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(54) Title: MICROFLUIDIC MEASUREMENTS OF THE RESPONSE OF AN ORGANISM TO A DRUG

(57) Abstract: Disclosed herein are methods and devices for rapid assessment of whether a microorganism present in a sample is susceptible or resistant to a treatment.

MICROFLUIDIC MEASUREMENTS OF THE RESPONSE OF AN ORGANISM TO A DRUG

CROSS-REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No. 62/075,648, filed November 5, 2014, which application is incorporated herein by reference in its entirety.

STATEMENT AS TO FEDERALLY SPONSORED RESEARCH

[0002] This invention was made with support under grant numbers EB012946, GM074961 and DP1OD003584 awarded by the National Institutes of Health (NIH) and under cooperative agreement number HR-0011-11-2-0006 by the Defense Advanced Research Projects Agency. The government has certain rights in the invention.

TECHNICAL FIELD

[0003] The present application relates to the field of microfluidics and to the fields of detection and amplification of biological entities.

BACKGROUND OF THE INVENTION

[0004] Antibiotic resistance constitutes a major imminent threat to public health worldwide. Statistics from the CDC(U. S. Department of Health and Human Services, 2013, 114) and WHO (Organization, 2014, 257) are alarming and emphasize that the current paradigm of liberal and non-selective antibiotic use cannot continue. Emergence of resistance in Gram-negative organisms, for which there are limited treatment options, is of particular concern. Over-use of broad-spectrum antibiotics in clinics is a major contributor to the evolution and spread of drug- resistant pathogens; however, combating resistance requires diagnostic development in all settings.

[0005] To improve care, clinical laboratories require rapid tests to identify the pathogen and determine its antimicrobial susceptibility. Laboratories currently conduct identification and antimicrobial susceptibility tests (AST) via culture-based methods, which take 24-48 hours. Because the physician does not know in a proximate timeframe what organism is present, the bacterial burden, or the susceptibility of the organism to various therapeutic approaches, clinicians often choose to treat with broad spectrum (e.g. 2nd and 3rd generation) antibiotics and can even treat sub-clinical infection or false positives. Overprescribing antibiotics is most acute among complex, recurrent, and specialty cases. These include febrile children, obstructed patients, such as men with BPH, nephrolithiasis, especially those with repeated instrumentation, women with infection during pregnancy, hospitalized patients, and immune-compromised patients. In these situations, clinicians tend to treat more aggressively. Accelerating turnaround will allow clinicians to improve patient care by administering the right antimicrobial regimen

without delays. Thus, to improve the problem of emerging antimicrobial resistance and to improve care, physicians in primary care settings require ultra-rapid tests to identify the pathogen and determine its antimicrobial susceptibility. What is needed, therefore, are devices and methods for rapidly and accurately diagnosing infection and antimicrobial resistance or susceptibility.

SUMMARY OF THE INVENTION

[0006] In one aspect, the invention provides devices and methods can rapidly identify a cell, including a cancer cell, or microorganism, including a pathogen, quantify their load, and diagnose their susceptibility or resistance to drugs, such as antibiotics. In some embodiments the devices can enable phenotypic detection and metabolic profiling of drug susceptibility or drug resistance using individual microorganisms or cells which can originate from various sample types, including clinical or environmental samples. These sample types can include, but are not limited to, blood, cerebral spinal fluid (CSF), saliva and urine and can also include environmental samples, such as from water or a hospital surface. In some embodiments, the devices enable incubation of cells with drugs, such as antibiotics, and then rapidly extract and quantify nucleic acids or other molecules in a contamination-free platform. The devices can use digital single- molecule measurements in microfluidics devices, which provide ultra-sensitive measurements that improve detection limits while providing quantitative data, important for differentiating pathogens from contaminants and enabling earlier differentiation between drug-resistant and susceptible organisms or cells. In some embodiments, these devices can differentiate the state of individual microorganisms or cells from a clinical sample, and understand the timing of their individual responses to drugs, such as for example antibiotics, providing ultra-fast drug- susceptibility measurements.

[0007] In some embodiments, the data associated with the sample comprises measurements from greater than one spatially-isolated compartment each of the compartments comprising a portion of the sample.

[0008] In some embodiments, the sample undergoes a nucleic acid amplification. In some embodiments, the nucleic acid amplification reaction is a loop mediated amplification (LAMP) reaction. In some embodiments, the nucleic acid amplification reaction is a PCR reaction. In some embodiments, the method is performed at about or at a temperature range of 55-65°C. In some embodiments, at least a portion of the sample is partitioned into an array comprising at least 2 or more. In some embodiments, the array is a SlipChip. In some embodiments, the nucleic acid that is amplified is RNA.

[0009] Also provided herein are methods for detecting and quantifying target molecules from a sample, comprising: providing a first sample comprising a target molecule from a first portion of a population of bacteria and a second sample comprising a target molecule from a second portion of said population of bacteria, wherein the first portion has been treated with an antibiotic, and wherein the second portion has not been treated with the antibiotic; distributing said first sample among a plurality of first analysis regions; distributing said second sample among a plurality of second analysis regions; contacting each of the first and second analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions; and detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said reaction.

[0010] In some embodiments, the threshold number is greater than zero, one, two, three, four, or five. In some embodiments, the distribution of each sample among the analysis regions is effected such that at least some of the analysis regions do not have the target molecule, and at least some of the analysis regions have only one target molecule. In some embodiments, the distribution of each sample among the analysis regions is effected such that at least one of the analysis regions contains only one target molecule. In some embodiments, the first sample comprises said reagent, and wherein contacting the plurality of first analysis regions with the reagent comprises said step of distributing said first sample among said plurality of first analysis regions. In some embodiments, the second sample comprises said reagent, and wherein contacting the plurality of second analysis regions with the reagent comprises said step of distributing said second sample among said plurality of second analysis regions.

[0011] In some embodiments, reaction comprises nucleic acid amplification. In some embodiments, the nucleic acid amplification is essentially isothermal. In some embodiments, the nucleic acid amplification is a polymerase chain reaction. In some embodiments, the nucleic acid amplification is a nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), loop mediated isothermal amplification (LAMP), strand displacement amplification (SDA), whole genome amplification, multiple displacement amplification, strand displacement amplification, helicase dependent amplification, nicking enzyme amplification reaction, recombinant polymerase amplification, reverse transcription PCR, or ligation mediated PCR.

[0012] In some embodiments, said analysis regions comprise wells. In some embodiments, the target molecule is a nucleic acid. In some embodiments, the reagent is a nucleic acid amplification reagent. In some embodiments, the method of detecting and quantifying target molecules from a sample further comprises exposing each of the analysis regions to conditions effective for amplification of the nucleic acid.

[0013] In some embodiments, the target molecule comprises a segment of a bacterial chromosome or plasmid. In some embodiments, the target molecule is less than 50 kDa, less than 100 kDa, less than 200 kDa, or less than 400 kDa from the origin of replication. In some embodiments, the target molecule comprises a sequence of a gene on said bacterial chromosome or plasmid. In some embodiments, the target molecule comprises mRNA. In some embodiments, the target molecule is amplified by an amplification reaction, said amplification reaction comprising primers complementary to a sequence of the target molecule. In some embodiments, the mRNA encodes recA or lexA.

[0014] In some embodiments, the reagent is disposed in a plurality of reagent regions. In some embodiments, contacting is effected by placing the plurality of reagent regions in fluid communication with the first or second plurality of analysis regions. In some embodiments, contacting comprises effecting relative motion between a substrate comprising the reagent regions with a substrate comprising the first and second plurality of analysis regions. In some embodiments, the method of detecting and quantifying target molecules from a sample further comprises analyzing the detection of the presence or the absence of the threshold number of target molecules in each plurality of analysis regions to determine a resistance or susceptibility to an antibiotic in the population of bacteria.

[0015] In some embodiments, the target molecule has been removed from the bacteria in the first sample, and wherein the time between initial exposure of the bacteria to antibiotic and the removal of the target molecules is less than the mean doubling time of the bacteria during a growth phase. In some embodiments, the detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 3 hours, 2 hours, or one hour after the end of exposure of the first sample to the antibiotic. In some embodiments, the detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 45 minutes, 30 minutes, 15 minutes, or 10 minutes after the end of exposure of the first sample to the antibiotic. In some embodiments, the detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 3 hours, less than 2 hours, or less than 1 hour after the end of exposure of the first sample to the antibiotic. In

some embodiments, the detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 45 minutes, less than 30 minutes, less than 15 minutes, or less than 10 minutes after the end of exposure of the first sample to the antibiotic.

[0016] In some embodiments, the bacteria from said first sample have been treated with antibiotic for a period of no more than 2 hours, no more than 1 hour, no more than 45 minutes, no more than 30 minutes, no more than 15 minutes, or no more than 10 minutes. In some embodiments, the time from the first exposure of the bacteria from the first sample to an antibiotic to the detection step is less than 3 hours, less than 2 hours, less than 1 hour after, less than 45 minutes, or less than 30 minutes.

[0017] In some embodiments, the first plurality of analysis regions comprises at least 10, 20, 30, 40, or 50 analysis regions. In some embodiments, the second plurality of analysis regions comprises at least 10, 20, 30, 40, or 50 analysis regions. In some embodiments, the distribution of the first and second sample into the first and second plurality of analysis regions is performed in parallel. In some embodiments, the first sample or second sample comprises less than 10,000, 5,000, 1,000, 500, 200, 100, 50, 20, or 10 target molecules.

[0018] Also provided herein is a method of determining a resistance or susceptibility to an antibiotic in a population of bacteria, comprising: providing a first sample comprising a target molecule from a first portion of a population of bacteria and a second sample comprising a target molecule from a second portion of said population of bacteria, wherein the first portion has been treated with an antibiotic, and wherein the second portion has not been treated with the antibiotic; distributing said first sample among a plurality of first analysis regions; distributing said second sample among a plurality of second analysis regions; contacting each of the first and second analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions; detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said reaction; and analyzing the results of the detection to determine a resistance or susceptibility to said antibiotic in the population of bacteria.

[0019] Also provided herein is a method of determining a resistance or susceptibility to an antibiotic in a population of bacteria, comprising: distributing a population of bacteria into a plurality of clonal isolation regions, the distribution being effected such that at least some of the

clonal isolation regions are statistically estimated to each contain a single isolated bacterium; expanding each of the single isolated bacteria to generate a plurality of clonal populations; distributing each of said plurality of clonal populations into at least one treatment region from a plurality of treatment regions and into at least one control region from a plurality of control regions; contacting the first plurality of treatment regions with an antibiotic, the plurality of control regions not contacted with the antibiotic; for each of the first plurality of treatment regions and each of the plurality of control regions, distributing one or more target molecules from each clonal population into a distinct plurality of analysis regions; contacting each of the analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions; and detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the distinct plurality of analysis regions and is not detected in some of the distinct plurality of analysis regions after said reaction.

[0020] In some embodiments, the method of determining a resistance or susceptibility to an antibiotic in a population of bacteria further comprises analyzing the results of the detection to determine a resistance or susceptibility to said antibiotic in at least some of said plurality of clonal populations.

[0021] Also provided herein are methods, comprising: providing a first sample comprising a target analyte from a first portion of a population of cells and a second sample comprising a target analyte from a second portion of said population of cells, wherein the first portion has been treated with a drug, and wherein the second portion has not been treated with the drug; distributing said first sample among a plurality of first analysis regions; distributing said second sample among a plurality of second analysis regions; contacting each of the first and second analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target analytes in each of the first and second analysis regions; and detecting the presence or absence of the threshold number of target analytes in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target analytes is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said reaction.

[0022] Also provided herein are kits comprising: a container comprising a plurality of analysis regions, a plurality of reagent regions comprising a component of a nucleic acid amplification,

wherein the container comprises a first layer and a second layer configured to move relative to the other between a first position, where the plurality of analysis regions and the plurality of reagent regions are isolated from each other, and a second position, wherein at least some of the plurality of analysis regions is in fluid communication with at least some of the plurality of reagent regions; and instructions for use for determining a resistance or susceptibility to an antibiotic in a population of bacteria.

[0023] In some embodiments, the component of a nucleic acid amplification comprises primers for amplifying recA or lexA mRNA. In some embodiments, the component of a nucleic acid amplification comprises primers for amplifying a nucleic acid target on a bacterial chromosome or plasmid less than 50kDa, less than 100 kDa, less than 200 kDa, or less than 400 kDa from the origin of replication. In some embodiments, the container comprises at least 10, 20, 30, 40, or 50 analysis regions. In some embodiments, the container comprises at least 10, 20, 30, 40, or 50 reagent regions.

[0024] In some embodiments, the antibiotic is selected from the group consisting of: aminoglycosides, cephalosporins, tetracyclines, sulfonamides, macrolides, vancomycin, and β-lactams. In certain of these embodiments, the instructions indicate that a decrease in nucleic acid target detection in a portion of the population of bacteria treated with said antibiotic as compared to a portion of the population of bacteria not treated with said antibiotic indicates that the population of bacteria is susceptible to said antibiotic.

[0025] In some embodiments, the antibiotic is selected from the group consisting of: HPUra, hydroxyurea, trimethoprim, ciprofloxacin, and MMC. In certain of these embodiments, the instructions indicate that an increase in nucleic acid target detection in a portion of the population of bacteria treated with said antibiotic as compared to a portion of the population of bacteria not treated with said antibiotic indicates that the population of bacteria is susceptible to said antibiotic.

[0026] Also provided herein is a device for processing a sample, comprising an incubation module, a sample preparation module, and a digital quantification module, wherein said device is configurable to place each module in fluid communication with the other modules; wherein said incubation module comprises an incubation chamber configured to incubate an organism with a drug, wherein said sample preparation module is configured to extract a nucleic acid from said organism; and wherein said digital quantification module comprises a plurality of reaction areas configured to perform digital detection of the presence or absence of said nucleic acid in said reaction area.

INCORPORATION BY REFERENCE

[0027] All publications, patents, and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication, patent, or patent application was specifically and individually indicated to be incorporated by reference.

[0028] Described herein are a number of devices and methods that can be used individually or in various combinations for applications including but not limited to those listed herein. Furthermore, they can be used in various combinations with previously disclosed devices and methods for previously described applications.

