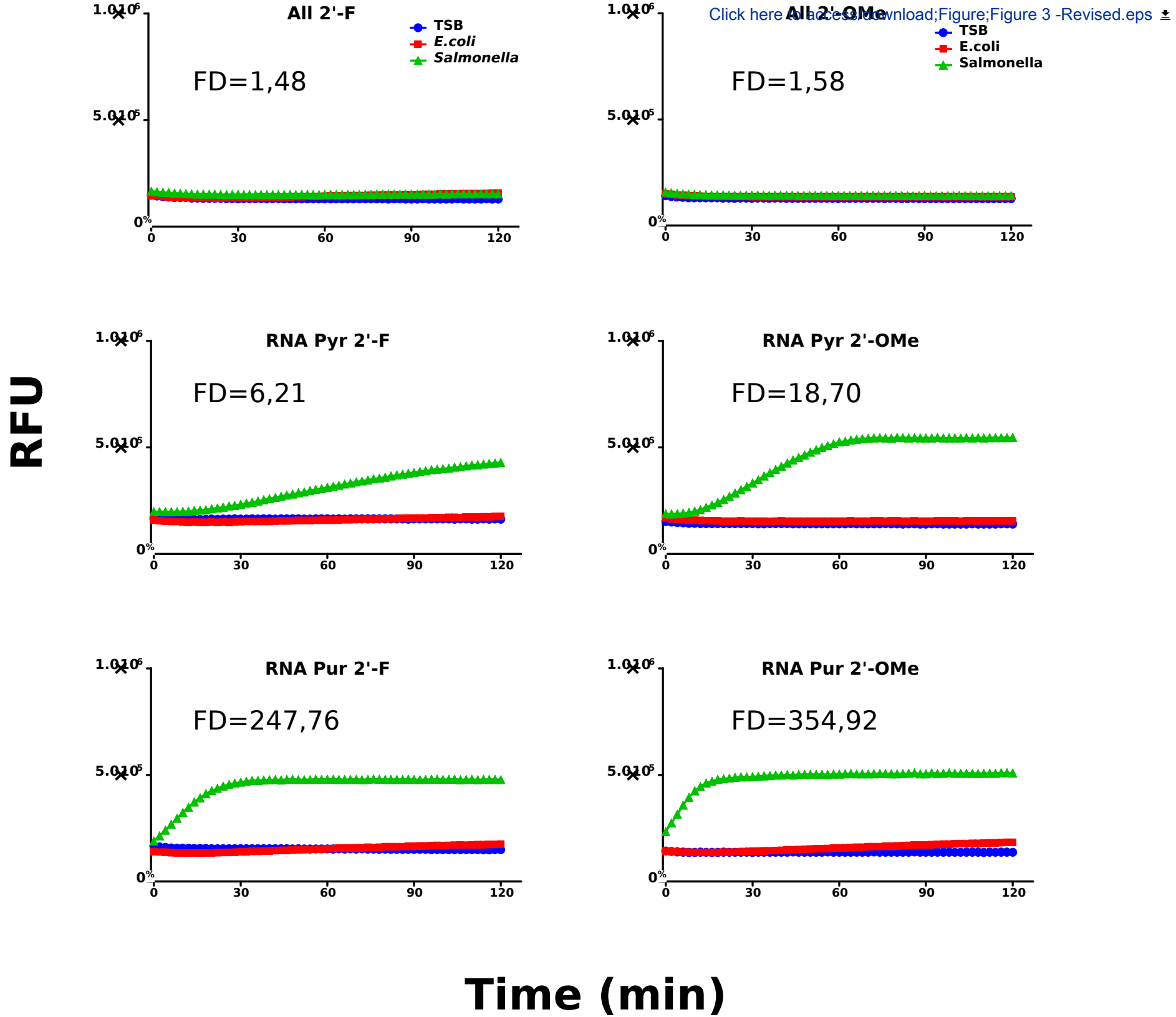

Table 1

Click here to access/download;Table;Table 1. List of sequences.xlsx

Probe name
DNA
DNA-Poly A
DNA-Poly T
DNA-Poly C
DNA -Poly G
RNA
RNA-Poly A
RNA-Poly U
RNA-Poly C
RNA-Poly G
All 2'-F
RNA Pyr-2'F
RNA Pur-2'F
All-2'OMe
RNA Pyr-2'OMe
RNA Pur-2'OMe

Uppercase red TACG = DNA

Lowercase blue uacg = RNA

mU/A/C/G = 2'-O-Methyl modifcation

fU/A/C/G= 2'-Fluoro modification

Unmodified= Does not contain nucleoside analog

Partially modified (chimeric sequence)= Contains nucleoside analogs

Fully modified= The entire sequences consists of nucleoside analgos

Sequence status	Sequence
Unmodified	FAM// TCTCGTACGTTC //TQ2
Unmodified	FAM// AAAAAAAAAAAA //TQ2
Unmodified	FAM// TTTTTTTTTTTT //TQ2
Unmodified	FAM// CCCCCCCCCCCC //TQ2
Unmodified	FAM// AGGGGGGGGGGG //TQ2
Unmodified	FAM// ucucguacguuc //TQ2
Unmodified	FAM// aaaaaaaaaaaa //TQ2
Unmodified	FAM// uuuuuuuuuuuu //TQ2
Unmodified	FAM// cccccccccccc //TQ2
Unmodified	FAM// ugggggggggggg //TQ2
Fully Modified	FAM// fUfCfUfCfGfUfAfCfGfUfUfC //TQ2
Partially Modified (chimeric sequence)	FAM// fUfCfUfCgfUafCgfUfUfC //TQ2
Partially Modified (chimeric sequence)	FAM// ucucfGufAcfGuuc //TQ2
Fully Modified	FAM// mUmCmUmCmGmUmAmCmGmUmUmC //TQ2
Partially Modified (chimeric sequence)	FAM// mUmCmUmCgmUamCgmUmUmC //TQ2
Partially Modified (chimeric sequence)	FAM// ucucmGumAcmGuuc //TQ2

Name of Material/ Equipment	Company
Black bottom, non-treated 96 well plate	Fisher Scientific
Cytation1	BioTek
Eppendorf tubes	Thermofisher
Escherichia coli	ATCC
Microbank cryogenic storage vial containing beads	Pro-Lab Diagnostics
Nucleic acid probes	Biomers.net
Phosphate Buffer Saline containing $MgCl_2$ and $CaCl_2$	Gibco™
Salmonella enterica subs. Enterica	ATCC
Tris-EDTA	Fisher Scientific
Tryptone Soya Agar with defibrinated sheep blood	Thermo Fisher Scientific
Tryptic Soy Broth	Sigma Aldrich

Catalog Number
10000631
CYT1FAV
11926955
25922
22-286-155
#
14040117
14028
10647633
10362223
22092

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Point-by-point response to editor concerns (Revision #2).
We want to thank the editor for his thorough revision of the manuscript. Editor comments are italicized below. Our responses are in normal font. The red text indicates the additions in the revised manuscript.

Editor´s Comments

1) *For the protocol section please ensure you answer the "how" question, i.e., how is the step performed? Please include all specific details associated with the step to be performed.For the software steps please include a click by click Instructions of how to perform the steps for the software used in your experiment.Once done please highlight 2.75 pages of the protocol text including headings and spacings for filming purpose.*

We have addressed these comments by revising the entire manuscript and we believed that all the "how" questions are now addressed accordingly with specific details for each case. The manuscript has been modified extensively and several paragraphs added (text in red) and two new supplementary files (button clicks) were added.

For filming purpose, 2.75 pages of the protocol text has been highlighted.

We have highlighted the text (yellow) as indicated by the editor for filming purposes.

2) *This part (library design) needs more detail for clarity.*

This comment has been considered. The various points made have been corrected in the re-written protocol as follow:

1. Oligonucleotide library design and preparation

1.1. Library design

1.1.1. Based on the following criteria design a library of oligonucleotide probes between 8 and 12-mer long to avoid secondary structure formation, with equal length for all probes:

1.1.1.1. Include at least one DNA and one RNA random sequence containing a combination of adenine (A), guanine (G), cytosine (C) and thymine (T)/uracil (U) **(**DNA and RNA probes in **Table 1)**.

1.1.1.2. Include polynucleotide sequences consisting of a single type of nucleotide to determine sequence dependence for a given nuclease activity (DNA-Poly A, DNA-Poly T, DNA-Poly C, DNA-Poly G, RNA-Poly A, RNA-Poly U, RNA-Poly C and RNA-Poly G in **Table 1**).

1.1.1.3. Using the same sequence of nucleotide residues as the initial DNA and RNA sequences, include sequences that contain nucleoside analogues harboring chemical modifications at the 2'-position of the ribose sugar, such as 2'-Fluoro and 2'-O-Methyl, as a stringent step for increasing the selectivity of the nucleases.

1.1.1.3.1. Include fully modified sequences consisting entirely of the 2'-Fluoro nucleoside analogues or the 2'-O-Methyl nucleoside analogues (All 2'-F and All 2'-OMe, **Table 1**)

1.1.1.3.2. Include chimeric sequences consisting of unmodified natural purines and 2'-Fluoro pyrimidine nucleoside analogues (RNA Pur-2'F, **Table 1**).

1.1.1.3.3. Include chimeric sequences consisting of unmodified natural purines and 2'-O-Methyl pyrimidine nucleoside analogues (RNA Pur-2'OMe, **Table 1**).

1.1.1.3.4. Include chimeric sequences consisting of unmodified natural pyrimidines and 2'-Fluoro purine nucleoside analogues (RNA Pyr-2'F, T**able 1**).

1.1.1.3.5. Include chimeric sequences consisting of unmodified natural pyrimidines and 2'-O-Methyl purine nucleoside analogues (RNA Pyr-2'OMe, **Table 1**).

3) *Is there specific sequence to be taken into consideration? In which bacteria … how was this done? How far away are each probe? How many probe per gene or per strain? Please include all specific details.*

The probe sequences in this study (substrates for nucleases) are not based on the genome of a given bacteria. We apologize for the misunderstanding. The following sentence and NOTE were rephrased for clarity purposes:

1.1.1.4. Include at least one DNA and one RNA random sequence containing a combination of adenine (A), guanine (G), cytosine (C) and thymine (T)/uracil (U) (**DNA and RNA probes in Table 1**).

And

NOTE: The DNA and RNA sequences described in **Table 1** have been useful as the initial substrates for classifying an unknown type of nuclease activity, either DNase or RNase. These two sequences are recommended as the starting point for any screening, as they have shown a wide capability to detect nuclease activity profiles in bacteria and tissue samples. If nuclease activity is not observed with these DNA and RNA sequences, the design of additional oligonucleotides is required.

4) *How do you identify natural DNA in the bacterial species? Do you use any software for the library design?*

No particular software is used for library design. We are not looking for bacterial DNA sequences. This comment is also addressed by the answer at the previous point #3.

5) *How many sequences per kind are included for one test? Also how do you determine the length of the sequences?*

This comment has been considered. The various points made have been corrected in the re-written protocol.

The length of probes is now described in more detail in the protocol (see below):

1.1.2. Based on the following criteria design a library of oligonucleotide probes between 8 and 12-mer long to avoid secondary structure formation, with equal length for all probes:

The sequences of the library included in each test/screening round are now specified in section 5 of the protocol:

5.1. First Round of Screening:

5.1.1. Perform the nuclease activity assay (as described in section 4) using DNA, RNA and polynucleotide probes.

5.1.2. Evaluate the preference for DNA chemistry or RNA chemistry by comparing the number and performance (FD value) of DNA and RNA candidate probes.