[0029] The present application incorporates the following applications by reference in their entireties for any and all purposes: United States Application 61/516,628, "Digital Isothermal Quantification of Nucleic Acids Via Simultaneous Chemical Initiation of Recombinase Polymerase Amplification (RPA) Reactions on Slip Chip," filed on April 5, 2011; United States Application 61/518,601, "Quantification of Nucleic Acids With Large Dynamic Range Using Multivolume Digital Reverse Transcription PCR (RT-PCR) On A Rotational Slip Chip Tested With Viral Load," filed on May 9, 2011; United States application 13/257,811, "Slip Chip Device and Methods," filed on September 20, 2011; international application PCT/US2010/028361, "Slip Chip Device and Methods," filed on March 23, 2010; United States Application 61/262,375, "Slip Chip Device and Methods," filed on November 18, 2009; United States Application 61/162,922, "Slip Chip Device and Methods," filed on March 24, 2009; United States Application 61/340,872, "Slip Chip Device and Methods," filed on March 22, 2010; United States Application 13/440,371, "Analysis Devices, Kits, And Related Methods For Digital Quantification Of Nucleic Acids And Other Analytes," filed on April 5, 2012; United States Application 13/467,482, "Multivolume Devices, Kits, Related Methods for Quantification and Detection of Nucleic Acids and Other Analytes," filed on May 9, 2012; United States Application 13/868,028, "Fluidic Devices and Systems for Sample Preparation or Autonomous Analysis," filed on April 22, 2013; United States Application 13/868,009, "Fluidic Devices for Biospecimen Preservation," filed on April 22, 2013; international application PCT/US2013/037658, "Fluidic Devices for Biospecimen Preservation," filed on April 22, 2013; international application PCT/US2013/037660, "Fluidic Devices and Systems for Sample Preparation or Autonomous Analysis," filed on April 22, 2013; United States application 13/869,856, "Slip-Induced Compartmentalization," filed April 24, 2013; international application PCT/US2013/063594, "Methods and Systems for Microfluidics Imaging and Analysis," filed on October 4, 2013; international application PCT/US2014/034728, "Parallelized Sample Handling," filed on April 18, 2014; international application

PCT/US2014/047092, "Digital Assay for Quantifying and Concentrating Analytes," filed on July 17, 2014; United States Application 62/038,036, "The Pumping Lid: Devices and Methods for Programmable Generation of Positive and Negative Pressures," filed on August 15, 2014; United States Application 62/050,647, "Digital Microfluidics Methods for Optimizing Isothermal Amplification Reactions," filed on Sept. 15, 2014; international application PCT/US2014/056401, "System and Method for Movement and Timing Control," filed on Sept. 18, 2014; and International Application No. PCT/US2014/060977 "Enhanced Nucleic Acid Identification and Detection" filed on October 16, 2014.

BRIEF DESCRIPTION OF THE DRAWINGS

[0030] The novel features of the invention are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present invention will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the invention are utilized, and the accompanying drawings of which:

[0031] **Figure 1** shows the mapped position of rDNA genes relative to the origin of replication in a Urinary Tract Infection (UTI) *Escherichia coli* isolates.

[0032] **Figure 2** shows circular representation of an example genome sequenced from an *E. coli* isolate from a UTI patient and the location of rRNA genes on the genome.

[0033] **Figure 3** illustrates the effect of a drug on DNA and RNA levels based on proximity to the origin of replication in susceptible cells.

[0034] **Figure 4** depicts the effects of no antibiotic, Antibiotic A, and antibiotic B on replication efficiency, replication fork stalls, and digital detection results for targets with different distances from the origin of replication from a single cell.

[0035] **Figure 5** depicts workflow of determining the resistance or susceptibility of a cell in a sample of urine to a drug according to an embodiment of the invention.

[0036] **Figures 6A, 6B, and 6C** show 3 different views of an integrated device comprising an incubation module, according to an embodiment of the invention.

[0037] **Figure 7** shows a photograph of blister packs, reagent packs, or other types of containers to be used with the integrated device, according to an embodiment of the invention.

[0038] **Figure 8** is a diagram of an operation module with one or more layers to be driven by a spring or motor, according to an embodiment of the invention.

[0039] **Figure 9** shows a flowchart for performing single–molecule and single-cell measurements to detect changes in individual cells from a heterogeneous population in response to drug treatment.

[0040] **Figure 10** depicts a flow chart for a point of care device that provides an assay and results from the assay on a sample in a single integrated device.

[0041] **Figure 11** shows the fold change in rDNA copy number for ciprofloxacin-susceptible *E. coli* treated with 2.5 ug/mL ciprofloxacin (right bar at each timepoint) and non-treated (left bar at each timepoint) samples measured at 10, 20, and 30 minutes as determined by qPCR.

[0042] **Figure 12A** shows the fold change in rDNA copy number for ciprofloxacin-susceptible *E.coli* treated with 2.5 ug/mL ciprofloxacin (right bar at each timepoint) and non-treated (left bar at each timepoint) samples measured at 0, 15, and 30 minutes as determined by qPCR. Figure 12B shows the same as determined by digital PCR on a SlipChip.

[0043] **Figure 13** shows the fold change in rDNA copy number for ciprofloxacin-susceptible *E.coli* treated with 0.75 ug/mL ciprofloxacin and non-treated samples measured at 15 minutes as determined by digital PCR on a SlipChip

[0044] **Figure 14A** shows the fold change in rDNA copy number for susceptible and resistant *E.coli* treated with tetracycline and non-treated samples measured at 30 minutes as determined by quantitative PCR. **Figure 14B** shows the fold change in rDNA copy number for susceptible and resistant *E.coli* treated with trimethoprim/sulfamethoxazoleand non-treated samples measured at 45 minutes as determined by quantitative PCR

[0045] **Figure 15** shows the relative distance of feoB, recA and DnaInhibitor protein gene from oriC in genomes of UTI *E. coli* isolates. The x axis represents each individual genome (organized arbitrarily).

[0046] **Figure 16** shows Cycle threshold (Ct) for quantification of DNA fragments copies number in UTI clinical isolates *E. coli* genomes, in susceptible and resistant of bacteria (A) after exposure ("treated") to a 2.5 ug/ml of ciprofloxacin for 15 minutes in BHI medium.

[0047] **Figure 17A** and **Figure 17B** show RNA quantification of FeoB and RecA genes expression in UTI clinical isolates susceptible (S) and resistant (R) to ciprofloxacin after 20 minutes of treatment with 2.5ug/mL of ciprofloxacin in BHI medium, versus 20 minutes of being in BHI medium without antibiotics.

[0048] **Figure 18** shows RNA quantification of FeoB and RecA genes expression in UTI clinical isolates susceptible and resistant to ciprofloxacin after 20 minutes of treatment with 10 ug/mL of ciprofloxacin in BHI medium, versus 20 minutes of being in BHI medium without antibiotics.

DETAILED DESCRIPTION OF THE INVENTION

[0049] Unless defined otherwise, all technical and scientific terms used herein have the same meaning as is commonly understood by one of skill in the art to which the claimed subject matter belongs. All patents, patent applications, published applications and publications, GENBANK sequences, websites and other published materials referred to throughout the entire disclosure herein, unless noted otherwise, are incorporated by reference in their entirety. In the event that there is a plurality of definitions for terms herein, those in this section prevail. Where reference is made to a URL or other such identifier or address, it is understood that such identifiers can change and particular information on the internet can come and go, but equivalent information is known and can be readily accessed, such as by searching the internet and/or appropriate databases. Reference thereto evidences the availability and public dissemination of such information.

[0050] As used herein, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. In this application, the use of the singular includes the plural unless specifically stated otherwise. As used herein, the use of "or" means "and/or" unless stated otherwise. Furthermore, use of the term "including" as well as other forms (e.g., "include", "includes", and "included") is not limiting.

[0051] As used herein, ranges and amounts can be expressed as "about" a particular value or range. About also includes the exact amount. Hence "about 10 degrees" means "about 10 degrees" and also "10 degrees." Generally, the term "about" can include an amount that would be expected to be within experimental error.

ASSAYS

[0052] The devices and methods described herein can be applied for assays for detection of drug susceptibility or resistance in an organism. The detection can be detection of a signal generated by an assay, for example, an assay to detect a nucleic acid or quantification of a nucleic acid associated with a resistance or susceptibility to a drug in an organism.

[0053] An assay can comprise conducting a reaction (e.g., amplification) on a nucleic acid from an organism exposed to a drug and comparing the results of the reaction (e.g., reaction outcome, positive or negative signal generation) to a reaction conducted on a nucleic acid from an organism that has not been exposed to the drug. This can reveal a susceptibility or a resistance of the organism to the drug.

[0054] In certain embodiments, the method comprises exposing a portion of a sample containing a microorganism to a drug. In some embodiments, the method further comprises extracting nucleic acid from the micro-organism. In some embodiments, the method comprises

performing a sequence-specific quantification of a nucleic acid from the microorganism. The quantification information is then used for determining or quantifying a resistance or susceptibility of a microorganism to a drug.

[0055] Assays can be conducted in a digital format, that is, assays can be conducted on a sample divided into partitions (i.e., analysis regions) such that some of the partitions provide no signal, while other partitions provide a signal. In some embodiments, the partitions contain one or zero target analytes (e.g., a target cell, a portion of a cell, or a target molecule, such as nucleic acid molecules or proteins). In some cases, some partitions can contain more than one target analyte. In some embodiments, the reaction efficiency is such that a threshold number of target analytes is required to achieve a positive signal. In these cases, the format is digital if some of the partitions contain a number of target analytes above the threshold, and some of the partitions contain a number of target analytes below the threshold. In some embodiments, the threshold can vary between partitions, such that some partitions with fewer target analytes generate a signal, while some partitions with more target analytes do not generate signal. In these cases, digital detection can still be performed based on a probabilistic threshold applied over a plurality of wells, as long as some partitions do not produce a signal, and some do.

[0056] In some cases, the majority of partitions contain one or zero target analytes. This digital or single molecule format can be used in conjunction with assays described herein, including identification, detection, genotyping, SNP detection, rare allele detection, and quantification of nucleic acids.

[0057] An assay can be conducted in less than or equal to about 600 minutes, 540 minutes, 480 minutes, 420 minutes, 360 minutes, 300 minutes, 240 minutes, 180 minutes, 120 minutes, 110 minutes, 100 minutes, 90 minutes, 80 minutes, 70 minutes, 60 minutes, 50 minutes, 40 minutes, 30 minutes, 20 minutes, 15 minutes, 10 minutes, 9 minutes, 8 minutes, 7 minutes, 6 minutes, 5 minutes, 4 minutes, 3 minutes, 2 minutes, or 1minute. An assay can have an accuracy of at least about 50%, 60%, 70%, 80%, 90%, 95%, 96%, 97%, 98%, 99%, 99.9%, or 99.99%. The rates of false positives can be below 10%, below 1%, below 0.1%, below 0.01%, below 0.001%, or below 0.0001%. The rates of false negatives can be below 10%, below 1%, below 0.1%, below 0.01%, below 0.001%, or below 0.0001%.

[0058] Assays can be used for detecting copy number variations (CNVs). CNVs are a form of structural variation, alterations of the DNA of a genome that changes the number of copies of one or more sections of the DNA. CNVs can correspond to relatively large regions of the genome that have been deleted or duplicated on certain chromosomes. Like other types of genetic variation, some CNVs have been associated with susceptibility or resistance to

disease. Gene copy number can be elevated in cancer cells. The methodology described herein also allows identifying genetic changes at chromosome level.

[0059] Antibiotic susceptibility testing method through measuring DNA replication (e.g. detecting CNV) is applicable to evaluation of response to antibiotics, and other agents impairing cell growth and genomic DNA replication in all the unicellular and multicellular organisms, including eukaryotes. The measurement of "relative chromosomal DNA replication rate" is a useful measurable parameter to distinguish between susceptible cells growing in the presence versus absence of a drug (e.g., an antibiotic) and resistant cells growing in the presence versus absence of a drug. In some embodiments, differences in these markers can be observed using the devices and methods disclosed herein within a time period that is shorter than the average time of division of a cell, enabling the detection of cellular replication in that cell earlier than methods of detection that are dependent on cell-division. Therefore, methods and devices described herein enable one to rapidly distinguish between cells that are drug resistant and drug susceptible. The methods and devices provided herein can also be applied to any drug-screening, including screening of human cells (such as e.g. in the monitoring of a tumor biopsy in response to treatment).

[0060] In one embodiment, copy number of rDNA within cells is measured after cells are incubated for short periods of time, with and without the presence of antibiotics, and the difference in the magnitude of this change is used to determine drug resistance and susceptibility of the cells. In some embodiments, the change in rDNA copy is determined using a nucleic acid amplification technique (such as for example qPCR or digital PCR or digital isothermal amplification), and the results used to determine resistance or susceptibility to the drug. In some embodiments, the method for determining drug susceptibility uses digital quantification. In some embodiments average DNA fragments copies originated from individual cells, are digitally quantified to measure proximal to origin/distant to origin selected gene rates.

[0061] In some embodiments, drug susceptibility testing is based on RNA, such as by comparing transcription levels. In some embodiments, drug susceptibility testing is based on RNA, such as by comparing transcription levels using digital quantification. In some embodiments, quantification strategies (such as e.g. NASBA, qRT-PCR, sequencing, nanostring, among others) can be appropriate. In some embodiments RNA from cells obtained from samples is quantified in a digital format. In some embodiments gene target expression levels in each individual cell are quantified through single cell measurements on a device, such as the devices described herein.

[0062] An assay can be used for quantitative detection of nucleic acids, such as recA mRNA. For example, a method can be used comprising the steps of taking a sample from the patient, accessing RNA in the sample or extracting RNA from the sample, using at least one RT- LAMP primer to reverse transcribe and amplify the mRNA in a qualitative and/or in a quantitative format, and testing for amplification to confirm presence of nucleic acids including but not limited to recA mRNA.

[0063] In some embodiments, relative RNA and/or DNA amplification is used. In some embodiments, relative RNA and/or DNA quantification is used. In some embodiments digital detection is used for RNA and/or DNA quantification. In some embodiments, multiple reliable RNA and / or DNA targets will be used simultaneously in multiplex format In some embodiments, RNA targets and/or their genes involved in the same physiological process or antibiotic response mechanism can be used.