5.1.3. Select type of nucleic acid chemistry rendering the greatest number of candidate probes (FD>3) and the best performing probes (FD value), as illustrated in Figure 1.

5.2. Second Round of Screening:

5.2.1. Perform the nuclease activity assay (as described in section 4) using fully modified probes and chimeric probes based on the nucleic acid chemistry selected in the first screening round.

5.2.2. Evaluate the preference towards sequences containing 2'-Fluoro and 2'-O-Methyl nucleoside analogues by comparing the results obtained for 2'-Fluoro and 2'-O-Methyl modified candidate probes.

5.2.3. Select the candidate type of nucleoside analog modification rendering the greatest number of candidate probes (FD>3) and the best performing probes (FD value), as illustrated in Figure 1.

6) *Are these against the bacterial genome? Which ones are chimeric in the table?*

An additional column has been added to Table 1 in order to clarify this point.

7) *What kind of bacteria?*
8) *Citation if any?*
9) *The abbreviation must be expanded during the first time use*
10) *Composition of the medium? Is the medium present on a Petri plate?*

These comments (7, 8, 9 and 10) have been addressed by adding a reference and

modifying point 2.1 in the protocol section:

2.1 To isolate individual bacterial colonies (*Salmonella* and *E.coli*), use the quadrant method[24] by streaking/rolling the bead directly onto a Petri dish containing Tryptic Soy Agar (TSA) medium supplemented with defibrinated sheep blood.

11) Of what? What volume of working dilution is prepared?

This comment has been addressed by editing the text in the protocol as follows:

4.1.1. Prepare a 20 µL working solution for each probe in 1.5 mL nuclease free microcentrifuge tubes by diluting (1:10 ratio) the stock solution (500 pmol/ µL) for a final working concentration of 50 pmol/ µL. For that purpose, mix 18 µL of Phosphate Buffer Saline (PBS) containing $MgCl_2$ and $CaCl_2$ with 2 µL of probe stock solution.

12) From step number?

13) How many bacterial supernatants are prepared? What is the difference? Are these different strains, species? Please bring out this clarity somewhere in the protocol

14) How many probes are prepared in this experiment? Do you add one probe per well? Please explain this part.

4.2.3. Carefully add 96 µL/tube of TSB sterile culture media, *Salmonella* supernatant or *E.coli* supernatant (from the step 3.4.). Subsequently, add 4 µL/tube of probe working solution accordingly. Perform this step at room temperature.

Note: 10 probes were used for the first round of the screening and 6 probes for the second round."

15) How? Using a pipette or plate mixer?

This information has been added by rephrasing the following sentence in the protocol

4.2.4. Mix thoroughly by pipetting up and down to obtain a homogenous solution. Avoid introducing air bubbles into the samples while mixing.

16) Do you mix all the probes or is it probe+ supernatant mixture?

This information has been clarified by modifying the following step in the protocol:

4.2.5. Load 95 µL of each solution (probe + supernatant or culture media) into a separate well of a black bottom, non-treated 96 well plate. Minimize the formation of bubbles in the wells upon loading by dispensing carefully with the tip close to the wall of the well.

17) Ensure how?

This comment has been addressed by rephrasing the protocol step as follows, to include the "how":

"Inspect visually the lid and check for pen markings or dust particle accumulation that may introduce measurement artifacts. Replace the lid for a new one if that is the case."

18) *Please include click by click instruction of how to perform this part. E.g., Click **open** to open the software. Then click **create** to create a new protocol. Manually enter 37 °C for the temperature… etc.*
19) *For all the sub steps please use complete sentences and show how it is done.*

We have addressed these comments by modified the supplementary figure 1, two supplementary figures with "button clicks" were added and we have rephrased the following paragraphs:

4.3. Measurement set up

4.3.3. Open the acquisition software (Gen5 3.05 or any other available compatible software) by clicking the software shortcut icon (Figure S1 A).

4.3.4. Select "Read Now" from the task manager window and choose "New…" to create the kinetic measurement protocol (Figure S1 B).

4.3.5. Click on "Set Temperature" in the dialog window titled "Procedure and select 37 °C. Confirm and save the settings by pressing "OK" (Figure S1C).

4.3.6. Click on "Start Kinetics" in the dialog window titled "Procedure". Then in the pop-up dialog window, titled "Kinetic Step", select 2 hours in the "Run Time" input box and 2 minutes in the "Interval" input box. Confirm and save the settings by pressing "OK" (Figure S1D).

4.3.7. Click on "Read" in the dialog window titled "Procedure". Then in the pop-up dialog window, titled "Read Method", select "Fluorescence Intensity" as a detection method, "Endpoint/Kinetic" as a read type and "Filters" as optics type. Confirm and save the settings by pressing "OK" (Figure S1E).

4.3.8. In the pop-up dialog window titled "Read Step", select "Green" from the "Filter Set". Confirm and save the settings by pressing "OK" (Figure S2A).

4.3.9. In the dialog window titled "Procedure", select "use lid" and click on "validate" to ensure that the created protocol is valid by receiving a pop-up dialog window (Figure S2B).

4.3.10. Select "protocol" in the menu bar and choose "procedure" (Figure S2C).

4.3.11. In the dialog window titled "Procedure", define the wells to be measured (Figure S2D).

4.3.12. Enter the name of the experiment in the file name input box (Figure S2E).

4.3.13. Load the plate with its lid into the plate reader. Ensure the plate is in the right orientation.

4.3.14. Start the acquisition by clicking "read new" button in the toolbar (Figure S2F).

4.4. Data Analysis

4.4.3. Click on one of the measured wells in the dialog window titled “Plate 1” (Figure S3A).

4.4.4. Click “select wells” and include all the measured wells in the “well selection dialog” window. Confirm and save the settings by pressing “OK”. (Figure S3B).

4.4.5. Select “data” in the dialog window titled “Plate 1” to visualize the tabulated results (Figure S3C).

4.4.6. Export the data to excel spread sheet by selecting “quick export” from the context menu (Figure S3C).

4.4.7. In the excel spread sheet label the data columns accordingly for each sample and probe.

4.4.8. Click “insert” in Excel menu bar. Select “line” from the toolbar and choose “line with markers” to generate kinetic graphs (x-axis: timeline of the reaction // y-axis: relative fluorescent units)

20) How is this done?

This comment has been addressed by including stepwise description of generating the graphs in the data analysis section.

21) Please include details of the screenings and how these were performed in your case. Third fourth and fifth screening set not described.

The third, fourth and fifth screening rounds are not described in the protocol as they were not performed experimentally, however, a mention to future rounds is made in the discussion and illustrated with the supplementary figures 4, 5 and 6.

22) How is this assessment performed?
23) How is this done?

These comments have been addressed by adding more details and steps to section 5 of the protocol (see below):

5.3. First Round of Screening:

5.3.1. Perform the nuclease activity assay (as described in section 4) using DNA, RNA and polynucleotide probes.

5.3.2. Evaluate the preference for DNA chemistry or RNA chemistry by comparing the number and performance (FD value) of DNA and RNA candidate probes.

5.3.3. Select type of nucleic acid chemistry rendering the most number of candidate probes (FD>3) and the best performing probes (FD value), as illustrated in Figure 1.

5.4. Second Round of Screening:

5.4.1. Perform the nuclease activity assay (as described in section 4) using fully modified probes and chimeric probes based on the nucleic acid chemistry selected in the first screening round.

5.4.2. Evaluate the preference towards sequences containing 2'-Fluoro and 2'-O-Methyl nucleoside analogues by comparing the results obtained for 2'-Fluoro and 2'-O-Methyl modified candidate probes.

5.4.3. Select the candidate type of nucleoside analog modification rendering the most number of candidate probes (FD>3) and the best performing probes (FD value), as illustrated in Figure 1.

24) *Please include this sentence somewhere in the introduction to bring out clarity in the protocol text.*

To address this comment, we have removed this sentence from the results section:

"We sought to identify the nuclease activity derived from Salmonella as a biomarker for the specific identification of this bacteria. Herein, we report on the methods for screening bacteria nuclease activity using kinetic analysis."

And substituted it for a similar sentence in the last paragraph of the introduction (line110), for clarity:

"As such, this approach was used to identify the nuclease activity derived from *Salmonella* for the specific identification of this bacteria. In the following protocol, we report on the methods for screening bacterial nuclease activity using kinetic analysis."

25) *How is this derived? What is Discretize time, TSB, E.coli and velocity and what do you infer from all these values. Need further description. Applicable to all the supplementary files.*

For clarity purposes, we have eliminated the term "Discretize" and more appropriate labels have been used to describe the supplementary files content. In addition, a more thorough legend description of the parameters included in supplementary files has been added as indicated below:

Supplementary file 1: Raw data from the first screening round. For each probe (labelled in red, on top), the acquisition time and the raw fluorescence values were reported for the three samples tested in this study (TSB, *E. coli* and *Salmonella*), along with the calculated rate value for each interval. The calculations were carried out as described in the methods section.

Supplementary file 2: Raw data from the second screening round. For each probe (labelled in red, on top), the acquisition time and the raw fluorescence values were reported for the

three samples tested in this study (TSB, *E. coli* and *Salmonella*), along with the calculated rate value for each interval. The calculations were carried out as described in the methods section

Supplementary file 3: Calculations based on the first screening round. For each probe (labelled in red, on top), the following calculations were performed for the three samples in this study (TSB, *E. coli* and *Salmonella*): maximum rate values, minimal and maximal interval time point, rate coefficient, fold difference values between *Salmonella* and *E.coli* over TSB and fold difference values between *Salmonella* and *E.coli (highlighted in yellow).* The calculations were carried out as described in the methods section and the calculation formulas and the step by step calculations are shown in the spreadsheet.

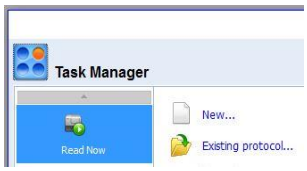
Supplementary file 4: Calculations based on the second screening round. For each probe (labelled in red, on top), the following calculations were performed for the three samples in this study (TSB, *E. coli* and *Salmonella*): maximum rate values, minimal and maximal interval time point, rate coefficient, fold difference values between *Salmonella* and *E.coli* over TSB and fold difference values between *Salmonella* and *E.coli (highlighted in yellow).* The calculations were carried out as described in the methods section and the calculation formulas and the step by step calculations are shown in the spreadsheet.