[0064] In some embodiments, this invention could be applied to drug resistance testing of tumor cells. In some embodiments, this invention be applied to drug resistance testing of cancerous cells. The devices describe herein are applicable for use with a variety of sample types, including clinical sample types, (such as, for example, in-patient vs out-patient, pre-treated vs treatment-naïve), infection levels (such as, for example, negative vs positive vs contaminated), and sample storage/handling (such as, for example, fresh vs borate-preserved vs refrigerated).

[0065] In some embodiments, a slow growing microorganism's drug resistance is possible to assess through a combination of staining and genetic markers using the devices and methods described herein. In some embodiments, a combination of genetic markers of cell growth and genetic markers of antibiotic susceptibility can be used to determine the genetic antibiotic resistance of slow-growing cells.

[0066] The assays, reactions, and techniques described herein can be performed on any suitable platform, including but not limited to tubes, capillary tubes, droplets, microfluidic devices (e.g., SlipChip devices), wells, well plates, microplates, microfluidic wells, microfluidic droplets, emulsions, solid supports (e.g., beads or microarrays), microchips, or gels (e.g., 2D gels, 3D gels) and reactions inside gels including "polonies" as in polony PCR on surfaces and in gels.

[0067] In one embodiment, an assay to determine the resistance or susceptibility of a cell is performed as follows: Cells are pre-cultured (e.g. at 37 °C) to a desired density (e.g. 10^1 cells/mL, 10^2 cells/mL, 10^3 cells/mL, 10^4 cells/mL, 10^5 cells/mL, 10^6 cells/mL, 10^7 cells/mL, 10^8 cells/mL, or 10^9 cells/mL) in various matrices (e.g. a Bacto Brain-Heart Infusion broth (BHI), a

mix of BHI and pooled human urine, and/or whole human urine) before being diluted and incubated with or without a drug. In some embodiments, incubation is performed at a desired temperature (e.g. 37˚C) and then treated with a desired concentration of antibiotics. Cells can be incubated with and without antibiotics for a period of time (e.g. <10 minutes, 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes, 60 minutes, or >60 minutes) before an aliquot of the culture or the original test sample is used for nucleic acid extraction. Nucleic acids can be extracted using standard methods (such as e.g. a one-step DNA extraction buffer or a one-step RNA extraction buffer (e.g. available from Epicentre), among others). In some embodiments, following extraction, nucleic acids are quantified using nucleic acid amplification techniques, such as e.g. quantitative PCR or digital PCR. To quantify 23S genes in Enterobacter, the following 23S primers specific for Enterobacter can be used: TGCCGTAACTTCGGGAGAAGGCA; TCAAGGACCAGTGTTCAGTGTC; 428 bp product. (See, Matsuda K, Tsuji H, Asahara T, Kado Y, Nomoto K. Sensitive Quantitative Detection of Commensal Bacteria by rRNA-Targeted Reverse Transcription-PCR. *Applied and Environmental Microbiology*. 2007;73(1):32-39. doi:10.1128/AEM.01224-06). The *E. coli* genome contains seven distinct operons with minor sequence differences, such as (such as (rrnA, rrnB, rrnC, rrnD. rrnE, rrnG (aka rrnF) and rrnH. The term "rDNA," as used herein, refers to rRNA operons sequences. This term includes both coding sequences and non-coding sequences/spacers. In some embodiments, this invention takes advantage of rDNA to provide both most conservative sequences and variable enough sequences to design primers targeting specific species. A typical operon coding for rRNA (such as GenBank: J01695.2) codes for 5S, 16S and 23S rRNA as well as tRNA, and has spacers in between these sequences. When using rDNA genes as replication markers, primers either for the coding parts of the rRNA operons, or for the spacers between them, or both, can be selected.

Samples

[0068] Disclosed herein are methods, devices and systems related to analysis of samples. In some embodiments, methods of the invention comprises obtaining a sample from an organism. A sample can be obtained from a subject (e.g., a patient or a pet) and can include blood, feces, urine, saliva or other bodily fluid. The sample can be obtained by the patient or by a medical professional. Examples of medical professionals include, but are not limited to, physicians, emergency medical technicians, nurses, first responders, psychologists, medical physics personnel, nurse practitioners, surgeons, dentists, and any other medical professional. The sample can be obtained from any bodily fluid, for example, amniotic fluid, aqueous humor, bile, lymph, breast milk, interstitial fluid, blood, blood plasma, cerumen (earwax), Cowper's fluid

(pre-ejaculatory fluid), chyle, chyme, female ejaculate, menses, mucus, saliva, urine, vomit, tears, vaginal lubrication, sweat, serum, semen, sebum, pus, pleural fluid, cerebrospinal fluid, synovial fluid, intracellular fluid, and vitreous humour. In an example, the sample is obtained by a blood draw, where the medical professional draws blood from a subject, such as by a syringe.

[0069] Samples can be collected in a sample collection container. In some embodiments the sample collection container is coded with information that can be detected. For example a detector can recognize a barcode. The barcode can have information about where a sample was collected or from which individual a sample was collected. A detector can take this information and use it to process or transmit data generated regarding a sample. For example a camera-phone can take a photo of a sample collection container. The camera-phone can recognize a barcode on the container which identifies a patient. The camera-phone can then link date generated regarding the sample to the patient from which the sample was obtained. The linked data can then be transmitted to the patient or to the patient's physician. In some embodiments a single image is generated of the sample collection container and a sample analysis unit.

[0070] Food samples can also be analyzed. Samples can be any composition potentially comprising a target organism. Sources of samples include, but are not limited to, geothermal and hydrothermal fields, acidic soils, sulfotara and boiling mud pots, pools, hot-springs and geysers where the enzymes are neutral to alkaline, marine actinomycetes, metazoan, endo and ectosymbionts, tropical soil, temperate soil, arid soil, compost piles, manure piles, marine sediments, freshwater sediments, water concentrates, hypersaline and super-cooled sea ice, arctic tundra, Sargasso sea, open ocean pelagic, marine snow, microbial mats (such as whale falls, springs and hydrothermal vents), insect and nematode gut microbial communities, plant endophytes, epiphytic water samples, industrial sites and ex situ enrichments. Additionally, a sample can be isolated from eukaryotes, prokaryotes, myxobacteria (epothilone), air, water, sediment, soil or rock, a plant sample, a food sample, a gut sample, a salivary sample, a blood sample, a sweat sample, a urine sample, a spinal fluid sample, a tissue sample, a vaginal swab, a stool sample, an amniotic fluid sample, a fingerprint, aerosols, including aerosols produced by coughing, skin samples, tissues, including tissue from biopsies, and/or a buccal mouthwash sample. Other sample types include samples for clinical testing (such as, for example, in-patient vs out-patient, pre-treated vs treatment-naïve), infection level testing (such as, for example, negative vs positive vs contaminated), and storage / handling testing (such as, for example, fresh vs borate-preserved vs refrigerated).

[0071] Samples can comprise organisms. Samples can comprise microorganisms. The number of microorganisms in a sample can be less than 10, less than 100, less than 1,000, less than 10^4, less than 10^5, or less than 10^6. In some embodiments, the sample is a processed sample (e.g., concentrated, filtered, etc.).

[0072] Samples can comprise target analytes from the organisms. Target analytes can comprise, for example, cells, portions of cells, polypeptides, or nucleic acids. Nucleic acids can be cell-free nucleic acids. Nucleic acids can be isolated from cells. Nucleic acids can be single or double stranded. Target analytes can comprise DNA or RNA. In some cases, the RNA is tRNA, mRNA, rRNA, trRNA, snRNA, snoRNA, smY, scaRNA, gRNA, RNase P, RNase MRP, aRNA, crRNA, incRNA, miRNA, piRNA, siRNA, tasi RNA, rasiRNA, 7SK, vRNA or any combination thereof. The DNA can be ssDNA, dsDNA, cDNA, or any combination thereof. In some cases, the DNA comprises a gene or a gene fragment. The gene or gene fragment can comprise a mutation. The mutation can comprise point mutations, insertions, deletions, amplifications, translocations, inversions, copy number variations, and/or other mutations. In some cases, the DNA comprises a non-coding region. The noncoding region can comprise functional sequences, regulatory elements, intrans, exons, pseudogenes, repeat sequences, transposons, viral elements, telomeres, genetic switches, transcription factor sites, operators, enhancers, silencers, promoters, insulators, and/or other regions. In some cases, the DNA comprises cDNA. In some cases, the DNA is from bacteria or viruses. In some cases, the DNA is collected from a cell. In some examples, the DNA is intracellular. In some cases, the DNA is extracellular.

[0073] Target analytes can comprise RNA. In some cases, the RNA comprises mRNA. In some cases, the RNA comprises noncoding RNA (ncRNA). The noncoding RNA can comprise transfer RNA (tRNA), ribosomal RNA (rRNA), transfer-messenger RNA (tmRNA), small nucleolar RNA (snoRNA), microRNA (miRNA), small interlering RNA (siRNA), small nuclear RNA (snRNA), piwi-interacting RNA (piRNA), long ncRNA (lncRNA), and/or other types of ncRNA. In some cases, the RNA is from bacteria or viruses. In some cases, the RNA is collected from a cell. In some examples, the RNA is intracellular. In some cases, the RNA is extracellular.

[0074] The terms "nucleic acid" and "nucleic acid molecule" as used interchangeably herein, refer to a molecule comprised of nucleotides, i.e., ribonucleotides, deoxyribonucleotides, or both. The term includes monomers and polymers of ribonucleotides and deoxyribonucleotides, with the ribonucleotide and/or deoxyribonucleotides being connected together, in the case of the polymers, via 5′ to 3′ linkages. However, linkages can include any of the linkages known in the nucleic acid synthesis art including, for example, nucleic acids comprising 5′ to 2′ linkages. The nucleotides used in the nucleic acid molecule can be naturally occurring or can be synthetically

produced analogues that are capable of forming base-pair relationships with naturally occurring base pairs. Examples of non-naturally occurring bases that are capable of forming base-pairing relationships include, but are not limited to, aza and deaza pyrimidine analogues, aza and deaza purine analogues, and other heterocyclic base analogues, wherein one or more of the carbon and nitrogen atoms of the purine and pyrimidine rings have been substituted by heteroatoms, e.g., oxygen, sulfur, selenium, phosphorus, and the like. Nucleic acids can be detected from a sample.

[0075] The term "oligonucleotide" as used herein refers to a nucleic acid molecule comprising multiple nucleotides. An oligonucleotide can comprise about 2 to about 300 nucleotides.

[0076] Target analytes can comprise genetic markers of cell growth and genetic markers of antibiotic susceptibility. Target analytes can include markers for drug resistance or susceptibility to tumor cells. Target analytes can include markers for oxidative stress that can be detected with, e.g., oxidation–sensitive dyes to assay cell viability in response to treatment with a drug. In some embodiments, target analytes comprise polypeptides or proteins. In some embodiments, target analytes include cell membrane or cell membrane associated molecules, wherein a change in the cell membrane or cell membrane associated molecule is linked to the response of an organism to a drug.

Organisms

[0077] The term "organism" refers to any organisms or microorganism, including bacteria, yeast, fungi, viruses, protists (protozoan, micro-algae), archaebacteria, plants and eukaryotes. Eukaryotes can be a single-celled eukaryotic cell. Bacteria include gram-positive and gram-negative bacteria. The term "organism" refers to living matter and viruses comprising nucleic acid that can be detected and identified by the methods of the invention. Organisms include, but are not limited to, bacteria, archaea, prokaryotes, eukaryotes, viruses, protozoa, mycoplasma, fungi, plants and nematodes. Different organisms can be different strains, different varieties, different species, different genera, different families, different orders, different classes, different phyla, and/or different kingdoms. Organisms can be isolated from environmental sources including soil extracts, marine sediments, freshwater sediments, hot springs, ice shelves, extraterrestrial samples, crevices of rocks, clouds, attached to particulates from aqueous environments, and can be involved in symbiotic relationships with multicellular organisms. Examples of such organisms include, but are not limited to *Streptomyces* species and uncharacterized/unknown species from natural sources. Organisms can include genetically engineered organisms or genetically modified organisms. Organisms can include transgenic plants. Organisms can include genetically modified crops. Any organism can be genetically

modified. Examples of organisms which can be genetically modified include plantains, yams, sorghum, sweet potatoes, soybeans, cassava, potatoes, rice, wheat, or corn.

[0078] Organisms can include bacterial pathogens such as: *Aeromonas hydrophila* and other species (spp.); *Bacillus anthracis; Bacillus cereus; Botulinum neurotoxin* producing species of *Clostridium; Brucella abortus; Brucella melitensis; Brucella suis; Burkholderia mallei* (formally *Pseudomonas mallei*); *Burkholderia pseudomallei* (formerly *Pseudomonas pseudomallei*); *Campylobacter jejuni; Chlamydia psittaci; Clostridium botulinum; Clostridium botulinum; Clostridium perfringens; Coccidioides immitis; Coccidioides posadasii; Cowdria ruminantium* (Heartwater); *Coxiella burnetii*; Enterovirulent *Escherichia* co//group (EEC Group) such as *Escherichia coli*—enterotoxigenic (ETEC), *Escherichia coli*—enteropathogenic (EPEC), *Escherichia coli*—O157:H7 enterohemorrhagic (EHEC), and *Escherichia coli*—enteroinvasive (EIEC); *Ehrlichia* spp. such as *Ehrlichia chaffeensis; Francisella tularensis; Legionella pneumophilia; Liberobacter africanus; Liberobacter asiaticus; Listeria monocytogenes*; miscellaneous enterics such as *Klebsiella, Enterobacter, Proteus, Citrobacter, Aerobacter, Providencia*, and *Serratia; Mycobacterium bovis; Mycobacterium tuberculosis; Mycoplasma capricolum; Mycoplasma mycoides* ssp *mycoides; Peronosclerospora philippinensis; Phakopsora pachyrhizi; Plesiomonas shigelloides; Ralstonia solanacearum* race 3, biovar 2; *Rickettsia prowazekii; Rickettsia rickettsii; Salmonella* spp.; *Schlerophthora rayssiae* varzeae; *Shigella* spp.; *Staphylococcus aureus; Streptococcus; Synchytrium endobioticum; Vibrio cholerae* non-O1; *Vibrio cholerae* O1; *Vibrio parahaemolyticus* and other *Vibrios; Vibrio vulnificus; Xanthomonas oryzae; Xylella fastidiosa* (citrus variegated chlorosis strain); *Yersinia enterocolitica* and *Yersinia pseudotuberculosis*; and *Yersinia pestis*.

[0079] A non-limiting list of pathogens with known drug-resistance properties that can be detected and analyzed with the methods and devices disclosed herein include *Clostridium difficile*, carbapenem-resistant *Enterobacteriaceae* (CRE), drug-resistant *Neisseria gonorrhoeae*, multidrug-resistant *Acinetobacter*, drug-resistant *Campylobacter*, fluconazole-resistant *Candida* (a fungus), extended spectrum β-lactamase producing *Enterobacteriaceae* (ESBLs), vancomycin-resistant *Enterococcus* (VRE), multidrug-resistant *Pseudomonas aeruginosa*, drug-resistant Non-typhoidal *Salmonella*, drug-resistant *Salmonella Typhi*, drug-resistant *Shigella*, methicillin-resistant *Staphylococcus aureus* (MRSA), drug-resistant *Streptococcus pneumoniae*, drug-resistant *M. tuberculosis*, vancomycin-resistant *Staphylococcus aureus* (VRSA), erythromycin-resistant Group A *Streptococcus*, and clindamycin-resistant Group B *Streptococcus*.

Antimicrobial / Antibiotic Compounds

[0080] Organisms are incubated with a drug to determine a response to indicate their resistance or susceptibility to the drug. In some embodiments, the drug includes compounds for the treatment of a tumor. In some embodiments, the drug is an antibiotic compound or an antimicrobial compound. As used herein, the term antimicrobial is meant to include any substance of natural, semisynthetic or synthetic origin that is used to kill or inhibit the growth of a microorganism. In preferred embodiments, antimicrobials do not harm a host of the microorganism. As used herein, the term "antimicrobial" and the term "antibiotic" are interchangeable. Examples of antimicrobial or antibiotic compounds include, but are not limited to: Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Tobramycin, Paromomycin, Streptomycin, Spectinomycin(Bs), Geldanamycin, Herbimycin, Rifaximin, Loracarbef, Ertapenem, Doripenem, Imipenem/Cilastatin, Meropenem, Cefadroxil, Cefazolin, Cefalotin /Cefalothin, Cefalexin, Cefaclor, Cefamandole, Cefoxitin, Cefprozil, Cefuroxime, Cefixime , Cefdinir, Cefditoren, Cefoperazone , Cefotaxime, Cefpodoxime, Ceftazidime , Ceftibuten, Ceftizoxime, Ceftriaxone , Cefepime, Ceftaroline fosamil, Ceftobiprole, Teicoplanin, Vancomycin, Telavancin, Dalbavancin, Oritavancin, Clindamycin, Lincomycin, Daptomycin, Azithromycin, Clarithromycin, Dirithromycin, Erythromycin, Roxithromycin, Troleandomycin, Telithromycin, Spiramycin, Aztreonam, Furazolidone, Nitrofurantoin(Bs), Linezolid, Posizolid, Radezolid, Torezolid, Amoxicillin, Ampicillin, Azlocillin, Carbenicillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Mezlocillin, Methicillin, Nafcillin, Oxacillin, Penicillin G, Penicillin V, Piperacillin, Penicillin G, Temocillin, Ticarcillin, Amoxicillin/clavulanate, Ampicillin/sulbactam, Piperacillin/tazobactam, Ticarcillin/clavulanate, Bacitracin, Colistin, Polymyxin B, Ciprofloxacin, Enoxacin, Gatifloxacin, Gemifloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Trovafloxacin, Grepafloxacin, Sparfloxacin, Temafloxacin, Mafenide, Sulfacetamide, Sulfadiazine, Silver sulfadiazine, Sulfadimethoxine, Sulfamethizole, Sulfamethoxazole, Sulfanilimide (archaic), Sulfasalazine, Sulfisoxazole, Trimethoprim-Sulfamethoxazole (Co-trimoxazole) (TMP-SMX), Sulfonamidochrysoidine (archaic), Demeclocycline, Doxycycline, Minocycline, Oxytetracycline, Tetracycline, Clofazimine, Dapsone, Capreomycin, Cycloserine, Ethambutol(Bs), Ethionamide, Isoniazid, Pyrazinamide, Rifampicin (Rifampin in US), Rifabutin, Rifapentine, Streptomycin, Arsphenamine, Chloramphenicol(Bs), Fosfomycin, Fusidic acid, Metronidazole, Mupirocin, Platensimycin, Quinupristin/Dalfopristin, Thiamphenicol, Tigecycline(Bs), Tinidazole, and Trimethoprim(Bs).

[0081] Different antibiotics or antimicrobials may have different mechanisms of action and can affect replication propagation and replication re-initiation differently. Some bacteriostatic antibiotics (such as aminoglycosides, cephalosporins, tetracyclines, sulfonamides, and macrolides) inhibit protein synthesis, while bactericidal antibiotics can act on the cell wall (eg, vancomycin and β-lactams),or bacterial DNA (eg, fluoroquinolones). Some antibiotics, such as trimethoprim and sulfamethoxazole interfere with a metabolic pathways and affect DNA replication indirectly, and some (nitrofurantoin) affect multiple process in a cell, including DNA replication.

[0082] In some embodiments, treatment conditions will be adjusted to intensify an effect of antibiotics on DNA replication for every antibiotics group. In some embodiments, treatment conditions will be adjusted to speed up observation of an effect of antibiotics on DNA replication for every antibiotics group. In some embodiments, the method described in this disclosure tests for antibiotic susceptibility though relative gene quantification.

Targets / Target Analytes

[0083] In some embodiments the methods and devices provided herein target analytes (e.g., markers) indicative of growth of the microorganism in response to the presence or absence of an antimicrobial. In some embodiments, the change in expression and in copy numbers of the of genes close to OriC (such as for example competence genes) is measured. Our methods and devices enable rapid and sensitive quantification of total gene copy number proximal to ori for each bacterial species of interest.

[0084] Slowly growing cells can replicate their chromosomes more slowly, allowing the differentiation between cells effected by the antibiotic, and those killed by it. In addition, cells with impaired metabolism can generate more or less of specific RNA targets. Selection and quantification of these targets is described herein.

[0085] In some embodiments, the target analyte of interest is DNA. In some embodiments, DNA regions, such as those represented in multiple copies in published bacterial genomes, are selected as targets (such as rDNA). In some embodiments, more than one target (such as a DNA fragment or gene) is selected for simultaneous quantification; these targets can be located close to the origin of replication and/or can involve both directions of replication. In some embodiments, more than one target (such as a DNA fragment or gene) is selected for simultaneous quantification; one or more of these targets is located close to the origin of replication and one or more targets is far from the origin of replication. By measuring the ratio of these targets at certain times (in some cases, before replication is complete), replication can be detected rapidly in some embodiments without the need to measure the number of cells

independently. This becomes valuable when very few cells are present in the sample, and or when very few cells are analyzed, and or when the accuracy with which the number of cells present in each part of a split sample is limited by Poisson statistics.

[0086] In some embodiments, detecting gene duplications due to antimicrobial treatment would allow detection of susceptible strains. In some embodiments, targets for a gene dosage approach can include Ori or genes positioned in proximity to OriC (such as e.g. within 50 kb, within 100 kb, , within 150 kb, within 200 kb, and/or within 300 kb from OriC. In some embodiments the DNA/gene dosage in drug-susceptible cells will increase rather than decrease.

[0087] In some embodiments, DNA targets represented in single or in multiple copies in published bacterial genomes are selected as targets (such as e.g. rDNA, feoB gene, rpoB gene, competence genes, among others). In some embodiments, more than one target (such as a DNA fragment or gene) is selected for simultaneous quantification; these can be preferentially located close to the origin of replication in both directions In some embodiments, the target of interest is ribosomal DNA, rDNA, sequence. Ribosomal gene sequences, while being well conserved among bacteria, can be used as a target for identification and/or group identification, such as by selecting specific probes and/or primers

[0088] In some rapidly growing bacterial species, it has been shown that ribosomal genes (and many genes associated with transcription and translation) tend to be preferentially located close to the origin of replication, suggesting the presence of evolutionary pressure, which is shown to be relevant to genome stability (Etienne Couturier, Eduardo P. C. Rocha. Replication-associated gene dosage effects shape the genomes of fast-growing bacteria but only for transcription and translation genes, 2006, Molecular microbiology, Volume 59, Issue 5, Pages 1506–1518 , DOI: 10.1111/j.1365-2958.2006.05046.) . These genes can be used as targets for quantification of replication, thus enabling the detection of organisms exposed to growth conditions over short time periods, such as a time period that is shorter than the cell replication period. Using these genes as targets for digital quantification, even incomplete DNA replication (that happens before cells divide) can be detected.

[0089] For example, in a subset of 11 available genome-sequenced *E. coli* strains linked to UTI infections the position of rDNA genes relative to the origin of replication were mapped. **Figure 1** shows the relative distance of rDNA from oriC in UTI *E. coli* isolates. Red dots (top and bottom row) illustrate the genome size by showing the distance from OriC to termination of replication in a circular chromosome for both replichores. The rare examples of having ribosomal genes at different distribution belong to the genomes of bacteria which underwent artificial selection in laboratory conditions, such as the genome fifth from the left on the graph,

which is not a UTI clinical isolate but *E. coli* from UTI patient gut selected for nitrofurantoin resistance (GenBank: CP007265.1). These show a trend of rDNA genes being clustered near the origin of replication.

[0090] Another example of rDNA clustering near the origin of replication is shown in Figure 2, showing a circular representation of an example genome sequenced from a UTI patient. Ribosomal RNA coding regions of the genome are marked with red. The origin of replication (OriC) is marked at the top of Figure 2. rDNA is preferentially clustered near the origin of replication.

[0091] In some embodiments, the method disclosed herein takes advantage of the fact that in at least some cells of interest, there can be a specific time order of chromosomal splitting. For example, in *E. coli*, chromosome dynamics determines splitting of the certain parts of the chromosomes in a specific time order. In some embodiments the quantification of gene targets that are replicated earlier will allow higher resolution or faster determination of resistant versus susceptible bacteria. In addition, the origin and terminus of replication divide the genome into replichores, having different strands as leading strands. In some embodiments, target genes on the leading and lagging strands can be selectively targeted to measure their different transcription levels when replication is impaired versus non-impaired.

[0092] In some embodiments, quantification of DNA fragments and genes located in proximity to the origin of replication (e.g., the origin itself or proximate genes such as gln udhA dnaB or some rDNA) is performed. In some embodiments, the difference in copy number per cell between antibiotic treated and non-treated cells can be detected by the methods and devices described herein (e.g., digital amplification).

[0093] In some embodiments, DNA targets located in proximity to the termination site of replication (ter) are quantified. Because they are the last parts of the genome to replicate, these regions tend to show lesser variation in copy number based on cell growth state, or the presence of antibiotics. In some embodiments, measurement of replication of DNA targets near ter can be used to standardize data obtained from other targets. For example, in some embodiments, at short drug exposure times, not sufficient to replicate the entire genome, these ter-proximal regions can be used as a measure of the loaded cell numbers while regions closer to ori can be used to access DNA replication and resistance to the drug; this is useful for example when the number of cells loaded is low (for example, less than 100 or less than 10) and therefore difficult to measure. In some embodiments, variations in copy number of these regions with and/or without treatment with a drug are used to distinguish resistant from susceptible bacteria. Drugs, such as antimicrobials, that cause the replication fork to fail can further amplify this effect. In some

embodiments, replication of a target nucleic acid sequence can be used to determine the resistance profile of genes in cells (such as bacteria) incubated in the presence of a drug (such as an antibiotic).

[0094] In some embodiments, the relative quantification of these targets with and/or without treatment with antibiotics will be used to determine antibiotic susceptibility. This can enable the very rapid differentiation between resistant and susceptible strains.

[0095] In some embodiments, such as when a cell's new replication fork starts from origin of replication, other older replication forks can continue replication independently on re-initiation event. (Fossum, S., E. Crooke, et al. (2007). "Organization of sister origins and replisomes during multifork DNA replication in *Escherichia coli*." The EMBO Journal 26(21): 4514-4522. Mohan C. Joshi, et al, 2010, "*Escherichia coli* sister chromosome separation includes an abrupt global transition with concomitant release of late-splitting intersister snaps", PNAS, vol. 108 no. 7,2765–2770). Thus, in some embodiments, a gene target used for drug resistance/susceptibility testing can be located distantly from the origin (e.g. oriC would still be replicated fast enough for determination of drug resistance/susceptibility).

[0096] By using targets close to the origin of replication, the relative copies number of those genes in total genetic material in the cells can be quantified to identify susceptible and resistant cells under antibiotic treatment earlier prior to completion of DNA replication or prior to completion of cell division. In some embodiments, the copy number of the target genes of the susceptible organism treated with a drug is less than the copy number of the target genes of the resistant organism or the non-treated organism as exposure to the drug in a susceptible organism decreases replication. However, some antibiotics cause certain regions of chromosomal DNA to increase in copy number in susceptible cells. Figure 3 illustrates the effect of susceptible cells to antibiotic dosing, specifically the relative DNA copy number in *E. coli* after treatment with trimethoprim as compared to the distance from the origin of replication (oriC), shown as the line that intersects with 0 at both ter regions. (Slager, J., M. Kjos, et al. (2014). "Antibiotic-Induced Replication Stress Triggers Bacterial Competence by Increasing Gene Dosage near the Origin." Cell 157(2): 395-406). Figure 3 shows that DNA copy number peaks close to the origin of replication after exposure to trimethoprim as compared to baseline level of zero.

[0097] Many antibiotics (such as quinolones) induce a replication fork stalling. As a direct consequence of replication fork stalling while DNA replication initiation continues, all antibiotics targeting DNA replication up-regulate origin-proximal genes copy number and induce a global changes in transcription in bacteria. This produces a result contrary to those antibiotics that slow DNA replication in susceptible organisms treated with a drug, providing an increase in

the number of positive results. Predicted results of the digital assay described herein for antibiotics that slow growth, and those that induce a replication fork stalling are shown in Figure 4. In Figure 4, "Antibiotic A" represents antimicrobials that result in a reduced replication efficiency in susceptible cells, thus lowering the number of gene copy near the origin of replication compared to resistant cells. "Antibiotic B" represents antimicrobials that result in a replication fork stalling in a cell, resulting in an increase in initiation of new replication near the origin of replication, thus increasing the copy number of genes near the origin of replication in susceptible cells as compared to resistant cells.