Supplemental Figures 1

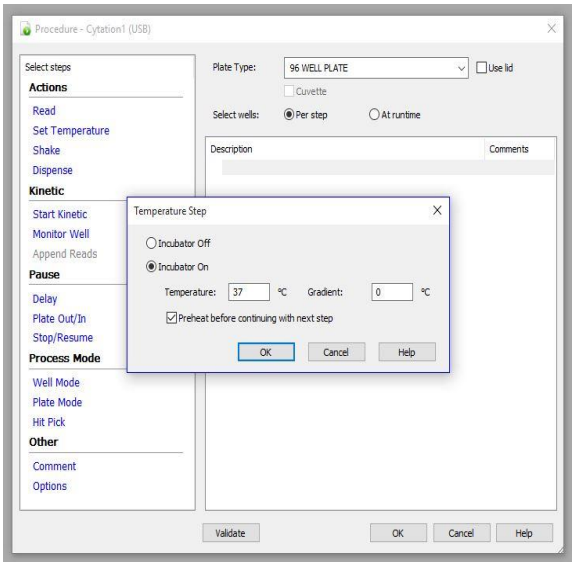
Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Figure 1.pdf

A)

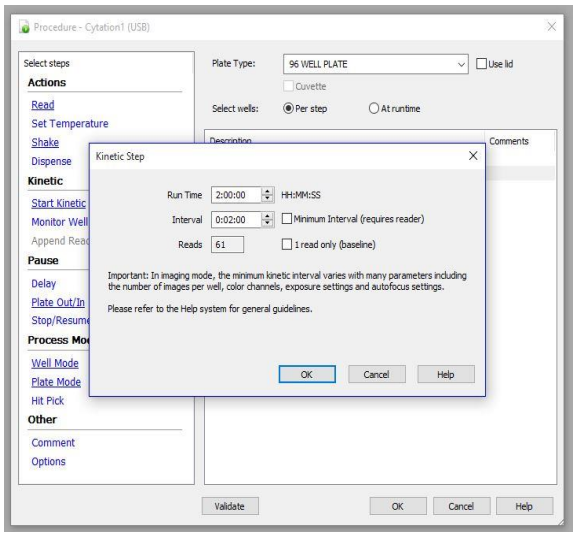
B)



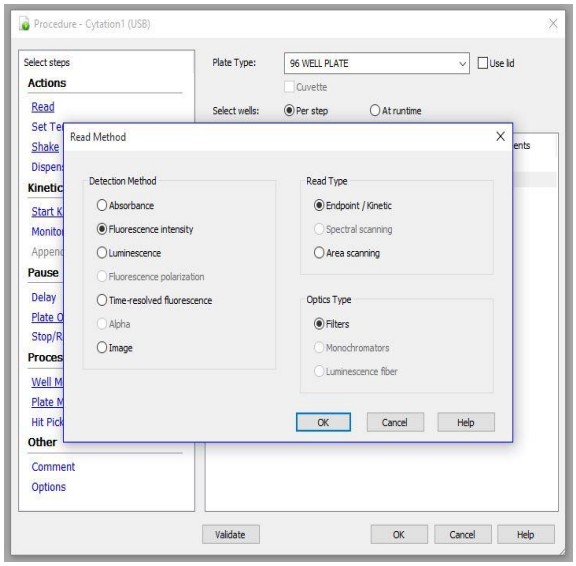
C)



D)



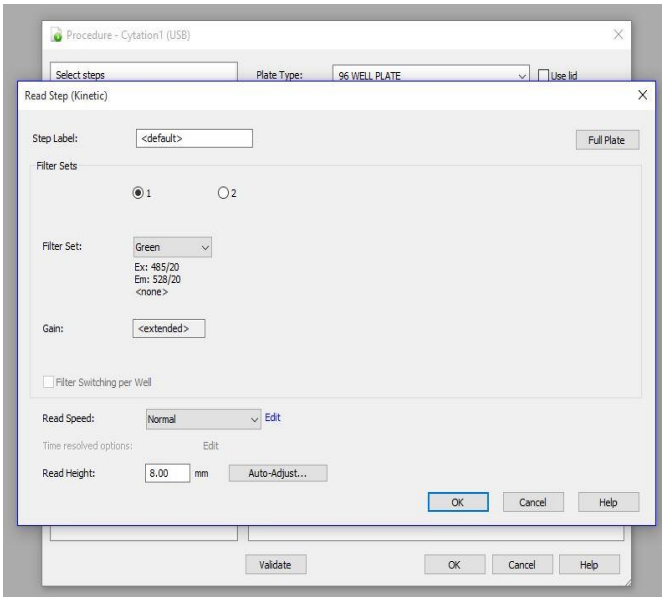
E)



Supplemental Figures 2

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary Figure 2.pdf

A)



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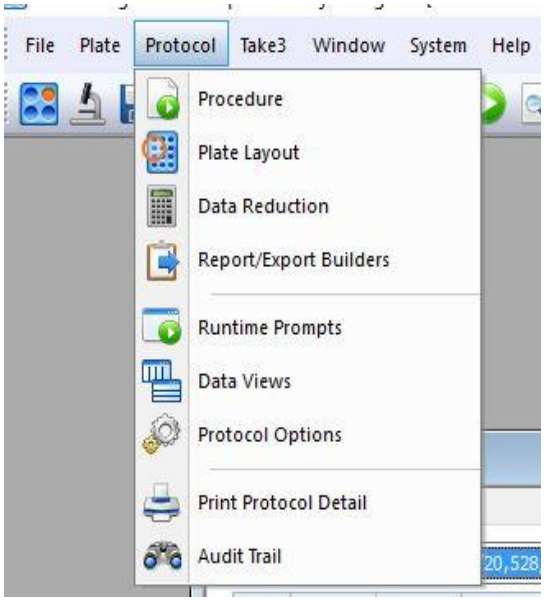

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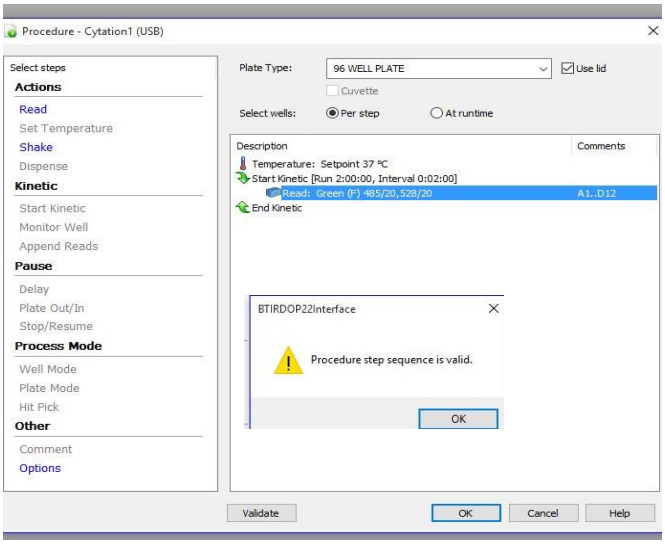

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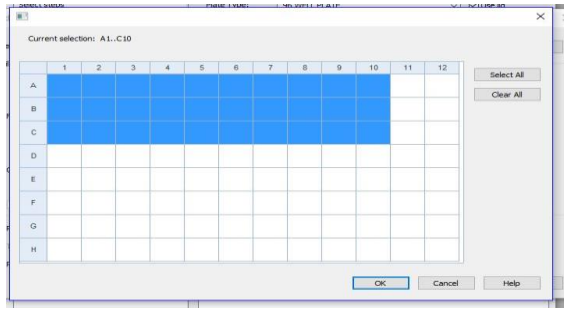
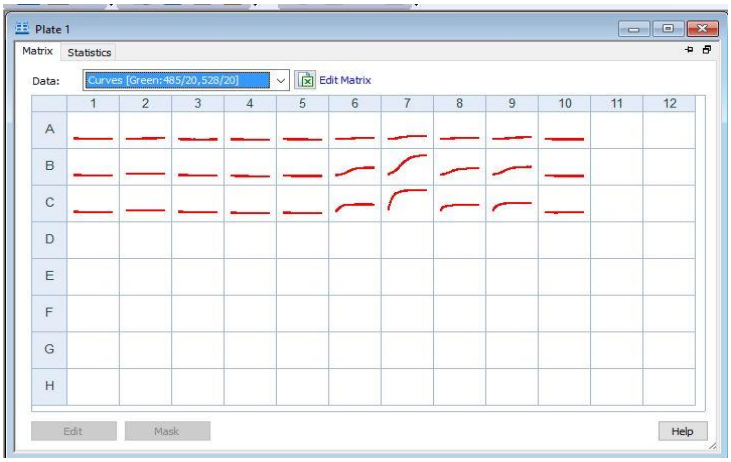


E)

F)

A)



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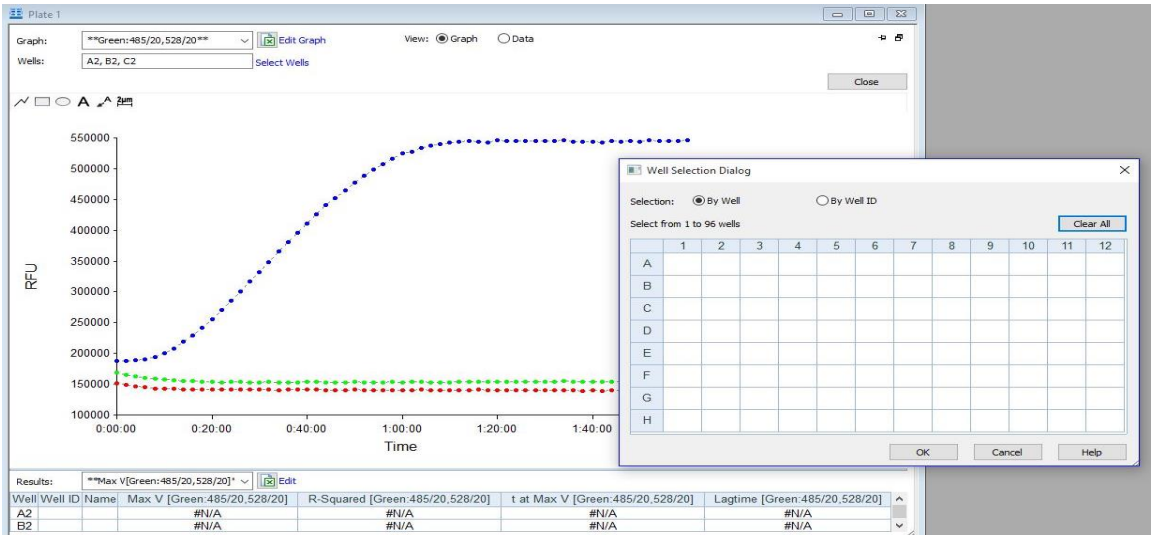

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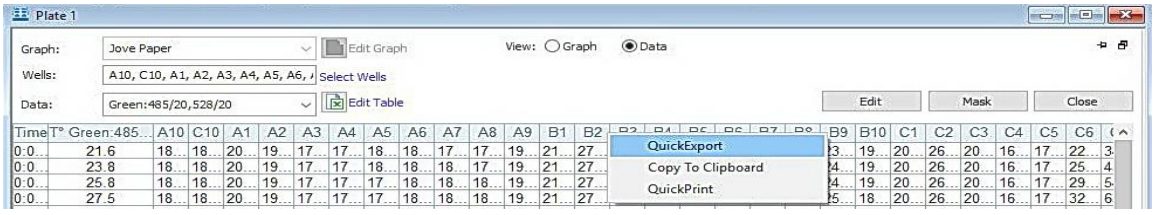
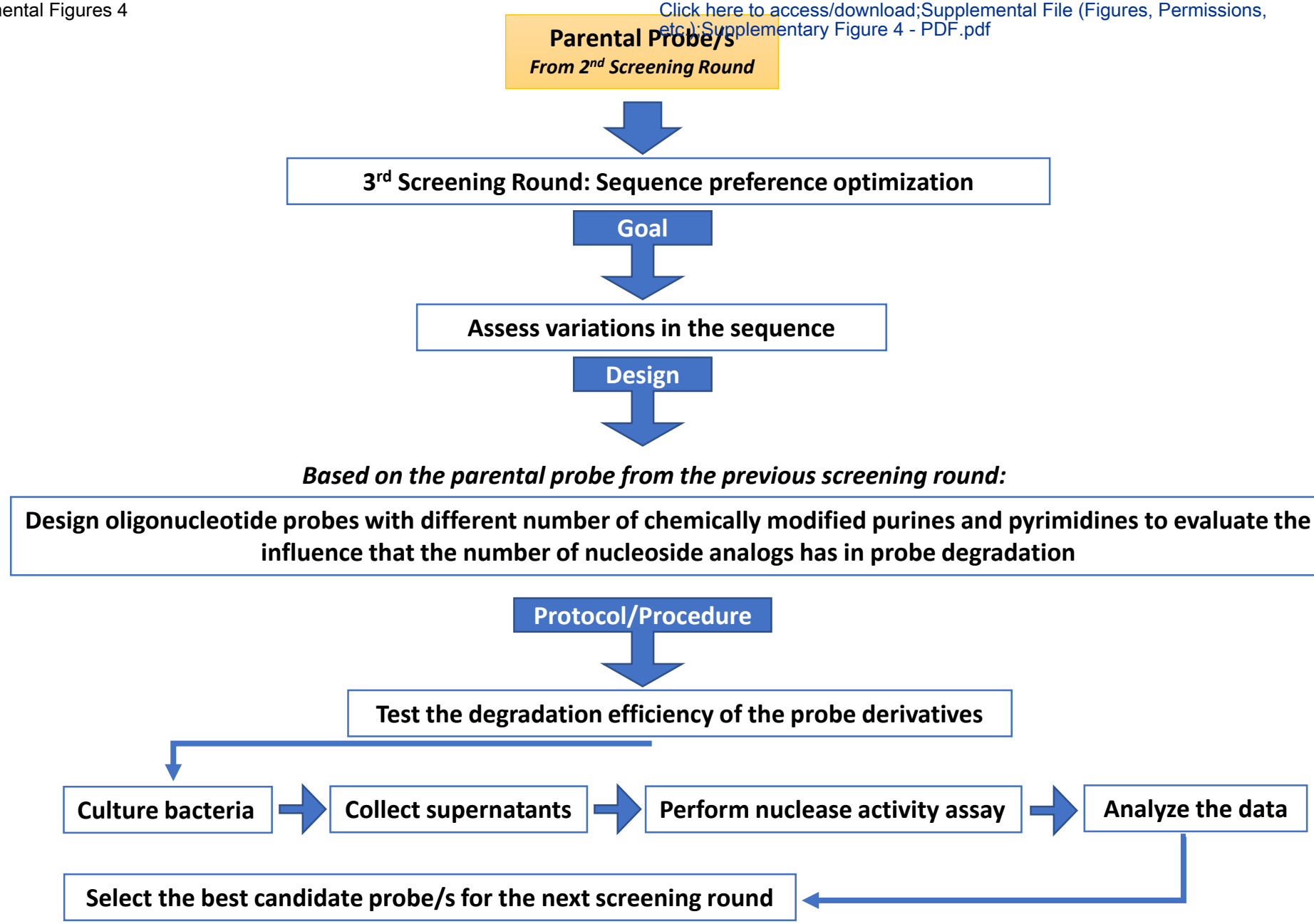
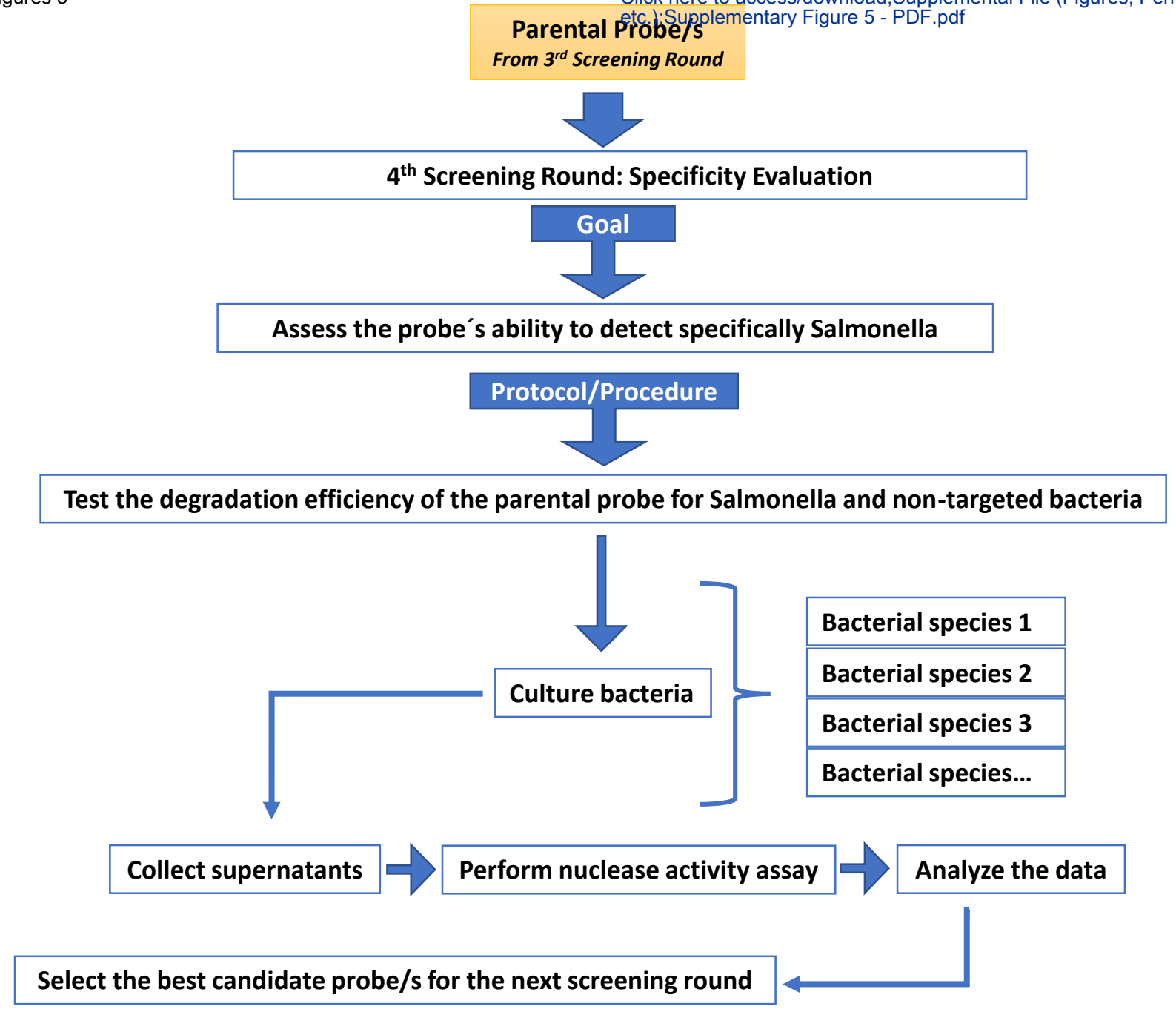
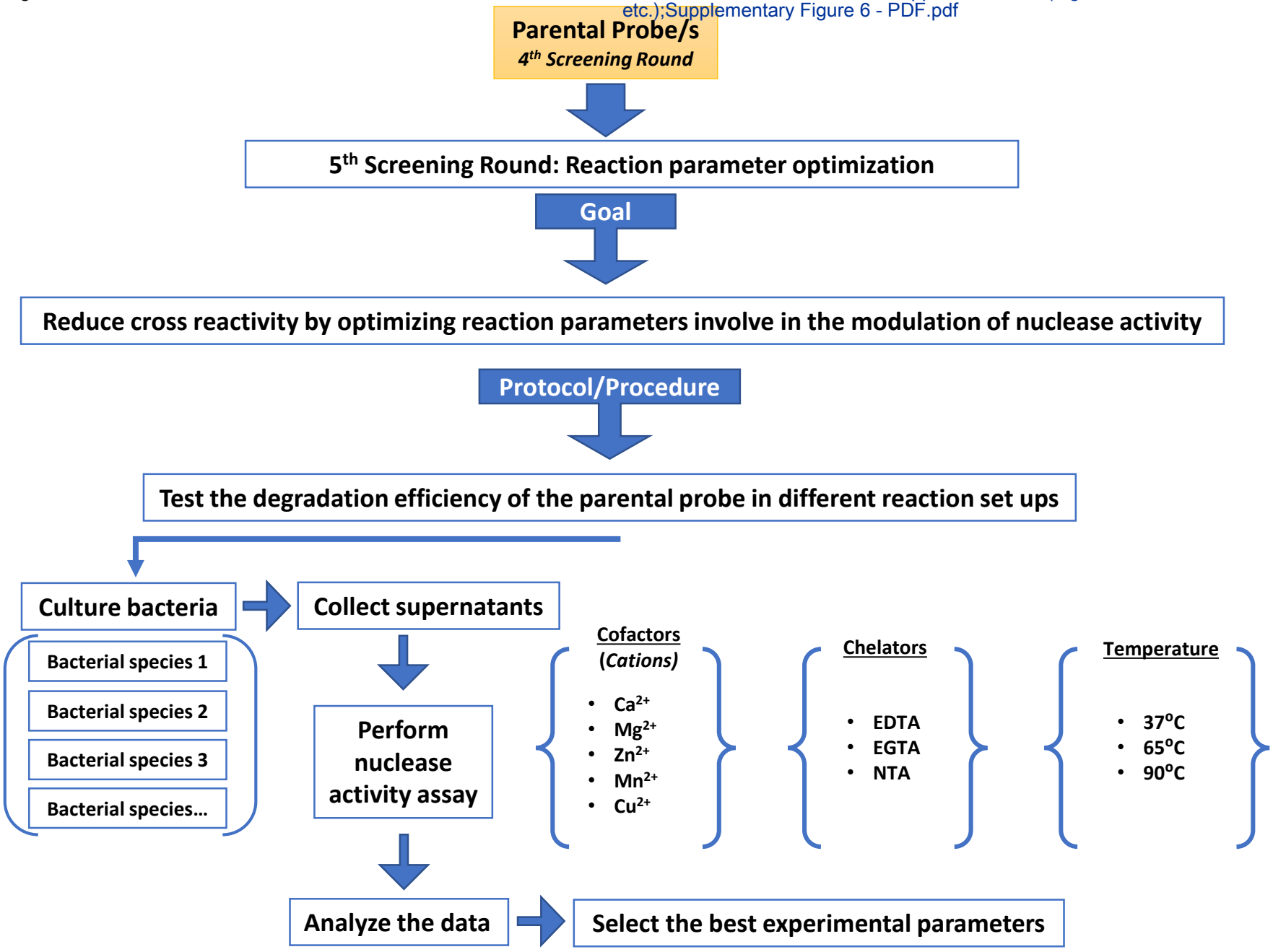




Supplemental Figures 5





Supplemental Table 1

Click here to access/download;Supplemental File (Figures, Permissions, etc.);Supplementary File 1 - Raw Data - Screening