[0098] "Antibiotic A," as represented in Figure 4, includes bacteriostatic antibiotics (such as aminoglycosides, cephalosporins, tetracyclines, sulfonamides, and macrolides) that inhibit protein synthesis, and bactericidal antibiotics that act on the cell wall (eg, vancomycin and β-lactams). For these antibiotics that slow DNA replication, in some embodiments, the antibiotic exposure is altered to induce oxidation stress in the cells, e.g., by controlling concentrations of Ferrous iron, pH and Magnesium ions, and ensuring aerobic conditions.

[0099] "Antibiotic B," as represented in Figure 4, includes antibiotics that cause certain regions of chromosomal DNA to increase in copy number in susceptible cells. For example, oriC-ter ratios are known to be affected by some antibiotics treatment (e.g., HPUra, hydroxyurea, trimethoprim, ciprofloxacin, and MMC) in *B. cereus*, *S. aureus*, and *E. coli* and *S. pneumoniae*. These antibiotics are known to impair chromosome replication, as trimethoprim affects DNA replication indirectly. Nitrofurantoin directly impairs many cell processes including replication. 6(p-Hydroxyphenylazo)-uracil (HPUra) is a selective inhibitor of gram-positive bacteria DNA. Hydroxycarbamide decreases the production of deoxyribonucleotides. Ciprofloxacin is a fluoroquinolone that stalls the replication fork and causes DNA fragmentation due to its interactions with DNA gyrase and topoisomerase. (Slager, J., M. Kjos, et al. (2014). "Antibiotic-Induced Replication Stress Triggers Bacterial Competence by Increasing Gene Dosage near the Origin." Cell 157(2): 395-406, and Tamayo M, Santiso R, Gosalvez J, Bou G, Fernández JL. Rapid assessment of the effect of ciprofloxacin on chromosomal DNA from *Escherichia coli* using an in situ DNA fragmentation assay. BMC Microbiology. 2009;9:69).

[00100] The resistance or susceptibility of a call to either type of antibiotic can be detected using the methods and devices described herein. Furthermore, digital PCR will provide a different result depending upon whether or not the genes copied before the replication fork stall are fragmented from the chromosomal or plasmid DNA. If they are not fragmented, they cannot separate into distinct wells, thus providing an additional level of resolution to characterize the cells. The DNA fragments liberated from the nucleoid under different antibiotics treatment are

estimated to be of 50 to 100 kb size (Tamayo M, Santiso R, Gosalvez J, Bou G, Fernández JL. Rapid assessment of the effect of ciprofloxacin on chromosomal DNA from *Escherichia coli* using an in situ DNA fragmentation assay. BMC Microbiology. 2009;9:69) similar to the presumed size of the DNA loops of the nucleoid and to the prominent DNA gyrase-mediated cleavage sites (Snyder M, Drlica K. DNA gyrase on the bacterial chromosome: DNA cleavage induced by oxolinic acid. J Mol Biol. 1979;131(2):287–302, Condemine, Smith CL. Transcription regulates oxolinic acid-induced DNA gyrase cleavage at specific sites on the *E. coli* chromosome. Nucleic Acids Res. 1990;18(24):7389–7396, and Hsu Y-H, Chung M-W, Li T-K. Distribution of gyrase and topoisomerase IV on bacterial nucleoid: implications for nucleoid organization. Nucleic Acids Res. 2006;34(10):3128–3138.).

[00101] In some embodiments, the method disclosed herein is used to detect genes and gene fragments that are locally amplified in short timeframes to distinguish drug resistant from drug susceptible cells. In some embodiments, the method makes use of the fact that some antibiotics cause the preferential amplification of DNA fragments located near origin of replication , such as some competence genes and the origin of replication itself. In some embodiments DNA fragments or genes located within 50-200 kb from the origin of replication in both replichores will be quantified in digital format from a small number of cells to determine their drug susceptibility. In some embodiments, the method makes use of the fact that some antibiotics cause accumulation of DNA fragments. This has been shown to be caused by antibiotics that cause the replication fork to stall. This can be analyzed through quantification of genes inside the accumulated region, outside the accumulated region, or a ratio between both.

[00102] In some embodiments, the change in the relative replication rates for selected positions on the chromosome can be detected in susceptible isolates after less than 20 minutes of exposure to a drug. In some embodiments, the difference in in the relative replication rates for selected positions on the chromosome can be discernable only after a certain time point, which can differ for different antibiotics types, organisms, cells and/or drugs. In some embodiments, the change in relative replication rates for selected positions on the bacterial chromosome can be detected in susceptible isolates after less than 5, 10, 15, 20, 25, 30, 45, 60, 90, 120, 150, or 180 minutes of exposure to a drug.

[00103] In some embodiments shift up protocol (for example one that adds nutrients and signaling molecules to stimulate growth and gene expression and DNA replication), can be used to ensure some control over the time of re-initiation of replication.

[00104] In some embodiments, changes in gene expression are used as markers of antimicrobial susceptibility or resistance in an organism. In some embodiments, drug susceptibility testing is

based on RNA, such as by comparing transcription levels. In some embodiments, drug susceptibility testing is based on RNA, such as by comparing transcription levels using digital quantification. In some embodiments, markers associated with a resistance or susceptibility in response to an antimicrobial can be measured.

[00105] In some embodiments, genes differently regulated in response to a drug or antibiotic can be used. The genes can include for example, the recA and LexA (Barczak, *et al.*, 2012, Proceedings of the National Academy of Sciences, 6217- 6222) genes induced by OxyR and SoxS in response to antibiotic-induced oxidative stress (Dwyer, *et al.*, 2014, Proceedings of the National Academy of Sciences, E2100-E2109).

[00106] In susceptible bacteria, many RNA targets have been shown to be either upregulated or downregulated in response to drug exposure over a short time. Many such genes also show significant changes in expression level as a response to variations in culture conditions and from isolate to isolate. Some genes expression (e.g., recA, involved in the SOS response) are up-regulated in several species in response to multiple antibiotics, whereas other transcriptional changes are organism and/or mechanism specific.

[00107] Changes in RNA targets RNA levels (through qPCR, micro arrays, RNAseq) can be difficult to detect using existing methods after a short time of drug / antibiotic treatment in bulk (such as by routine reverse transcription and isothermal or PCR amplification) due to the presence of non-specific nucleic acids from the host's cells or from other microorganisms, as well as due to the presence of inhibitors from the clinical sample, and due to the short time of treatment. In some embodiments, binary, digital and multivolume digital formats can be used to isolate targets in volumes where local concentration is high, enabling the fast and reliable quantification even in such samples.

[00108] In some embodiments, DNA targets with a higher copy number present in cells will be used to enable the detection of resistance profiles even in very low numbers of cells (such as in blood or cerebral spinal fluid). For example, while in genome of most of the *E. coli* strains and isolates there are ~7 copies of rDNA, due to the presence of at least two, but potentially numerous replication forks, one could expect to have , in some cases, from 12 to 35 rDNA copies per cell, depending on its growth rate (*E. coli* and *Salmonella*, cellular and molecular biology. Frederic C. Neidhardt, editor in Chief. v2. ASM press, Washington DC. 1996). In some embodiments, using genes with higher copy number in the cell will enable the increased statistical resolution of quantification to determine antibiotic resistance when very low numbers of cells are present. In some embodiments, this is quantification is performed by amplification. In some embodiments, this quantification makes use of digital amplification methods.

[00109] In some embodiments, additional targets are evaluated from publically available studies such as those published by the Broad Institute. In some embodiments, targets for analysis are generated from analysis of DNA/RNA-seq data. In some embodiments, quantification strategies (such as e.g. NASBA, qRT-PCR, sequencing, nanostring, among others) can be used. In some embodiments RNA from cells obtained from samples in a digital format is quantified. In some embodiments gene target expression levels in each individual cell is quantified through single cell measurements.

[00110] An exemplary list of RNA targets that can be used for multiplexed and/or individual use for evaluating a cells' response to a drug (e.g., ciprofloxacin) are provided in Table 1

Table 1

Gene	**Response to Ciprofloxacin**
EcHS_A0999_dmsA_anaerobic_dimethyl_sulfoxide_reductase,_A_subunit	Same or Downregulated
EcHS_A1247_pepT_peptidase_T	Same or Downregulated
EcHS_A1333_narK_nitrite_extrusion_protein_1	Same or Downregulated
EcHS_A1334_narG_nitrate_reductase,_alpha_subunit	Same or Downregulated
EcHS_A1335_narH_nitrate_reductase,_beta_subunit	Same or Downregulated
EcHS_A1557_fdnG_formate_dehydrogenase,_nitrate_inducible,_alpha_subunit,_selenocysteine-containing	Same or Downregulated
EcHS_A1755_-_hypothetical_protein	Same or Downregulated
EcHS_A1987_flhC_transcriptional_activator_FlhC	Same or Downregulated
EcHS_A1988_flhD_transcriptional_activator_FlhD	Same or Downregulated
EcHS_A2346_napF_ferredoxin-type_protein	Same or Downregulated
EcHS_A2342_napH_quinol_dehydrogenase_membrane_component	Significantly Downregulated
EcHS_A2343_napG_quinol_dehydrogenase_periplasmic_component	Significantly Downregulated
EcHS_A2380_glpT_sn-glycerol-3-phosphate_transporter	Significantly Downregulated
EcHS_A2381_-_hypothetical_protein	Significantly Downregulated
EcHS_A2382_glpA_sn-glycerol-3-phosphate_dehydrogenase_subunit_A	Significantly Downregulated

EcHS_A2597_aegA1_oxidoreductase_Fe-S_binding_subunit	Significantly Downregulated
EcHS_A3117_ansB_L-asparaginase_II	Significantly Downregulated
EcHS_A3561_nirB_nitrite_reductase_[NAD(P)H],_large_subunit	Significantly Downregulated
EcHS_A3562_nirD_nitrite_reductase_small_subunit	Significantly Downregulated
EcHS_A3605_feoA_ferrous_iron_transport_protein_A	Significantly Downregulated
EcHS_A3606_feoB_ferrous_iron_transport_protein_B	Significantly Downregulated
EcHS_A3607_-_hypothetical_protein	Significantly Downregulated
EcHS_A3616_malT_transcriptional_regulator_MalT	Significantly Downregulated
EcHS_A4395_frdD_fumarate_reductase_subunit_D	Significantly Downregulated
EcHS_A1940_-_DNA_damage-inducible_protein_YebG	Significantly Upregulated
EcHS_A2835_recA_recombinase_A	Significantly Upregulated
EcHS_A4283_lexA_LexA_repressor	Significantly Upregulated
EcHS_A4284_dinF_DNA-damage-inducible_SOS_response_protein	Significantly Upregulated
EcHS_A4300_uvrA_excinuclease_ABC_subunit_A	Significantly Upregulated
EcHS_A2341_napB_citrate_reductase_cytochrome_c-type_subunit	Requires Evaluation
EcHS_A2344_napA_nitrate_reductase_catalytic_subunit	Requires Evaluation
EcHS_A3610_gntX_gluconate_periplasmic_binding_protein	Requires Evaluation
EcHS_A3612_-_hypothetical_protein	Requires Evaluation
EcHS_A3821_secB_preprotein_translocase_subunit_SecB	Requires Evaluation
EcHS_A4404_rsgA_ribosome-associated_GTPase	Requires Evaluation
EcHS_A4424_-_23S_rRNA_(guanosine-2'-O-)-methyltransferase	Requires Evaluation

23S rRNA	Requires Evaluation
EcHS_A3544_-_hypothetical_protein	Requires Evaluation
EcHS_A0467_phoB_transcriptional_regulator_PhoB	Requires Evaluation
EcHS_A0468_phoR_phosphate_regulon_sensor_protein	Requires Evaluation
EcHS_A1067_sulA_SOS_cell_division_inhibitor	Requires Evaluation
EcHS_A3855_DNA-damage-inducible protein D_DinD	Requires Evaluation
EcHS_A2357_two-component response regulator RscB	Requires Evaluation
EcHS_A1184_dinI_DNA_damage-inducible_protein_I	Requires Evaluation
EcHS_A4417_FtsH_protease_regulator_HflC	Requires Evaluation

[00111] In some embodiments of the devices, reactive species can be used to detect an oxidative stress in every single cell. Because non-viable cells cannot maintain a reducing environment in the cytoplasm, oxidation-sensitive dyes make up the majority of viability assays.

[00112] In some embodiments the devices and methods disclosed herein can be used to detect an increase in expression of genes up- regulated by OxyR and SoxR transcription factors as an evidence of antibiotic-induced oxidative stress response. These genes examples are such as for example sodA (encoding Mn- cofactored superoxide dismutase) and acrAB (encoding a multidrug efflux pump), soxS (secondary transcription factor), and KatG and Ahp genes, OxyS, RecA.

[00113] In some embodiments RNA targets and/or their genes involved in Fe ions transport and regulation are selected. In some embodiments, RNA targets and/or their genes involved in oxidation stress response are selected.

[00114] FeoB is a ferrous iron uptake system belonging to the Ferrous Iron Uptake (FeoB) transporter family (Kammler M, Schön C, Hantke K. Characterization of the ferrous iron uptake system of *Escherichia coli*. Journal of Bacteriology. 1993;175(19):6212-6219). FeoB is one of the numerous genes which transcription is reported to be down-regulated in response to ciprofloxacin treatment in RNAseq analysis of *E. coli* isolates total RNA expression. (Shishkin, A. A., G. Giannoukos, et al. (2015). "Simultaneous generation of many RNA-seq libraries in a single reaction." Nat Meth 12(4): 323-325). FeoB transcription is known to be under repression by Fur. In some embodiments, targets are selected out of those genes activated or repressed by Fur.

[00115] FeoB expression is also activated by RstAB two component system. In some embodiments, targets will be selected out of those genes activated or repressed by the RastAB two component system.

[00116] In some embodiments, the pH and Mg2+ concentrations of medium used to expose cells to antibiotic will be increased or decreased in conjunction with methods described herein to enhance the cellular response to antibiotics. In some embodiments bacterial cells are treated in relatively low Mg 2+, low pH (mild acidic conditions), and different selected controlled levels of Fe, to increase amplitude of changes in FeoB expression in response to antibiotics.

[00117] In some embodiments, the methods described herein comprise the steps of 1) pre-incubating cells in low Mg and low Fe (2+) and low pH (mild acidic conditions), and 2) adding antibiotic together with high Fe(2+). In some embodiments, the method comprises 1) pre-incubating cells low Mg and high Fe (2+) and low pH (mild acidic conditions), and 2) adding antibiotic.