	DNA			
Time (minutes)	Fluorescence (RFU) TSB	Rate	Fluorescence (RFU) E. coli	Rate
0	188344		166567	
2	189311	8	166942	3
4	187864	-12	167066	1
6	186154	-14	166003	-9
8	186055	-1	164443	-13
10	184270	-15	164457	0
12	183300	-8	162176	-19
14	182155	-10	160558	-13
16	180831	-11	159956	-5
18	179960	-7	158734	-10
20	178750	-10	157295	-12
22	177076	-14	156572	-6
24	176169	-8	155153	-12
26	175570	-5	154870	-2
28	174079	-12	153120	-15
30	173593	-4	153193	1
32	172876	-6	152492	-6
34	171586	-11	152010	-4
36	171542	0	151762	-2
38	171186	-3	151417	-3
40	170633	-5	151308	-1
42	171017	3	151502	2
44	170770	-2	151041	-4
46	169291	-12	151689	5
48	169878	5	151229	-4
50	169887	0	151789	5
52	169529	-3	151160	-5
54	169429	-1	151507	3
56	168925	-4	151946	4
58	168551	-3	151700	-2
60	167903	-5	152054	3
62	167347	-5	152804	6
64	167585	2	152504	-3
66	168049	4	153068	5
68	168082	0	152881	-2
70	167244	-7	153399	4
72	167127	-1	153242	-1
74	166508	-5	154029	7
76	167102	5	153689	-3
78	165682	-12	154108	3
80	165752	1	154275	1
82	165353	-3	155160	7
84	165715	3	154522	-5
86	165557	-1	155109	5
88	165713	1	155208	1
90	164841	-7	155462	2
92	164727	-1	155422	0

94	165667	8	156135	6
96	165411	-2	156126	0
98	164405	-8	156545	3
100	165389	8	156576	0
102	164034	-11	156882	3
104	164285	2	157176	2
106	164436	1	157354	1
108	164881	4	157708	3
110	163037	-15	157843	1
112	162619	-3	158575	6
114	162648	0	158006	-5
116	163061	3	159337	11
118	162886	-1	158833	-4
120	162746	-1	159572	6

Fluorescence (RFU) Salmonella	Rate	Fluorescence (RFU) TSB	Rate
172931		228723	
172507	-4	230285	13
173118	5	228108	-18
172618	-4	227507	-5
172159	-4	228037	4
170985	-10	225820	-18
170377	-5	226939	9
169393	-8	224825	-18
168614	-6	222930	-16
167260	-11	223978	9
166835	-4	222675	-11
166205	-5	221742	-8
165185	-9	220977	-6
164321	-7	221796	7
162979	-11	219067	-23
163014	0	220790	14
162553	-4	219161	-14
162116	-4	218093	-9
162143	0	219782	14
161229	-8	219065	-6
162036	7	219129	1
161242	-7	218972	-1
161326	1	218705	-2
161324	0	217316	-12
161278	0	217865	5
160904	-3	218017	1
161015	1	216988	-9
160504	-4	216991	0
160346	-1	217336	3
160496	1	217623	2
160578	1	216090	-13
160285	-2	215856	-2
160898	5	216864	8
160582	-3	216176	-6
160695	1	216462	2
160809	1	215796	-6
160126	-6	215447	-3
160943	7	214341	-9
160473	-4	216531	18
160083	-3	215936	-5
160539	4	214794	-10
160326	-2	215004	2
159911	-3	214803	-2
159927	0	215037	2
160120	2	213981	-9
159694	-4	214004	0
159840	1	214783	6

159925	1	215124	3
159654	-2	214231	-7
160101	4	214132	-1
159723	-3	214659	4
160246	4	212691	-16
159778	-4	213931	10
160299	4	212833	-9
159800	-4	213600	6
160580	7	213213	-3
159874	-6	213201	0
160048	1	212564	-5
160211	1	213800	10
160150	-1	213416	-3
159452	-6	213382	0

DNA Poly A

Fluorescence (RFU) E. coli	Rate	Fluorescence (RFU) Salmonella	Rate
172785		239934	
173074	2	241318	12
172837	-2	240547	-6
172451	-3	241124	5
172252	-2	240171	-8
171900	-3	239938	-2
171979	1	238929	-8
170968	-8	238327	-5
170237	-6	237900	-4
170375	1	236933	-8
170614	2	236514	-3
170066	-5	236885	3
169858	-2	234776	-18
170159	3	235049	2
169786	-3	234993	0
171062	11	233862	-9
171685	5	233090	-6
170853	-7	233433	3
172327	12	234357	8
172255	-1	233958	-3
173345	9	233469	-4
174630	11	233293	-1
174757	1	234029	6
175814	9	232918	-9
176562	6	233806	7
176850	2	232378	-12
178551	14	233572	10
179074	4	233440	-1
179978	8	233695	2
180408	4	233391	-3
180775	3	232700	-6
181742	8	232892	2
182776	9	233016	1
183803	9	232974	0
184637	7	232128	-7
185754	9	233199	9
185617	-1	232244	-8
186555	8	232999	6
187656	9	232670	-3
188452	7	232007	-6
188602	1	232464	4
188977	3	232336	-1
190104	9	232291	0
190485	3	232666	3
191291	7	232164	-4
192317	9	232279	1
192499	2	232466	2

193057	5	231979	-4
192936	-1	231869	-1
195100	18	231890	0
195608	4	232375	4
196123	4	231739	-5
196789	6	232084	3
196901	1	231678	-3
197710	7	232167	4
198025	3	232079	-1
198661	5	231802	-2
200446	15	232483	6
200575	1	231555	-8
200681	1	231758	2
201624	8	232075	3

DNA Poly T			
Fluorescence (RFU) TSB	Rate	Fluorescence (RFU) E. coli	Rate
170614		158154	
170840	2	158099	0
170310	-4	157603	-4
169240	-9	157447	-1
168854	-3	155499	-16
167199	-14	155422	-1
167088	-1	153819	-13
164925	-18	153335	-4
164722	-2	152270	-9
164341	-3	150823	-12
163071	-11	149785	-9
162604	-4	149297	-4
161095	-13	148536	-6
160264	-7	148088	-4
160128	-1	146600	-12
159103	-9	146125	-4
158782	-3	145983	-1
157641	-10	145804	-1
158386	6	145929	1
158210	-1	145567	-3
157682	-4	145840	2
157679	0	146087	2
157408	-2	146597	4
156773	-5	146165	-4
157016	2	146075	-1
157414	3	146262	2
156709	-6	146416	1
156917	2	147070	5
156964	0	146785	-2
156501	-4	147363	5
155831	-6	147899	4
155327	-4	147472	-4
155898	5	147841	3
155953	0	147921	1
156650	6	148170	2
155227	-12	148238	1
155761	4	149643	12
154774	-8	148468	-10
155804	9	148628	1
155005	-7	149375	6
154766	-2	149586	2
154940	1	149953	3
155055	1	150050	1
154488	-5	150213	1
154856	3	150747	4
154067	-7	150428	-3
153931	-1	151448	9

155023	9	151864	3
154229	-7	151971	1
154115	-1	152342	3
154050	-1	152112	-2
153302	-6	152420	3
154856	13	153001	5
153559	-11	153192	2
154046	4	153375	2
153780	-2	153051	-3
154145	3	153940	7
153847	-2	154647	6
153755	-1	154212	-4
153487	-2	155115	8
153635	1	154850	-2

Fluorescence (RFU) Salmonella	Rate	Fluorescence (RFU) TSB	Rate
194351		182567	
195511	10	182797	2
194548	-8	180454	-20
193636	-8	180442	0
193084	-5	179613	-7
191984	-9	177429	-18
190778	-10	176859	-5
189981	-7	174790	-17
189707	-2	174026	-6
188796	-8	173284	-6
187338	-12	171306	-16
185930	-12	169477	-15
185042	-7	168822	-5
184039	-8	168861	0
184278	2	167199	-14
184249	0	165797	-12
182573	-14	164912	-7
182820	2	163900	-8
182245	-5	164247	3
182212	0	164007	-2
182175	0	163453	-5
181944	-2	163580	1
181908	0	163098	-4
181573	-3	162543	-5
181181	-3	163680	9
181181	0	162349	-11
181458	2	162958	5
181456	0	162300	-5
180589	-7	162476	1
181278	6	163020	5
181093	-2	161921	-9
180194	-7	162184	2
180318	1	162718	4
181250	8	162599	-1
180473	-6	162255	-3
180501	0	162417	1
180163	-3	162554	1
180362	2	162023	-4
180285	-1	162587	5
180079	-2	162132	-4
180357	2	162356	2
180129	-2	162011	-3
179825	-3	161960	0
180129	3	161542	-3
179589	-5	162435	7
179685	1	161374	-9
179117	-5	162013	5

179836	6	162646	5
180021	2	161871	-6
178878	-10	162475	5
179867	8	163000	4
179158	-6	162409	-5
178902	-2	162446	0
178798	-1	162896	4
179342	5	163431	4
179320	0	162336	-9
179253	-1	161689	-5
179258	0	162498	7
179116	-1	162079	-3
178776	-3	163348	11
178866	1	162181	-10

DNA Poly C

Fluorescence (RFU) E. coli	Rate	Fluorescence (RFU) Salmonella	Rate
169340		143321	
170541	10	143073	-2
169926	-5	142992	-1
168918	-8	142506	-4
168439	-4	141604	-8
167265	-10	140917	-6
165740	-13	140606	-3
164717	-9	139369	-10
164082	-5	138687	-6
163018	-9	137774	-8
161052	-16	136779	-8
160166	-7	136271	-4
159573	-5	135624	-5
157752	-15	134656	-8
157145	-5	134054	-5
157112	0	133539	-4
155792	-11	132639	-8
155119	-6	132564	-1
155499	3	132426	-1
154914	-5	132044	-3
154170	-6	132130	1
154590	4	131783	-3
154327	-2	131561	-2
153781	-5	131292	-2
153408	-3	131460	1
153692	2	131032	-4
152977	-6	131244	2
152621	-3	130976	-2
153090	4	130783	-2
152742	-3	130266	-4
152276	-4	130532	2
152461	2	130594	1
153742	11	130493	-1
151818	-16	130770	2
152030	2	130694	-1
152251	2	130024	-6
151975	-2	130254	2
152006	0	130238	0
152198	2	130276	0
151402	-7	130492	2
151689	2	130190	-3
151966	2	130087	-1
152068	1	130321	2
151538	-4	129972	-3
151440	-1	129877	-1
151806	3	130169	2
151209	-5	130143	0

151427	2	129766	-3
151313	-1	130134	3
151797	4	129428	-6
151002	-7	129635	2
151083	1	129342	-2
150659	-4	129955	5
150741	1	129718	-2
150798	0	129958	2
150678	-1	129792	-1
150165	-4	129519	-2
150839	6	129869	3
150911	1	129584	-2
150008	-8	129309	-2
150303	2	129774	4

DNA Poly G			
Fluorescence (RFU) TSB	Rate	Fluorescence (RFU) E. coli	Rate
145073		136318	
145460	3	136220	-1
144347	-9	136625	3
143041	-11	135932	-6
143179	1	135241	-6
140729	-20	134984	-2
141263	4	133853	-9
138934	-19	133486	-3
138327	-5	132212	-11
137329	-8	131182	-9
136259	-9	130813	-3
134316	-16	129870	-8
133511	-7	129255	-5
133723	2	128903	-3
132443	-11	128051	-7
131972	-4	127468	-5
131004	-8	127090	-3
130355	-5	127082	0
130247	-1	126469	-5
130036	-2	126455	0
129273	-6	126485	0
129925	5	126591	1
129209	-6	125916	-6
128310	-7	125880	0
128565	2	125628	-2
129025	4	125468	-1
128145	-7	125759	2
128633	4	125256	-4
128134	-4	125446	2
127825	-3	125388	0
128037	2	124970	-3
127281	-6	125185	2
127258	0	124615	-5
127942	6	125073	4
127594	-3	124836	-2
127390	-2	124999	1
126851	-4	125047	0
126687	-1	124557	-4
127111	4	124870	3
126510	-5	124835	0
126507	0	124705	-1
126278	-2	124335	-3
126368	1	124246	-1
125870	-4	124613	3
126716	7	124333	-2
126341	-3	124306	0
125785	-5	124298	0

126008	2	123628	-6
125596	-3	124121	4
125821	2	124158	0
125689	-1	124014	-1
125012	-6	123825	-2
126251	10	123895	1
125286	-8	123856	0
125372	1	123937	1
124573	-7	123877	-1
124597	0	123645	-2
124361	-2	124225	5
125074	6	123625	-5
124591	-4	123352	-2
124704	1	123439	1

Fluorescence (RFU) Salmonella	Rate	Fluorescence (RFU) TSB	Rate
218623		166547	
149426	-577	165960	-5
148712	-6	164186	-15
148423	-2	163234	-8
147258	-10	162911	-3
146586	-6	161689	-10
145922	-6	161757	1
144562	-11	162183	4
143421	-10	161774	-3
142607	-7	161853	1
141495	-9	161560	-2
140194	-11	161664	1
139812	-3	162014	3
138558	-10	161493	-4
137717	-7	161609	1
136740	-8	161654	0
136544	-2	161963	3
136196	-3	161628	-3
135396	-7	161876	2
135645	2	162054	1
135514	-1	161800	-2
134820	-6	161483	-3
134527	-2	162766	11
134228	-2	163468	6
134287	0	162160	-11
133842	-4	162930	6
133668	-1	162291	-5
133660	0	162202	-1
133810	1	162044	-1
132951	-7	162170	1
133247	2	162459	2
133356	1	162197	-2
132790	-5	162799	5
132706	-1	162461	-3
132504	-2	161653	-7
133024	4	162277	5
132228	-7	161815	-4
132748	4	162159	3
132441	-3	161966	-2
132177	-2	161931	0
131883	-2	162491	5
131751	-1	162219	-2
131403	-3	162239	0
131906	4	162269	0
131830	-1	162161	-1
131441	-3	162517	3
131147	-2	161775	-6

131560	3	162646	7
131750	2	162315	-3
130996	-6	162158	-1
130925	-1	161806	-3
131244	3	161767	0
130652	-5	162178	3
131102	4	161800	-3
131174	1	162584	7
130685	-4	162781	2
130651	0	162270	-4
130387	-2	161790	-4
130111	-2	162351	5
130364	2	162106	-2
130080	-2	161248	-7

RNA			
Fluorescence (RFU) E. coli	Rate	Fluorescence (RFU) Salmonella	Rate
167057		218729	
164354	-23	265939	393
163530	-7	313032	392
162055	-12	358510	379
162226	1	393272	290
162842	5	418375	209
163942	9	432313	116
166446	21	439046	56
168756	19	443083	34
170398	14	446824	31
172480	17	450867	34
175090	22	454943	34
177761	22	454820	-1
179627	16	457204	20
182125	21	458759	13
184658	21	459353	5
187782	26	459985	5
189768	17	462219	19
192890	26	464221	17
194759	16	464467	2
197342	22	463237	-10
199826	21	463882	5
201432	13	464687	7
203215	15	465582	7
204529	11	465719	1
207000	21	467521	15
207838	7	468515	8
209972	18	468059	-4
211436	12	467481	-5
212242	7	467539	0
212973	6	468211	6
214025	9	468781	5
214435	3	467878	-8
215651	10	469239	11
215559	-1	468534	-6
216147	5	469004	4
216439	2	470301	11
217483	9	469043	-10
217434	0	468645	-3
218044	5	470484	15
217248	-7	468796	-14
218218	8	469495	6
218171	0	469037	-4
218234	1	469360	3
217986	-2	470001	5
218725	6	469601	-3
217767	-8	468121	-12

217887	1	469208	9
218375	4	470031	7
218673	2	469086	-8
218514	-1	469576	4
218611	1	470240	6
219623	8	469419	-7
218798	-7	469705	2
219371	5	469015	-6
218645	-6	468772	-2
219048	3	468754	0
218678	-3	468190	-5
218883	2	469229	9
219700	7	468615	-5
219004	-6	470134	13

RNA Poly A			
Fluorescence (RFU) TSB	Rate	Fluorescence (RFU) E. coli	Rate
218085		159328	
217815	-2	160330	8
215975	-15	163581	27
213980	-17	167490	33
213068	-8	170849	28
212250	-7	175491	39
211315	-8	180965	46
210426	-7	186724	48
210099	-3	192012	44
209662	-4	198183	51
209877	2	204114	49
209112	-6	210129	50
209430	3	215681	46
208680	-6	221374	47
208108	-5	225966	38
208987	7	231503	46
208652	-3	235474	33
208586	-1	239162	31
207901	-6	242897	31
207870	0	247254	36
207846	0	248943	14
207929	1	251924	25
208594	6	253874	16
208880	2	255211	11
209116	2	257047	15
208321	-7	259834	23
207639	-6	259903	1
208913	11	260595	6
208141	-6	260998	3
208381	2	263356	20
208709	3	262313	-9
208299	-3	263127	7
208080	-2	263734	5
208283	2	264084	3
208021	-2	264648	5
208630	5	265370	6
207901	-6	264895	-4
208925	9	265363	4
208171	-6	266021	5
208163	0	265664	-3
208432	2	266797	9
207925	-4	266333	-4
208435	4	266799	4
208632	2	267803	8
208391	-2	267125	-6
208538	1	268671	13
207363	-10	267606	-9

208469	9	268240	5
208713	2	268439	2
208142	-5	269223	7
207873	-2	269017	-2
208373	4	269809	7
208582	2	270189	3
208571	0	269716	-4
208407	-1	269888	1
208123	-2	270277	3
208440	3	270314	0
207635	-7	270782	4
207974	3	270866	1
207877	-1	270642	-2
207359	-4	271784	10

Fluorescence (RFU) Salmonella	Rate	Fluorescence (RFU) TSB	Rate
393590		153940	
549175	1297	153734	-2
694638	1212	153945	2
809910	961	152906	-9
897661	731	151994	-8
954283	472	152204	2
991894	313	151316	-7
1018452	221	151349	0
1034442	133	151010	-3
1047954	113	150660	-3
1062025	117	150653	0
1070270	69	150143	-4
1082666	103	149972	-1
1092879	85	150208	2
1098342	46	150257	0
1108765	87	150196	-1
1116399	64	149634	-5
1123445	59	150374	6
1129011	46	150148	-2
1134141	43	150110	0
1140069	49	150114	0
1142735	22	150002	-1
1143307	5	149825	-1
1150762	62	149902	1
1153339	21	149892	0
1155627	19	149999	1
1159280	30	149887	-1
1159027	-2	149896	0
1163972	41	149790	-1
1163914	0	150068	2
1164968	9	150101	0
1169084	34	150356	2
1172913	32	149624	-6
1169040	-32	149923	2
1170827	15	150107	2
1171543	6	150034	-1
1175561	33	150095	1
1171329	-35	150178	1
1171571	2	149687	-4
1173957	20	150095	3
1172287	-14	149967	-1
1175674	28	150481	4
1173827	-15	150444	0
1175708	16	150538	1
1174449	-10	150093	-4
1176201	15	149961	-1
1175068	-9	150103	1

1175447	3	150590	4
1176233	7	150631	0
1177360	9	150043	-5
1180983	30	150330	2
1175827	-43	150390	1
1178896	26	150008	-3
1177089	-15	150158	1
1177233	1	149998	-1
1178326	9	149895	-1
1175445	-24	149759	-1
1179447	33	149855	1
1180983	13	150114	2
1177013	-33	150634	4
1176117	-7	150011	-5

RNA Poly U

Fluorescence (RFU) E. coli	Rate	Fluorescence (RFU) Salmonella	Rate
145176		286570	
146977	15	364416	649
147341	3	418078	447
149084	15	449827	265
150943	15	467249	145
152954	17	478087	90
154897	16	484238	51
156511	13	491103	57
159102	22	494566	29
161516	20	498392	32
164034	21	501959	30
167050	25	505461	29
169956	24	507727	19
172032	17	509378	14
173534	13	511872	21
175634	18	514783	24
176491	7	516202	12
177383	7	516967	6
179245	16	519913	25
179781	4	521085	10
180674	7	524228	26
181346	6	524117	-1
182402	9	523819	-2
182931	4	525311	12
182801	-1	526959	14
182909	1	526413	-5
183407	4	529584	26
183358	0	528258	-11
183577	2	528262	0
183997	4	529417	10
184161	1	529672	2
184009	-1	530812	10
184688	6	530959	1
184725	0	530595	-3
185497	6	531382	7
185660	1	533810	20
185309	-3	531653	-18
185465	1	531302	-3
186042	5	532161	7
185983	0	532424	2
185281	-6	532492	1
185518	2	533503	8
186068	5	534777	11
185824	-2	534456	-3
186815	8	532837	-13
186381	-4	534077	10
185835	-5	534517	4

187220	12	533189	-11
187734	4	534380	10
186710	-9	533336	-9
187878	10	533292	0
187170	-6	533996	6
186976	-2	535651	14
187842	7	535415	-2
187964	1	535104	-3
187722	-2	535078	0
188095	3	535317	2
187801	-2	535500	2
187986	2	534685	-7
187553	-4	535827	10
188338	7	534977	-7

RNA Poly C			
Fluorescence (RFU) TSB	Rate	Fluorescence (RFU) E. coli	Rate
178518		178270	
179119	5	177680	-5
178943	-1	177463	-2
179007	1	177357	-1
178845	-1	177584	2
178904	0	177883	2
178659	-2	179255	11
179094	4	180147	7
178428	-6	182322	18
178920	4	183813	12
178505	-3	186191	20
178960	4	188751	21
178025	-8	192167	28
178778	6	194644	21
178617	-1	197601	25
178948	3	200125	21
179105	1	203429	28
178841	-2	206002	21
178951	1	209907	33
178307	-5	211906	17
179114	7	214867	25
178660	-4	217765	24
178994	3	221134	28
178863	-1	223511	20
179680	7	225934	20
179169	-4	228012	17
179087	-1	230595	22
179375	2	233599	25
179458	1	235889	19
179753	2	237726	15
179607	-1	239186	12
179930	3	241526	20
179206	-6	243329	15
179775	5	244840	13
179335	-4	246299	12
179017	-3	247520	10
179803	7	248834	11
179531	-2	249501	6
179705	1	251560	17
179202	-4	252386	7
179528	3	252821	4
179700	1	253801	8
179657	0	254108	3
179601	0	255291	10
179423	-1	255861	5
180086	6	255975	1
179876	-2	256529	5

179612	-2	257524	8
179822	2	257693	1
179544	-2	257149	-5
179450	-1	258088	8
179531	1	257249	-7
179360	-1	258124	7
179789	4	257943	-2
179996	2	258056	1
179182	-7	258341	2
179429	2	258426	1
180464	9	258975	5
179107	-11	258685	-2
179823	6	258517	-1
179640	-2	258900	3