[00118] In some embodiments, tuning of dissolved oxygen, PH, Fe2+, silver ions concentrations, Mg2+ concentrations, and other salts concentrations is performed to optimize conditions for the fastest/most reliable AST through measuring FeoB expression levels. These variations can be combined with the gene expression targets mentioned in this document. This will enable the higher resolution determination of antibiotic susceptibility by quantification of these targets. In some embodiments air/oxygen is provided to the bacteria incubated in a presence of antibiotics. In some embodiments, oxidative stress is enhanced in the bacteria via addition of peroxides.

[00119] In some embodiments, the target analyte can comprises an individual target or a group of targets, e.g. RNA from a group of genes coding for the proteins with the similar function (such as group of genes mentioned above and linked to iron metabolism) responding to pH, Mg^{2+} and oxidative stress. In some embodiments, the target analyte will comprise RNA from a group of genes relevant to oxidative stress (oxidation stress).

[00120] In some embodiments the recA gene and/or recA RNA are quantified to determine antibiotic susceptibility. In some embodiments, other genes in the SOS response are used as targets for quantification to determine antibiotic susceptibility. In some embodiments FeoB RNA and/or recA RNA are quantified to determine antibiotic susceptibility. In some embodiments FeoB RNA and recA RNA are quantified, detecting their ratio to determine antibiotic susceptibility and resistance. In some embodiments other genes up regulated and down regulated in response to antibiotics are quantified together and their ratio is detected to determine antibiotic susceptibility or resistance in a microorganism.

[00121] In some embodiments, the devices use a combination of precursor rRNA and ribosomal RNA markers for detecting resistant and susceptible cells or microorganisms with real-time RT-PCR on single cells. In some embodiments, the devices can integrate a combination of markers for universal drug-susceptibility and/or specific drug-resistance detection.

SAMPLE PROCESSING

[00122] In some embodiments, the microorganism is exposed to a drug to assay its response to the drug and determine whether the microorganism is resistant or susceptible to the drug. In some embodiments, isolates can be pre-cultured in or pre-exposed to a variety of matrices (such as for example bacterial culture media or human urine, among others), and can be subsequently incubated in the presence or absence of various drugs (e.g. antibiotics, such as ciprofloxacin, nitrofurantoin, trimethoprim, tetracycline, and sulfamethoxazole, and others.) where exposure to multiple drugs and / or multiple additives is performed on the same device substantially simultaneously. In some embodiments, microorganisms do not substantially divide during exposure to a drug. In some embodiments, microorganisms confined as single cells, or being in the small group and / or aggregate of cells, respond to a drug faster than an average population.

[00123] Devices and methods described herein can include those when some of the microorganisms confined as single cells, or as a small group and / or aggregate of cells, such as for example fewer than 3 cells, fewer than 10 cells, fewer than 30 cells, fewer than 100 cells in the same compartment. The volume of the compartment can be in the range of 100 fL to 1 nL, 1 nL to 100 nL, or 100 nL to 500 nL.

[00124] In some embodiments, digital assays can use the confinement of single cells from a sample into clonal isolation regions. In some embodiments, each of the isolated cells are cultured in the clonal isolation regions to generate a plurality of clonal populations from the sample. In some embodiments, the isolated cells are treated with a drug before or after expansion. In some embodiments, after culture and expansion from an isolated cell, the clonal population is divided into two or more treatment regions. As such, at least one portion of the clonal population can be treated with a drug, while another portion of the clonal population is not treated with a drug. Then, the assays described herein can be performed to determine whether the cells in the clonal population are susceptible or resistant to one or more drugs.

[00125] In some embodiments, specific culturing conditions are used to speed up a microorganism's response to a drug (e.g. quorum sensing molecules, gas partial pressures, temperature, etc). In some embodiments, the microorganism is exposed gases or gas mixtures, e.g., containing H_2S, CO, and NO. Such gases are known, for example, to affect susceptibility of

microorganisms to antibiotics. Such gases can be diluted with a gas mixture which could be anaerobic, aerobic, or microoxic. Such gas mixture can contain CO_2.

[00126] In some embodiments, co-culturing of the microorganism with eukaryotic cell and/or other microorganism is used to speed up a microorganism's response to a drug.

[00127] In some embodiments, measures to control the average number and/or location and/or time of initiation of replication in culture are performed. For example, cells can be placed into high- or low-nutrient conditions prior to exposure to antibiotics. In some embodiments, the acclimatization of cells to changes in growing conditions (such as, for example, mixing infected blood with nutrient rich media) will cause variation in the rate of DNA replication. In some embodiments, this effect can be mitigated by the modification of growth media. In some embodiments, data analyses can allow the capture of changes in replication rates over this noise. In some embodiments, the effects of media on replication can be used to enhance the effect of a drug treatment.

[00128] In some embodiments, cells are exposed to antibiotics as follows: Cells are pre-cultured at 37˚C to a density of up to 10^9 cells/mL in various matrices including Bacto Brain-Heart Infusion broth (BHI), a mix of BHI and pooled human urine, and whole human urine before being diluted and incubated with or without antibiotics. The antibiotics used for treatment include ciprofloxacin, nitrofurantoin, trimethoprim, tetracycline, and sulfamethoxazole. Incubations were performed at 37 ˚C at starting concentrations ranging from 10^5 - 10^8 cells/mL, and then treated with concentrations of antibiotics ranging from 0.75 ug/mL - 156 ug/mL depending on the treatment. Cells were incubated with and without antibiotics for a period of time including 10 minutes, 15 minutes, 20 minutes, 30 minutes, 45 minutes, or 60 minutes before an aliquot of the culture was used for nucleic acid extraction.

[00129] In some embodiments, the microorganism is exposed to a drug for a time less than 4 hours, less than 3 hours, less than 2 hours, less than 1 hour, less than 30 minutes, less than 15 minutes, less than 10 minutes, less than 5 minutes, less than 3 minutes, or less than 1 minute.

[00130] The nucleic acids can be extracted before analysis. The exact protocol used to extract nucleic acids depends on the sample and the exact assay to be performed. Extracting nucleic acids from target bacteria usually involves a cell lysis step followed by nucleic acid purification. The cell lysis step disrupts the cell and nuclear membranes, releasing the genetic material. This is often accomplished using a lysis detergent, such as sodium dodecyl sulfate, which also denatures the large amount of proteins present in the cells.

[00131] The nucleic acids can then be purified with an alcohol precipitation step, usually ice-cold ethanol or isopropanol, or via a solid phase purification step, typically on a silica matrix in a

column, resin or on paramagnetic beads in the presence of high concentrations of a chaotropic salt, prior to washing and then elution in a low ionic strength buffer. An optional step prior to nucleic acid precipitation is the addition of a protease which digests the proteins in order to further purify the sample.

[00132] In some embodiments, nucleic acids are extracted using standard methods including a one-step DNA extraction buffer or a one-step RNA extraction buffer (available from Epicentre). Following extraction, nucleic acids were quantified using nucleic acid amplification techniques including quantitative PCR and digital PCR.

[00133] In some embodiments, the microorganisms of the sample are lysed. In some embodiments, inhibitors are removed from the sample. In some embodiments, inhibitors in the sample are inactivated. In some embodiments, the sample is exposed to conditions or reagents for preventing degradation of the nucleic acid. In some embodiments, the sample undergoes ribosomal RNA depletion. IN some embodiments, unwanted RNA is removed from the sample. IN some embodiments, microorganisms in the sample are treated with a reagent that binds to and prevents amplification of free nucleic acids, such as PMA and EMA. In some embodiments, the sample is irradiated to initiate photochemical reaction to prevent the amplification of unwanted nucleic acids. In some embodiments, extracted nucleic acids are further purified prior to quantification.

[00134] In some embodiments sample preparation before the quantification reaction takes less than two hours, less than one hour, less than 30 minutes, less than 15 minutes, less than 10 minutes, less than 5 minutes, less than 3 minutes , less than 1 minute, or less than 30 seconds

[00135] In some embodiments techniques such as denaturation, restriction digestion, fragmentation, digestion of replicated DNA fragments are used before digital quantification. This enables enhanced isolation of individual genes into individual volumes for detection and amplification. In some embodiments, a restriction digest in between genes is used to facilitate isolation into individual volumes of DNA molecules carrying target genes. For example, in some embodiments, using as the target rDNA in *E. coli* with 7 copies per genome, a digital experiment can only show one positive volume per large genome fragment. When the genome is denatured , fragmented, and/or digested, each gene could be isolated into individual volumes, giving 24 to 70 template-positive volumes for analyses in a device from a single genome.

[00136] In some embodiments agents inhibiting amplification of nucleic acids from dead cells will be used (e.g., propidium monoazide (PMA) or ethidium monoazide). In some embodiments, cells will be treated with such agents inhibiting amplification of nucleic acids from dead cells

prior to incubation with antibiotics. In some embodiments, cells will be treated with such agents inhibiting amplification of nucleic acids from dead cells after incubation with antibiotics.

[00137] In some embodiments, a restriction digest of chromosomal DNA is done prior to quantification, such as to separate fragments of DNA containing multiple replicated copies of the target gene while preserving the fragments of interest to be suitable for detection and quantification.

[00138] In some embodiments, one or more denaturation steps are done, such as to duplicate the number of positive templates (for example if in a given sample *E. coli* cells could have from 12 to 35 copies of rDNA genes under certain conditions (depending on a growth rate, individual ages and individual states) – and yield for example 24-70 fragments after denaturation; this can in some cases improve visualization in digital approaches.)

[00139] In some embodiments, denaturation or digestion can be used to separate DNA at replication forks—pieces of newly replicated DNA can partition into different wells—such as to increase the resolution between inhibited cells and actively replicating cells.

[00140] In some embodiments, digital assays can use the confinement of a few or single target nucleic acid molecules into individual reaction volumes. Prior to the completion of replication, denaturing agents (such as e.g. heat, urea, Guanidinium Chloride, acids, bases, mechanical strain, enzymes, restriction enzymes, among others) can be used to enhance the separation of target DNA strands into independent volumes. This can be of particular use for example in cases where multiple target regions are present within a single genome.

[00141] In some embodiments, denaturation of a whole chromosomal DNA is performed before performing digital quantification.

Amplification

[00142] In some embodiments, a nucleic acid amplification reaction is performed to amplify the target analyte (e.g., a target nucleic acid). Amplification reaction can include polymerase chain reaction (PCR), nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), loop mediated isothermal amplification (LAMP), strand displacement amplification (SDA), whole genome amplification, multiple displacement amplification, strand displacement amplification, helicase dependent amplification, nicking enzyme amplification reaction, recombinant polymerase amplification, reverse transcription PCR or ligation mediated PCR. Amplification or detection methods for nucleic acids can include but are not limited to PCR, RT-PCR, or other methods including isothermal amplification methods. Isothermal nucleic acid amplification methods can include but are not limited to strand displacement amplification (SDA), transcription mediated amplification, nucleic acid sequence based amplification

(NASBA), recombinase polymerase amplification (RPA), rolling circle amplification, ramification amplification, helicase-dependent isothermal DNA amplification, loop mediated isothermal amplification (LAMP), methods based on both signal amplification and target amplification such as branched-DNA-based detection methodologies, hybridization chain reaction, or nucleic acid- based logic gates and DNA circuits (see, e.g., Qian and Winfree, Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades, Science 2011; 6034: 1196-1201).

[00143] The amplification reaction assay can be PCR. PCR is well known in this field and comprehensive description of this type of reaction is provided in E. van Pelt-Verkuil et al., Principles and Technical Aspects of PCR Amplification, Springer, 2008.

[00144] PCR is a powerful technique that amplifies a target DNA sequence against a background of complex DNA. If RNA is to be amplified (by PCR), it must be first transcribed into cDNA (complementary DNA) using an enzyme called reverse transcriptase. Afterwards, the resulting cDNA is amplified by PCR.

[00145] PCR is an exponential process that proceeds as long as the conditions for sustaining the reaction are acceptable. The components of the reaction are:

1. pair of primers—short single strands of DNA with around 10-30 nucleotides complementary to the regions flanking the target sequence
2. DNA polymerase—a thermostable enzyme that synthesizes DNA
3. deoxyribonucleoside triphosphates (dNTPs)—provide the nucleotides that are incorporated into the newly synthesized DNA strand
4. buffer—to provide the optimal chemical environment for DNA synthesis.

[00146] In embodiments using PCR, the components of the reaction can be in contact with sample. The components of the reaction can be added to a container that holds the sample. The components of the reaction can be present in a container, and the sample can be added. In some embodiments, a kit can comprise a plurality of small containers, at least one container holding the components of a PCR reaction. A kit can comprise a SlipChip and the components of the reaction.

[00147] PCR typically involves placing these reactants in a small tube (~10-50 microlitres) containing the extracted nucleic acids. The tube is placed in a thermal cycler; an instrument that subjects the reaction to a series of different temperatures for varying amounts of time. The standard protocol for each thermal cycle involves a denaturation phase, an annealing phase, and an extension phase. The extension phase is sometimes referred to as the primer extension phase. In addition to such three-step protocols, two-step thermal protocols can be employed, in which

the annealing and extension phases are combined. The denaturation phase typically involves raising the temperature of the reaction to 90-95° C. to denature the DNA strands; in the annealing phase, the temperature is lowered to ~50-60° C. for the primers to anneal; and then in the extension phase the temperature is raised to the optimal DNA polymerase activity temperature of 60-72° C. for primer extension. This process is repeated cyclically around 20-40 times, the end result being the creation of millions of copies of the target sequence between the primers.

[00148] The amplification reaction assay can be a variant of PCR. The amplification reaction assay can be selected from the group of variants to the standard PCR protocol such as multiplex PCR, linker-primed PCR, direct PCR, tandem PCR, real-time PCR and reverse-transcriptase PCR, amongst others, which have been developed for molecular diagnostics.

[00149] The amplification reaction assay can be multiplex PCR. Multiplex PCR uses multiple primer sets within a single PCR mixture to produce amplicons of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information can be gained from a single test-run that otherwise would require several experiments.

[00150] In some embodiments, a multiplexed PCR reaction is performed where a plurality of primer sets are added to a reaction mixture and each amplify their specified target within the same volume, for example. In other embodiments a sample is split into a plurality of smaller volumes into which single primer sets are introduced.