Fluorescence (RFU) Salmonella	Rate	Fluorescence (RFU) TSB	Rate
268574		135194	
353349	706	134380	-7
427451	618	132964	-12
487538	501	131960	-8
527427	332	130387	-13
557395	250	129728	-5
574929	146	128997	-6
586194	94	128406	-5
595077	74	127018	-12
600516	45	126757	-2
605097	38	126006	-6
611089	50	126009	0
613160	17	125396	-5
616127	25	125016	-3
621260	43	125198	2
624270	25	125445	2
626047	15	124742	-6
628112	17	124283	-4
631328	27	124327	0
632572	10	123820	-4
633659	9	123904	1
635891	19	123768	-1
638219	19	123586	-2
636212	-17	124135	5
638763	21	123869	-2
638502	-2	123608	-2
639752	10	123730	1
641386	14	124017	2
641948	5	123484	-4
640986	-8	123724	2
642687	14	123636	-1
643854	10	123312	-3
643286	-5	123497	2
645374	17	123368	-1
643756	-13	123425	0
643919	1	123061	-3
643980	1	123227	1
645687	14	123698	4
645759	1	123065	-5
647827	17	123185	1
647281	-5	123470	2
646616	-6	122976	-4
647453	7	123073	1
646480	-8	123120	0
646183	-2	123181	1
646756	5	123209	0
649572	23	123058	-1

647982	-13	123758	6
646032	-16	123284	-4
646652	5	122929	-3
648050	12	122996	1
648377	3	122901	-1
648981	5	122570	-3
647406	-13	123090	4
647226	-2	122964	-1
646748	-4	122554	-3
647619	7	123396	7
646661	-8	122816	-5
649753	26	122991	1
648106	-14	123056	1
648100	0	122333	-6

RNA Poly G			
Fluorescence (RFU) E. coli	Rate	Fluorescence (RFU) Salmonella	Rate
160942		256678	
159551	-12	137905	-990
158218	-11	136195	-14
157322	-7	135677	-4
155365	-16	134523	-10
154471	-7	133490	-9
153676	-7	132964	-4
153457	-2	133267	3
152143	-11	133159	-1
151497	-5	132911	-2
151142	-3	133459	5
150615	-4	133354	-1
150619	0	134263	8
150518	-1	134404	1
150132	-3	134608	2
150307	1	135473	7
150050	-2	135858	3
149548	-4	137094	10
149464	-1	137511	3
150033	5	138053	5
149499	-4	138110	0
149961	4	139299	10
150223	2	139869	5
150306	1	139945	1
149858	-4	140769	7
150079	2	141203	4
149789	-2	141392	2
149867	1	142140	6
149772	-1	142470	3
149984	2	142984	4
150025	0	143179	2
149848	-1	143585	3
149376	-4	144204	5
149720	3	144559	3
150064	3	144514	0
149992	-1	144941	4
149596	-3	145315	3
150084	4	145632	3
150218	1	146118	4
149360	-7	146138	0
149392	0	146734	5
150140	6	146557	-1
150013	-1	147191	5
149678	-3	147055	-1
149337	-3	147926	7
149652	3	147974	0
149867	2	148568	5

149456	-3	148077	-4
149278	-1	148498	4
149007	-2	148701	2
149497	4	149449	6
149347	-1	148902	-5
149354	0	149180	2
149037	-3	150341	10
149348	3	149573	-6
149918	5	150033	4
149321	-5	149919	-1
149500	1	149848	-1
149193	-3	150280	4
149542	3	150044	-2
149235	-3	150584	5

Supplemental Table 2

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	All 2'Ome			
Time (minutes)	Fluorescence (RFU) TSB	Rate	Fluorescence (RFU) E. coli	Rate
0	140397		149124	
2	136641	-31	146466	-22
4	134217	-20	144120	-20
6	132693	-13	141692	-20
8	131179	-13	140970	-6
10	130309	-7	139369	-13
12	130280	0	138674	-6
14	130231	0	137723	-8
16	130101	-1	137820	1
18	129978	-1	136918	-8
20	129532	-4	136600	-3
22	128868	-6	136883	2
24	128656	-2	136989	1
26	129035	3	136986	0
28	128723	-3	136761	-2
30	128582	-1	136366	-3
32	128860	2	136328	0
34	129107	2	135838	-4
36	128300	-7	136062	2
38	129136	7	135991	-1
40	128772	-3	136145	1
42	128331	-4	136064	-1
44	128594	2	136076	0
46	128590	0	136066	0
48	128339	-2	135475	-5
50	127983	-3	136079	5
52	128281	2	135606	-4
54	127747	-4	136345	6
56	127779	0	135652	-6
58	128263	4	135717	1
60	127492	-6	135973	2
62	127937	4	136032	0
64	127856	-1	135807	-2
66	127958	1	135991	2
68	128178	2	135913	-1
70	127622	-5	135758	-1
72	128435	7	135714	0
74	127712	-6	135912	2
76	127024	-6	136294	3
78	127962	8	136267	0
80	128186	2	135874	-3
82	126972	-10	135683	-2
84	126883	-1	135755	1
86	127650	6	135788	0
88	127443	-2	135456	-3
90	127043	-3	135926	4
92	126889	-1	136094	1

94	127057	1	135554	-5
96	127329	2	135719	1
98	127108	-2	135729	0
100	127100	0	135778	0
102	126848	-2	135486	-2
104	127482	5	135801	3
106	127461	0	136034	2
108	127409	0	135766	-2
110	126583	-7	135947	2
112	127306	6	136051	1
114	126705	-5	135679	-3
116	126781	1	135885	2
118	126688	-1	136124	2
120	126540	-1	135600	-4

Fluorescence (RFU) Salmonella	Rate	Fluorescence (RFU) TSB	Rate
157979		151869	
154306	-31	148921	-25
151839	-21	146046	-24
150149	-14	144766	-11
148712	-12	143054	-14
147197	-13	143005	0
146504	-6	142329	-6
145438	-9	141879	-4
145000	-4	141911	0
144820	-2	141902	0
144699	-1	141910	0
143928	-6	141312	-5
144199	2	141506	2
144025	-1	141233	-2
143713	-3	141532	2
143392	-3	140925	-5
143349	0	140781	-1
143141	-2	140452	-3
143001	-1	141224	6
143814	7	141131	-1
143326	-4	140907	-2
143346	0	141002	1
143518	1	140601	-3
142471	-9	140570	0
142809	3	140606	0
142536	-2	140754	1
142792	2	140619	-1
142700	-1	139998	-5
142144	-5	140475	4
142833	6	140122	-3
142710	-1	140174	0
142965	2	140222	0
142083	-7	141191	8
142252	1	139883	-11
142296	0	140329	4
142050	-2	140538	2
142213	1	140594	0
142467	2	140540	0
142273	-2	140893	3
142030	-2	140001	-7
142699	6	140545	5
142024	-6	139751	-7
141731	-2	140486	6
141767	0	140045	-4
141942	1	139532	-4
142066	1	139721	2
141983	-1	140170	4

141902	-1	140139	0
141522	-3	139722	-3
141676	1	139120	-5
141724	0	139613	4
142057	3	139441	-1
141637	-4	139590	1
141172	-4	140278	6
141790	5	139528	-6
141101	-6	139686	1
140984	-1	139215	-4
141175	2	138711	-4
141179	0	140062	11
141973	7	140197	1
141537	-4	139555	-5

RNA Pyr 2'Ome

Fluorescence (RFU) E. coli	Rate	Fluorescence (RFU) Salmonella	Rate
168505		188132	
165670	-24	188003	-1
162815	-24	188591	5
160614	-18	190247	14
158512	-18	194550	36
157772	-6	200309	48
155904	-16	208177	66
155381	-4	218504	86
155047	-3	229206	89
154296	-6	241538	103
153569	-6	255242	114
153084	-4	269889	122
153538	4	285345	129
153688	1	301004	130
152849	-7	316607	130
153024	1	332425	132
153442	3	347945	129
153155	-2	365745	148
152224	-8	380610	124
152558	3	396101	129
153683	9	410557	120
153454	-2	426167	130
152696	-6	441111	125
152934	2	452127	92
153012	1	464180	100
153296	2	477229	109
152964	-3	488146	91
152922	0	498784	89
153197	2	507748	75
153481	2	516630	74
153267	-2	525382	73
153544	2	528041	22
153689	1	534326	52
153062	-5	538072	31
153216	1	540419	20
153221	0	542106	14
153721	4	544187	17
153696	0	544924	6
153584	-1	544247	-6
153754	1	542902	-11
153774	0	545865	25
153275	-4	545224	-5
153514	2	544604	-5
153856	3	544692	1
153306	-5	544628	-1
153487	2	544613	0
153430	0	544371	-2

154533	9	545871	13
153786	-6	543633	-19
154018	2	544065	4
154130	1	544035	0
153337	-7	543075	-8
154127	7	545111	17
153668	-4	543865	-10
154135	4	545574	14
154569	4	543580	-17
154004	-5	545734	18
154172	1	545579	-1
154207	0	545383	-2
154155	0	545216	-1
153967	-2	545961	6

RNA Pur 2'Ome			
Fluorescence (RFU) TSB	Rate	Fluorescence (RFU) E. coli	Rate
142987		141662	
140473	-21	140020	-14
138405	-17	139074	-8
137397	-8	137412	-14
136369	-9	136832	-5
136284	-1	136121	-6
137032	6	135710	-3
136897	-1	135588	-1
136393	-4	135819	2
136331	-1	135966	1
137018	6	136834	7
136835	-2	137222	3
136541	-2	138044	7
136634	1	139371	11
137324	6	139944	5
136412	-8	141187	10
137294	7	142259	9
137027	-2	142795	4
137116	1	144148	11
136674	-4	145035	7
137331	5	146116	9
137301	0	147873	15
137569	2	148278	3
137362	-2	149985	14
137334	0	150474	4
136997	-3	151569	9
136904	-1	152578	8
137382	4	153543	8
137556	1	154483	8
137141	-3	155654	10
137208	1	156563	8
136929	-2	157910	11
137516	5	158739	7
136874	-5	159818	9
137501	5	160769	8
137262	-2	162052	11
137908	5	163101	9
137460	-4	163402	3
137505	0	164831	12
137394	-1	165354	4
137141	-2	166810	12
136785	-3	167946	9
136970	2	168783	7
137625	5	169932	10
137386	-2	171403	12
136621	-6	171688	2
137517	7	172428	6

137402	-1	173657	10
137218	-2	174618	8
137109	-1	174839	2
136853	-2	175535	6
136794	0	177955	20
137486	6	177733	-2
137136	-3	178063	3
137166	0	179052	8
136361	-7	180010	8
137127	6	180650	5
136982	-1	180947	2
137243	2	181618	6
137154	-1	183883	19
136861	-2	183333	-5

Fluorescence (RFU) Salmonella	Rate	Fluorescence (RFU) TSB	Rate
234103		146252	
274093	333	143029	-27
315541	345	140144	-24
358061	354	138215	-16
395000	308	136050	-18
424464	246	135405	-5
446152	181	134803	-5
461562	128	133019	-15
469685	68	132747	-2
478809	76	132395	-3
481441	22	132416	0
485154	31	131727	-6
487529	20	131594	-1
490312	23	130986	-5
490248	-1	131238	2
491859	13	130236	-8
493417	13	130574	3
495823	20	130430	-1
496551	6	130789	3
499050	21	130361	-4
499034	0	130318	0
501132	17	130388	1
499287	-15	130465	1
501782	21	129917	-5
501998	2	130328	3
502599	5	129891	-4
502471	-1	129667	-2
502643	1	129779	1
501101	-13	129858	1
503421	19	129723	-1
503057	-3	129530	-2
504729	14	130285	6
506711	17	129581	-6
504566	-18	129233	-3
504871	3	129983	6
504381	-4	129513	-4
505323	8	129678	1
506399	9	129971	2
506106	-2	129468	-4
505302	-7	129514	0
505816	4	129361	-1
506003	2	129064	-2
506936	8	129283	2
509611	22	129541	2
505040	-38	128868	-6
505785	6	128894	0
508690	24	129156	2