[00151] The amplification reaction assay can be linker-primed PCR, also known as ligation adaptor PCR. Linker-primed PCR is a method used to enable nucleic acid amplification of essentially all DNA sequences in a complex DNA mixture without the need for target-specific primers. The method firstly involves digesting the target DNA population with a suitable restriction endonuclease (enzyme). Double-stranded oligonucleotide linkers (also called adaptors) with a suitable overhanging end are then ligated to the ends of target DNA fragments using a ligase enzyme. Nucleic acid amplification is subsequently performed using oligonucleotide primers which are specific for the linker sequences. In this way, all fragments of the DNA source which are flanked by linker oligonucleotides can be amplified.

[00152] The amplification reaction assay can be direct PCR. Direct PCR describes a system whereby PCR is performed directly on a sample without any, or with minimal, nucleic acid extraction. With appropriate chemistry and sample concentration it is possible to perform PCR with minimal DNA purification, or direct PCR. Adjustments to the PCR chemistry for direct PCR include increased buffer strength, the use of polymerases which have high activity and processivity, and additives which chelate with potential polymerase inhibitors.

[00153] The amplification reaction assay can be tandem PCR. Tandem PCR utilizes two distinct rounds of nucleic acid amplification to increase the probability that the correct amplicon is amplified. One form of tandem PCR is nested PCR in which two pairs of PCR primers are used to amplify a single locus in separate rounds of nucleic acid amplification. The amplification reaction assay can be nested PCR. The first pair of primers hybridize to the nucleic acid sequence at regions external to the target nucleic acid sequence. The second pair of primers (nested primers) used in the second round of amplification bind within the first PCR product and produce a second PCR product containing the target nucleic acid, that can be shorter than the first one. The logic behind this strategy is that if the wrong locus were amplified by mistake during the first round of nucleic acid amplification, the probability is very low that it would also be amplified a second time by a second pair of primers and thus increases specificity.

[00154] The amplification reaction assay can be real-time PCR. The amplification reaction assay can be quantitative PCR. Real-time PCR, or quantitative PCR, is used to measure the quantity of a PCR product in real time. By using a fluorophore-containing probe or fluorescent dyes along with a set of standards in the reaction, it is possible to quantify the starting amount of nucleic acid in the sample. This is particularly useful in molecular diagnostics where treatment options can differ depending on the pathogen load in the sample.

[00155] The amplification reaction assay can be reverse-transcriptase PCR (RT-PCR). Reverse-transcriptase PCR (RT-PCR) is used to amplify DNA from RNA. Reverse transcriptase is an enzyme that reverse transcribes RNA into complementary DNA (cDNA), which is then amplified by PCR. RT-PCR can be used in expression profiling, to determine the expression of a gene or to identify the sequence of an RNA transcript, including transcription start and termination sites. It can be used to amplify RNA viruses such as human immunodeficiency virus or hepatitis C virus.

[00156] The amplification reaction assay can be isothermal. Isothermal amplification is a form of nucleic acid amplification which does not rely on the thermal denaturation of the target nucleic acid during the amplification reaction and hence may not require multiple rapid changes in temperature. Isothermal nucleic acid amplification methods can therefore be carried out inside or outside of a laboratory environment. A number of isothermal nucleic acid amplification methods have been developed, including but not limited to Strand Displacement Amplification (SDA), Transcription Mediated Amplification (TMA), Nucleic Acid Sequence Based Amplification (NASBA), Recombinase Polymerase Amplification (RPA), Rolling Circle Amplification (RCA), Ramification Amplification (RAM), Helicase-Dependent Isothermal DNA Amplification (HDA), Circular Helicase-Dependent Amplification (cHDA), Loop-

Mediated Isothermal Amplification (LAMP), Single Primer Isothermal Amplification (SPIA), Signal Mediated Amplification of RNA Technology (SMART), Self-Sustained Sequence Replication (3SR), Genome Exponential Amplification Reaction (GEAR) and Isothermal Multiple Displacement Amplification (IMDA). Further examples of such amplification chemistries are described in, for example, ("Isothermal nucleic acid amplification technologies for point-of-care diagnostics: a critical review, Pascal Craw and Wamadeva Balachandrana Lab Chip, 2012, 12, 2469-2486, DOI: 10.1039/C2LC40100B,") incorporated here in its entirety by reference. Isothermal amplification methods that operate at temperatures lower than PCR operating temperatures can be used, e.g., to improve compatibility of restriction enzymes with the amplification process if the restriction enzyme is not sufficiently stable under typical PCR operating temperatures.

[00157] Furthermore, detection methods based on both signal amplification and target amplification, such as branched-DNA-based detection methodologies, can be used in this approach. For example, for branched-DNA-based detection methodologies, using an enzyme that can cleave the target in a position located between two positions used for binding of the capture extender and the label extender (e.g., as described in Tsongalis, Branched DNA Technology in Molecular Diagnostics, Am J Clin Pathol 2006; 126: 448-453), can reduce the signal obtained in the assay when a restriction enzyme recognizes and cleaves the target.

[00158] The amplification reaction assay can be Strand Displacement Amplification (SDA). Strand Displacement Amplification (SDA) can rely on the ability of certain restriction enzymes to nick the unmodified strand of hemi-modified DNA and the ability of a 5′-3′ exonuclease-deficient polymerase to extend and displace the downstream strand. Exponential nucleic acid amplification can then achieved by coupling sense and antisense reactions in which strand displacement from the sense reaction serves as a template for the antisense reaction. The use of nickase enzymes which do not cut DNA in the traditional manner but produce a nick on one of the DNA strands, such as N. Alw1, N. BstNB1 and Mly1, for example, can be used in this reaction. SDA has been improved by the use of a combination of a heat-stable restriction enzyme (Ava1) and heat-stable Exo-polymerase (Bst polymerase). This combination has been shown to increase amplification efficiency of the reaction from 10^8 fold amplification to 10^{10} fold amplification so that it is possible using this technique to amplify unique single copy molecules.

[00159] The amplification reaction assay can be Transcription Mediated Amplification (TMA). The amplification reaction assay can be Nucleic Acid Sequence Based Amplification (NASBA). Transcription Mediated Amplification (TMA) and Nucleic Acid Sequence Based Amplification (NASBA) can use an RNA polymerase to copy RNA sequences but not corresponding genomic

DNA. The technology can use two primers and two or three enzymes, RNA polymerase, reverse transcriptase and optionally RNase H (if the reverse transcriptase does not have RNase activity). One primer can contain a promoter sequence for RNA polymerase. In the first step of nucleic acid amplification, this primer hybridizes to the target ribosomal RNA (rRNA) at a defined site. Reverse transcriptase can create a DNA copy of the target rRNA by extension from the 3′ end of the promoter primer. The RNA in the resulting RNA:DNA duplex can be degraded by the RNase activity of the reverse transcriptase if present or the additional RNase H. Next, a second primer binds to the DNA copy. A new strand of DNA is synthesized from the end of this primer by reverse transcriptase, creating a double-stranded DNA molecule. RNA polymerase recognizes the promoter sequence in the DNA template and initiates transcription. Each of the newly synthesized RNA amplicons re-enters the process and serves as a template for a new round of replication.

[00160] The amplification reaction assay can be Recombinase Polymerase Amplification (RPA). In Recombinase Polymerase Amplification (RPA), the isothermal amplification of specific DNA fragments is achieved by the binding of opposing oligonucleotide primers to template DNA and their extension by a DNA polymerase. Heat is not always required to denature the double-stranded DNA (dsDNA) template. Instead, RPA can employ recombinase-primer complexes to scan dsDNA and facilitate strand exchange at cognate sites. The resulting structures are stabilized by single-stranded DNA binding proteins interacting with the displaced template strand, thus preventing the ejection of the primer by branch migration. Recombinase disassembly leaves the 3′ end of the oligonucleotide accessible to a strand displacing DNA polymerase, such as the large fragment of Bacillus subtilis Pol I (Bsu), and primer extension ensues. Exponential nucleic acid amplification is accomplished by the cyclic repetition of this process.

[00161] The amplification reaction assay can be Helicase-dependent amplification (HDA). Helicase-dependent amplification (HDA) mimics the in vivo system in that it uses a DNA helicase enzyme to generate single-stranded templates for primer hybridization and subsequent primer extension by a DNA polymerase. In the first step of the HDA reaction, the helicase enzyme traverses along the target DNA, disrupting the hydrogen bonds linking the two strands which are then bound by single-stranded binding proteins. Exposure of the single-stranded target region by the helicase allows primers to anneal. The DNA polymerase then extends the 3′ ends of each primer using free deoxyribonucleoside triphosphates (dNTPs) to produce two DNA replicates. The two replicated dsDNA strands independently enter the next cycle of HDA, resulting in exponential nucleic acid amplification of the target sequence.

[00162] The amplification reaction assay can be Rolling Circle Amplification (RCA). Other DNA-based isothermal techniques include Rolling Circle Amplification (RCA) in which a DNA polymerase extends a primer continuously around a circular DNA template, generating a long DNA product that consists of many repeated copies of the circle. By the end of the reaction, the polymerase generates many thousands of copies of the circular template, with the chain of copies tethered to the original target DNA. This allows for spatial resolution of target and rapid nucleic acid amplification of the signal. Up to 10^{12} copies of template can be generated in 1 hour. Ramification amplification is a variation of RCA and utilizes a closed circular probe (C-probe) or padlock probe and a DNA polymerase with a high processivity to exponentially amplify the C-probe under isothermal conditions.

[00163] The amplification reaction assay can be Loop-mediated isothermal amplification (LAMP). LAMP offers high selectivity and employs a DNA polymerase and a set of four specially designed primers that recognize a total of six distinct sequences on the target DNA. An inner primer containing sequences of the sense and antisense strands of the target DNA initiates LAMP. The following strand displacement DNA synthesis primed by an outer primer releases a single-stranded DNA. This serves as template for DNA synthesis primed by the second inner and outer primers that hybridize to the other end of the target, which produces a stem-loop DNA structure. In subsequent LAMP cycling one inner primer hybridizes to the loop on the product and initiates displacement DNA synthesis, yielding the original stem-loop DNA and a new stem-loop DNA with a stem twice as long. The cycling reaction continues with accumulation of many copies of target in less than an hour. The final products are stem-loop DNAs with several inverted repeats of the target and cauliflower-like structures with multiple loops formed by annealing between alternately inverted repeats of the target in the same strand.

[00164] In some embodiments, the amplification is a one-step digital reverse-transcription loop-mediated isothermal amplification (dRT-LAMP) reaction for quantifying mRNA with all reactions performed. LAMP produces a bright fluorescence signal through replacement of manganese with magnesium in calcein. In some embodiments, this fluorescence can then be detected and counted using a commercial cell phone camera.

[00165] Nucleic acid-based logic gates and DNA circuits can be used for nucleic acid amplification. The use of restriction enzymes with nucleic acid-based logic gates and DNA circuits can reduce or stop the intrinsic leakage problem for DNA networks. Combining the molecular recognition ability of both restriction enzymes and DNA networks, restriction enzyme logic gates can be highly active components for the design and construction of biocomputational

devices (see e.g., Qian and Winfree, Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades, Science 2011; 6034: 1196-1201).

[00166] In some embodiments the amplification employed can take place in a variety of different mediums, such as for example, aqueous solution, polymeric matrix, solid support, etc

Detection

[00167] Assay results can comprise a readout or detection mechanism chosen from a range of readouts used to detect progress or results of reactions, including but not limited to optical techniques, electrical techniques or magnetic techniques. Examples include but are not limited to electrochemical readouts, optical readouts, including for example fluorescence readouts, colorimetric readouts, chemiluminescence, electrical signals, quenching, probe binding, probe hybridization, metal labeling, contrast agent labeling, absorbance, mass spectrometry, sequencing, lateral flow strips, and the generation of a heterogeneous substance (e.g., precipitation, gas bubble).

[00168] A readout mechanism can comprise fluorescence. For example fluorescent dye can be used to label nucleic acids; reactions with more nucleic acid product can yield more fluorescence signal. Fluorescent dyes can include but are not limited to ethidium bromide, berberine, proflavine, daunomycin, doxorubicin, thalidomide, YOY0-1, SYBR Green I, SYBR Green II, oxazole yellow (YO), thiazole orange (TO), PicoGreen (PG), TOTO, TO-PRO, SYTOX, SYTO, other cyanine dyes, and calcein. The fluorescence intensity can be measured at an end-point or in real-time, allowing measurement of the reaction progress. For example, a given level of fluorescence can be set as the threshold for a positive signal from a digital or quasi-digital compartment.

[00169] In some cases, signal can be generated from molecules with reporter moieties and affinity moieties that are applied to digital units to bind to captured target analyte. The reporter molecule or reporter moiety can be fluorescent. The digital units or capture regions can be washed to remove unbound reporter. In some cases, the reporter molecule can be calcein or calcein with cetyl trimethyl ammonium bromide (calcein-CTAB). In some cases, the reporter can be an intercalating dye. Target analytes can be labeled with enzymes which can produce an electrical signal, for example by electro-activating a substrate molecule which can be oxidized and reduced. Labeling can occur by binding with an affinity agent, for example as in a sandwich assay. Labeling can occur by intercalating dyes.

[00170] A readout mechanism can comprise mass spectrometry. For example, nucleic acids of different sizes (e.g. from restriction digestion or ligation) can be distinguished and/or counted by mass spectrometry. Alternatively, a readout mechanism can operate without mass spectrometry.

[00171] A readout mechanism can comprise electrophoresis, including gel electrophoresis. For example, nucleic acids of different sizes (e.g. from restriction digestion or ligation) can be identified or distinguished by electrophoresis. Alternatively, a readout mechanism can operate without electrophoresis.

[00172] A readout mechanism can comprise sequencing. Sequencing, or sequence determination techniques, can be performed by methods including but not limited to Sanger sequencing, Illumina (Solexa) sequencing, pyrosequencing, next generation sequencing, Maxam-Gilbert sequencing, chain termination methods, shotgun sequencing, or bridge PCR; next generation sequencing methodologies can comprise massively parallel signature sequencing, polony sequencing, SOLiD sequencing, Ion Torrent semiconductor sequencing, DNA nanoball sequencing, Heliscope single molecule sequencing, single molecule real time (SMRT) sequencing, nanopore DNA sequencing, tunneling currents DNA sequencing, sequencing by hybridization, sequencing with mass spectrometry, microfluidic Sanger sequencing, microscopy- based techniques, RNA polymerase sequencing or in vitro virus high-throughput sequencing.