506805	-16	129365	2
507071	2	129037	-3
509395	19	128900	-1
507134	-19	128956	0
507479	3	128896	-1
507921	4	129161	2
508022	1	129799	5
506892	-9	128361	-12
507227	3	128813	4
507891	6	128901	1
507680	-2	128483	-3
509284	13	129347	7
509292	0	129002	-3
508573	-6	128498	-4

All 2'F			
Fluorescence (RFU) E. coli	Rate	Fluorescence (RFU) Salmonella	Rate
145186		166384	
142707	-21	163588	-23
141107	-13	160974	-22
139121	-17	159038	-16
137659	-12	157415	-14
136508	-10	155505	-16
136126	-3	154812	-6
134416	-14	153492	-11
134830	3	152521	-8
134388	-4	152294	-2
134307	-1	151969	-3
133988	-3	151740	-2
133994	0	150723	-8
134401	3	150177	-5
135091	6	150314	1
135240	1	150617	3
135362	1	149769	-7
135690	3	149897	1
135792	1	149714	-2
136727	8	149636	-1
137588	7	149627	0
138127	4	150102	4
138168	0	149821	-2
138562	3	150129	3
138950	3	150378	2
140048	9	150265	-1
140306	2	150378	1
141130	7	150605	2
141176	0	149932	-6
141258	1	150300	3
141649	3	150581	2
142714	9	150467	-1
142906	2	150794	3
143432	4	150894	1
144421	8	150513	-3
144507	1	150250	-2
144372	-1	150962	6
145150	6	151538	5
146172	9	150752	-7
147109	8	151896	10
147652	5	151487	-3
147705	0	151775	2
148482	6	151426	-3
148346	-1	152310	7
148944	5	151947	-3
149100	1	151920	0
149919	7	152003	1

150428	4	152237	2
150956	4	152293	0
151181	2	151916	-3
151602	4	152527	5
152452	7	151974	-5
152994	5	152997	9
153120	1	153363	3
153702	5	153218	-1
153905	2	152991	-2
154631	6	153552	5
154500	-1	153097	-4
155657	10	153495	3
155934	2	153623	1
156449	4	153443	-2

RNA Pyr 2'F			
Fluorescence (RFU) TSB	Rate	Fluorescence (RFU) E. coli	Rate
165840		158088	
163204	-22	155159	-24
162153	-9	153430	-14
161758	-3	152435	-8
160733	-9	150896	-13
161419	6	149241	-14
161860	4	148507	-6
161758	-1	148790	2
161927	1	148167	-5
162311	3	148211	0
162782	4	148726	4
162017	-6	148407	-3
163002	8	148698	2
162791	-2	148604	-1
164674	16	149336	6
162942	-14	149858	4
163486	5	150910	9
164081	5	151015	1
163485	-5	151261	2
164306	7	151968	6
164030	-2	152291	3
163458	-5	153278	8
163928	4	153901	5
163641	-2	153828	-1
163967	3	155180	11
164488	4	156106	8
163525	-8	155896	-2
163368	-1	156428	4
164185	7	157331	8
164273	1	157740	3
163734	-4	158169	4
163527	-2	158467	2
163625	1	159417	8
163302	-3	159735	3
163991	6	160658	8
163798	-2	162218	13
163885	1	162098	-1
163919	0	162006	-1
163999	1	162480	4
163622	-3	163709	10
163878	2	163672	0
163898	0	164580	8
163320	-5	165208	5
163595	2	165946	6
162984	-5	167082	9
163575	5	167213	1
163320	-2	167462	2

163506	2	167464	0
163153	-3	168431	8
163306	1	169759	11
163499	2	170028	2
163590	1	170932	8
162852	-6	170995	1
163502	5	170643	-3
163568	1	172091	12
162921	-5	172318	2
162840	-1	172768	4
163272	4	173074	3
162872	-3	173769	6
163694	7	174505	6
163006	-6	175336	7

Fluorescence (RFU) Salmonella	Rate	Fluorescence (RFU) TSB	Rate
199035		165470	
197885	-10	162320	-26
198363	4	159186	-26
197857	-4	156995	-18
198436	5	155617	-11
199220	7	155562	0
200688	12	155354	-2
203204	21	154831	-4
205081	16	154416	-3
207260	18	154426	0
210824	30	154150	-2
215578	40	154042	-1
218589	25	153933	-1
224578	50	154022	1
227900	28	153769	-2
232610	39	153572	-2
238197	47	153718	1
242319	34	153429	-2
247645	44	153269	-1
253626	50	152818	-4
259023	45	153472	5
264440	45	152864	-5
270447	50	153314	4
275920	46	152854	-4
280593	39	152390	-4
286589	50	152565	1
290389	32	152506	0
297827	62	151887	-5
301926	34	152254	3
306417	37	152094	-1
311829	45	152263	1
317593	48	151848	-3
322201	38	152162	3
327687	46	151308	-7
332539	40	152638	11
337842	44	151508	-9
342179	36	152149	5
346586	37	151488	-6
351665	42	151667	1
355300	30	150511	-10
359592	36	151032	4
365162	46	150429	-5
370062	41	150646	2
373310	27	150733	1
378125	40	150432	-3
381616	29	150285	-1
386547	41	150104	-2

389402	24	150294	2
395345	50	149708	-5
397436	17	149642	-1
399248	15	149680	0
402458	27	149118	-5
406841	37	149496	3
409103	19	149361	-1
412639	29	149057	-3
416772	34	149058	0
417476	6	148983	-1
421179	31	148254	-6
423618	20	148480	2
426659	25	148643	1
429119	21	148813	1

RNA Pur 2'F			
Fluorescence (RFU) E. coli	Rate	Fluorescence (RFU) Salmonella	Rate
139720		189692	
137706	-17	214552	207
136728	-8	240742	218
135163	-13	267930	227
134051	-9	295782	232
133413	-5	323462	231
133046	-3	349064	213
133220	1	372253	193
132872	-3	392530	169
133810	8	411502	158
133926	1	425479	116
134344	3	436958	96
135484	10	447046	84
136027	5	455065	67
137203	10	461262	52
138214	8	465747	37
138995	7	469853	34
140348	11	473676	32
140625	2	473961	2
142162	13	475368	12
142725	5	476661	11
144239	13	478123	12
145535	11	475964	-18
145945	3	478837	24
146828	7	480092	10
148129	11	477635	-20
149325	10	477816	2
149863	4	477819	0
151415	13	479844	17
152314	7	478954	-7
152589	2	480053	9
153843	10	480025	0
155002	10	478847	-10
155572	5	478599	-2
156450	7	478940	3
157779	11	480244	11
158582	7	477371	-24
159815	10	479085	14
159711	-1	480363	11
160597	7	479411	-8
162108	13	479127	-2
162535	4	478768	-3
162869	3	478735	0
163235	3	478657	-1
164303	9	480091	12
164813	4	478523	-13
165992	10	477763	-6

166426	4	478261	4
167726	11	479398	9
168135	3	479860	4
167796	-3	478311	-13
169418	14	479504	10
170536	9	480244	6
170902	3	477260	-25
170738	-1	478394	9
171811	9	479622	10
172442	5	476788	-24
172832	3	479346	21
173579	6	478960	-3
173736	1	479199	2
174512	6	477678	-13

Probe	DNA		
	TSB	*E. coli*	*Salmonella*
Maximum rate values (Rmax)	8	11	7
Interval (min)	98	114	72
Interval (max)	100	116	74
Rate coefficient (Rmax/interval time) (FU/s/min)	0.082828283	0.096449275	0.09326484
Fold difference with respect to media	1	1.164448568	1.126002339
Fold difference between salmonella and E. coli			**0.966983317**

NOTE: The maximum rate values were obtianed by selecting the maximum values from the supplementary file 1

DNA Poly A			DNA Poly T		
TSB	*E. coli*	*Salmonella*	TSB	*E. coli*	*Salmonella*
18	18	12	13	12	10
74	96	0	102	70	0
76	98	2	104	72	2
0.243333333	0.185910653	11.53333333	0.125728155	0.164906103	9.666666667
1	0.764016382	47.39726027	1	1.311608389	76.88545689
		62.03696858			**58.61921708**

DNA Poly C			DNA Poly G		
TSB	*E. coli*	*Salmonella*	TSB	*E. coli*	*Salmonella*
11	11	5	10	5	4
116	62	102	102	112	68
118	64	104	104	114	70
0.090384615	0.169444444	0.049595469	0.100242718	0.042772861	0.062801932
1	1.874704492	0.54871583	1	0.426692951	0.626498696
		0.292694573			**1.468265867**

RNA			RNA Poly A		
TSB	*E. coli*	*Salmonella*	TSB	*E. coli*	*Salmonella*
11	26	393	11	51	1297
42	30	0	52	16	0
44	32	2	54	18	2
0.248643411	0.839784946	393.4166667	0.200314465	3.025	1296.541667
1	3.377467126	1582.252533	1	15.10125589	6472.531397
		468.4731114			**428.6088154**

RNA Poly U			RNA Poly C		
TSB	*E. coli*	*Salmonella*	TSB	*E. coli*	*Salmonella*
6	25	649	9	33	706
32	20	0	112	34	0
34	22	2	114	36	2
0.186868687	1.196825397	648.7166667	0.076327434	0.929761905	706.4583333
1	6.404633205	3471.510811	1	12.18122843	9255.628019
		542.0311671			**759.8271447**

RNA Poly G		
TSB	*E. coli*	*Salmonella*
7	6	10
110	80	32
112	82	34
0.063213213	0.076954733	0.312121212
1	1.217383654	4.937594472
		4.05590666

Probe	All 2'Ome		
	TSB	*E. coli*	*Salmonella*
Maximum rate values (Rmax)	8	6	7
Interval (min) (min)	76	52	36
Interval (max) (min)	78	54	38
Rate Coefficient (Rmax/interval time) (FU/s/min)	0.101515152	0.116194969	0.183108108
Fold difference with respect to media	1	1.144607153	1.803751513
Fold difference between salmonella and E. coli			**1.57586951**

NOTE: The maximum rate values were obtianed by selecting the maximum values from the supplementary file 2

RNA Pyr 2'Ome			RNA Pur 2'Ome		
TSB	*E. coli*	*Salmonella*	TSB	*E. coli*	*Salmonella*
11	9	148	7	20	354
114	38	32	90	100	4
116	40	34	92	102	6
0.097898551	0.240384615	4.494949495	0.082051282	0.199669967	70.86666667
1	2.455446108	45.91436198	1	2.433477723	863.6875
		18.6989899			**354.9190083**

All 2'F			RNA Pyr 2'F		
TSB	*E. coli*	*Salmonella*	TSB	*E. coli*	*Salmonella*
7	10	10	16	13	62
114	114	76	26	68	52
116	116	78	28	70	54
0.062608696	0.08384058	0.123809524	0.58117284	0.188405797	1.169496855
1	1.33912037	1.977513228	1	0.324182041	2.012304733
		1.476725522			**6.207329463**

RNA Pur 2'F		
TSB	*E. coli*	*Salmonella*
11	14	232
66	100	6
68	102	8
0.165422886	0.133828383	33.15714286
1	0.809007668	200.4386681
		247.7586754