[00173] The signal can be electromagnetic. The signal can comprise the presence or absence of a physical object, such as a bead. Captured target analytes can be labeled with a fluorescent agent or a contrast agent. Target analytes can be labeled with enzymes which can produce a fluorescent signal. Target analytes can be labeled with enzymes which can produce a color change in a substrate, producing a colorimetric signal. In some cases, signal can be generated from reporter molecules that are bound to affinity molecules and applied to digital units to bind to captured target analyte.

[00174] Sequencing reads can be used to identify reaction products, and the number of sequencing reads generated for a given nucleic acid product can be used to evaluate the reaction. For example, a given number of sequencing reads can be set as the threshold for a positive signal from a digital or quasi-digital compartment. Alternatively, a readout mechanism can operate without sequencing.

[00175] Multiplexed signal detection ensure that in multiplexed signal detection there is the ability to distinguish the amplification of many signals within the same volume as well as the ability to distinguish different signals from different volumes.

Binary Quantification / Digital Amplification

[00176] In some embodiments, the methods and assays described herein use digital and binary quantification methods; digital methods enable quantification of targets in samples containing low concentrations of cells (such as for example, when a sample (e.g. blood) has low

concentrations of a pathogen (e.g. 1 bacterial cell per mL or 10 cells / mL) in a range that is still clinically relevant or even represent a life threatening illness). In some embodiments, the digital methods described herein can be used to ensure reliable quantification resolution between susceptible and resistant cells.

[00177] The process of binary quantification begins with a sample that can contain a target analyte. The target analyte can be a molecule to be quantified or searched for, for instance a particular nucleic acid, a particular nucleic acid sequence, a gene, or a protein, for example. The sample can be partitioned into many separate reaction volumes. In some embodiments, the reaction volumes are separate analysis regions. In some embodiments, the separate reaction volumes are physically separated in separate wells, chambers, areas on the surface of a slide, droplets, beads, or aliquots, for example. In some embodiments, the separate reaction volumes can be in the same container, for instance, the target analyte can be affixed to a substrate or attached to a bead. The reaction volumes can be on beads, on the surface of a slide, or attached to a substrate. The sample is distributed to many separate reaction volumes such that some, but not all of the reaction volumes generate a positive signal.

[00178] The sample is distributed to many separate reaction volumes such that each individual reaction volume contains a number of target analytes either below or above the threshold value for generating a positive signal. Generation of a positive signal from a reaction volume can depend on the number or concentration of target analytes captured, trapped, or bound by that reaction volume. In some cases, a threshold number of target analytes captured, trapped, or bound by a reaction volume allows a positive signal to be generated from that reaction volume. The threshold number of target analytes to allow positive signal generation can be at least about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50 or more target analytes. The threshold number of target analytes to allow positive signal generation can be at most about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, or 50 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 1 to 19 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 2 to 19 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 3 to 19 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 4 to 19 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 5 to 19 target analytes. In some cases,

the threshold number of target analytes to allow positive signal generation can be from 6 to 19 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 7 to 19 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 8 to 19 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 9 to 19 target analytes. In some cases, the threshold number of target analytes to allow positive signal generation can be from 10 to 19 target analytes. In some cases, a threshold concentration of analytes captured, trapped, or bound by a reaction volume allows a positive signal to be generated from that reaction volume. The threshold concentration of target analytes to allow positive signal generation can be at least about zero zeptomolar (zM), 1 zM, 10 zM, 100 zM, 1 attomolar (aM), 10 aM, 100 aM, 1 femtomolar (fM), 10 fM, 100 fM, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM, 1 mM, 10 mM, 100 mM, 1 M, or more. The threshold concentration of target analytes to allow positive signal generation can be at most about 1, fM, 10 fM, 100 fM, 1 pM, 10 pM, 100 pM, 1 nM, 10 nM, 100 nM, 1 μM, 10 μM, 100 μM, 1 mM, 10 mM, 100 mM, 1 M, or less. The threshold number or concentration of target analytes to allow positive signal generation from a reaction volume can be controlled. Inhibitors can be used with reaction volumes to control the threshold number or concentration. For example, the number or concentration of target analytes can be required to be higher than the number or concentration of inhibitors in a reaction volume in order for a signal to be produced from that reaction volume.

[00179] In some cases, the probability of a positive signal being generated from a reaction volume depends on the number or concentration of target analytes captured, trapped, or bound by that reaction volume. The probability of a positive signal being generated from a reaction volume can be controlled. For example, the efficiency of a signal generating reaction can be controlled, thereby controlling the probability of signal generation; a lower efficiency reaction can result in a lower probability of signal generation for a given number or concentration of target analytes.

[00180] In some embodiments, the sample is distributed to many separate reaction volumes such that each individual reaction volume contains either zero individual occurrences of the target analyte, or one or more individual occurrences of the target analyte. One or more molecules can mean a non-zero number of molecules. One or more molecules can mean one molecule. In some embodiments, one or more molecules can mean one molecule, two molecules, three molecules, four molecules… etc. In some embodiments, each separate reaction volume is contained in a well. In some embodiments, the sample is distributed such that each reaction

volume, on average comprises less than one individual molecule of the target analyte. In some embodiments, the sample is distributed such that most reaction volumes comprise either zero or one molecules of the target analyte. Next, a qualitative "yes or no" test can be done to determine whether or not each reaction volume contains one or more target analytes by reading the pattern of discrete positive and negative reaction volumes. A positive reaction volume can be a reaction volume determined to contain one or more target analytes. A positive reaction volume can be a reaction volume determined to have a signal that correlates to the presence of one or more target analytes. A positive reaction volume can be a reaction volume determined to have a signal above a threshold that correlates to the presence of one or more target analyte. In some embodiments, a positive reaction volume is quantified as 1, or a simple multiple of 1 such as 2, 3, etc. while a negative reaction volume is quantified as 0, or less than a threshold. In some embodiments, a positive reaction volume is quantified as 1 and a negative reaction volume is quantified as 0. A negative reaction volume can be a reaction volume determined to contain zero target analyte. A negative reaction volume can be a reaction volume that does not have a signal that correlates to the presence of one or more target analyte. A negative reaction volume can be a reaction volume that does not have a signal above the threshold that correlates to the presence of one or more target analyte. The determination and/or designation of each reaction volume as a positive or a negative reaction volume can be referred to as a binary assay or a digital assay. This "yes or no test" or test like this can be referred to as a binary assay. This qualitative analysis of which reaction volume are negative reaction volume and which reaction volume are positive reaction volume can then be translated into a quantitative concentration of target analyte in the sample using Poisson analysis. A high dynamic range can be achieved through using many reaction volumes. A high dynamic range can be achieved by using a device that has reaction volume of different sizes. A high dynamic range can be achieved by partitioning the sample into many wells and/or into wells of different sizes.

[00181] This overall process can be called binary quantification of nucleic acids. This process can be called counting numbers of target analyte. In some embodiments, binary quantification is the process of partitioning a sample into a plurality of reaction volume such that each reaction volume contains either zero or a non-zero number of target analyte; determining and/or designating which reaction volume are positive reaction volume and which reaction volume are negative reaction volume with respect to the target analyte; and translating the information about positive and negative reaction volume into information about the quantity or concentration of the target analyte in the sample. In some embodiments, the absolute number of target analyte is determined. In some embodiments, the translation of the information about which reaction

volume are positive reaction volume and which reaction volume are negative reaction volume to information about the amount, absolute number of molecules, or concentration of the target analyte in the sample is called digital quantification of the target analyte. In some embodiments, the target analyte is a nucleic acid. In some embodiments, the binary quantification of nucleic acids is achieved. In some embodiments, binary quantification of a nucleic acid target analyte is determined wherein the sample is partitioned into several reaction volumes, wherein the reaction volumes are on a SlipChip.

[00182] In some embodiments, a binary quantification of target analyte in a sample can be achieved without spatially separating the sample into multiple reaction volumes. In these embodiments, the target analyte can be counted by informational separation. In some embodiments, target analyte in the sample undergo a binary quantification through a process wherein the target analyte are tagged with a pool of information-carrying molecules, amplified or copied, and the number of distinct information-carrying molecules that were amplified or copied is counted in to get a quantification of the starting number of target analyte (see e.g. WO 2012148477). In some embodiments, the information-carrying molecule can be a pool of chemical barcodes. In some embodiments, the information-carrying molecule can be a set of nucleic acid sequences.

[00183] Digital analyses can be achieved using the polymerase chain reaction (PCR), recombinant polymerase amplification (RPA), and loop mediated amplification (LAMP) as a way of quantifying RNA or DNA concentrations. Amplifications such as RPA and LAMP, which can use isothermal chemistries, can be well suited for home and limited-resource setting use. LAMP chemistry in particular is an attractive candidate for use in a home or limited-resource setting platform as it can have a relatively broad temperature tolerance range, can work with simple and cheap chemical-based heaters and phase-change materials, and can have a fluorescence gain with positive wells.

Robustness

[00184] Robustness can be the degree to which a series of repeated quantitative measurements provides a set of similar measurements under varying experimental conditions. For example a cell phone camera can be used to successfully perform similar measurements on a SlipChip under a variety of conditions found in the real world. Similar measurements can be identical measurements. Similar measurements can be the same diagnosis. Similar measurements can be the same answer. Similar measurements can mean more than one measurement within experimental error of each other. Similar measurements can yield a consistent outcome with statistical significance. Similar measurements can be of similar numerical size, for instance

within 5%, 10%, 15%, 20%, 25%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 200%, 1,000% of each other. Robust assays can produce similar measurements more often than 25%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, 99.5%, 99.9%, 99.99%, for example, of instances measured under a given set of conditions.

[00185] Different types of assays can be robust assays. A nucleic acid amplification and quantification assay can be robust. An assay to detect a protein or other target such as a cell, exosome, liposome, bacteria, virus, etc. can be robust. A LAMP assay can be robust. A RT-LAMP assay can be robust. A dRT-LAMP assay can be robust. A binary LAMP reaction can be robust. A binary, two-step LAMP reaction can be robust. A PCR reaction can be robust. A qPCR assay can be robust. A quantitative nucleic acid amplification reaction can be robust. A qualitative nucleic acid amplification reaction can be robust. A method to diagnosis a health outcome based on the amplification of a nucleic acid sequence can be robust. A process within a SlipChip can be robust. The imaging and analysis of a SlipChip after a LAMP reaction can be a robust process.

[00186] The absolute efficiency of dRT-LAMP can be increased over 10-fold, e.g. from ~2 % to ~28 %, by i) using a more efficient reverse transcriptase, ii) introducing RNase H to break up the DNA-RNA hybrid, and iii) adding only the BIP primer during the RT step. dRT-LAMP can be compatable with a plastic SlipChip device and used this two-step method to quantify HIV RNA. The dRT-LAMP quantification results were in some cases very sensitive to the sequence of the patient's HIV RNA.

[00187] Assays can be robust with respect to experimental variables. An assay can be robust with respect to a given temperature range. An assay can be robust of over a temperature range. Some non-limiting ranges, over which an assay can be robust include 1 °C, 2 °C, 3 °C, 4 °C, 5 °C, 6 °C, 7 °C, 8 °C, 9 °C, 10 °C, 11 °C, 12 °C, 16 °C, 20 °C, 24 °C, 28 °C, 32 °C, 40 °C, 50 °C, 60 °C,80 °C, 100 °C, 150 °C ,200 °C, 250 °C, or 300 °C, for example. The temperature range of which an assay is robust can be centered on temperature on an absolute temperature scale. Some non-limiting temperatures that could be the center of the temperature range that an assay is robust to include -40 °C,-30 °C,-20 °C, -10 °C, 0 °C, 10 °C, 20 °C, room temperature, 25 °C, 30 °C, 35 °C, body temperature, 37 °C,40 °C, 45 °C, 50 °C, 55 °C, 60 °C, 65 °C, 70 °C, 80 °C, 90 °C,100 °C, 110 °C, 150 °C, or 200 °C, for example. In some embodiments, a binary LAMP assay is used to amplify and subsequently image and quantify a nucleic acid sequence in a sample. In these embodiments, the assay can be a robust quantification of a nucleic acid sequence with over a temperature range of 9 °C centered at about 60 °C. A binary LAMP assay used to amplify and subsequently image and quantify a nucleic acid sequence in a sample can be robust over the

temperature range from about 55 °C to about 66 °C. In some embodiments, a SlipChip can be imaged and the data can be processed to give robust findings over a range of a temperature from about 5 °C to about 70 °C.

[00188] An assay can be robust with respect to time. An assay can give consistent results over a range of time points. An assay can require only end-point readout. A binary DNA amplification experiment can require only end-point readout. The endpoint read out can be obtained near the completion of amplification, or at a time after this time point. A robust DNA amplification assay can give consistent results at a time point near the end of the reaction and/or at a timepoint after the reaction is complete. A non-limiting range of reaction time that an assay could be robust over includes 0.01 min, 0.1 min, 0.5 min, 1 min, 2 min, 3 min, 4 min, 5 min, 6 min, 7 min, 8 min, 9 min, 10 min, 12 min, 14 min, 16 min, 20 min, 24 min, 28 min, 32 min, 40 min, 45 min, 50 min, 1.0 hour, 2 hour, 3 hour, 4 hour, 5 hour, 6 hour, 8 hour, 10 hour, 12 hour, 16 hour, 18 hour, 1 day, 2 day, 3 day, 7 days, 1 month, or 1 year, for example. In some cases, binary DNA amplification experiments do not require exact knowledge of time. The output of a binary DNA amplification can be robust to variation in reaction time beyond the optimal reaction time. In some embodiments, a d-LAMP assay on a SlipChip is robust over a 20 minute time period between 40 minutes and 60 minutes after the LAMP reaction begins, for example.

[00189] An assay can be robust with respect to variations in atmospheric humidity. In some embodiments, an assay can be robust regardless of the atmospheric humidity. In some embodiments, an assay can be robust over a range of atmospheric humidity. The range of humidity can be from about 0% to 100% relative humidity. The range of atmospheric humidity at which an assay can be robust can be from about 0 to about 40 grams water per cubic meter of air at about 30°C. In some embodiments, an assay can be robust from about 0% humidity to about 40%, 50%, 60%, 70%, 80%, 90%, or 100% humidity, for example. In some embodiments, an assay can be robust over a humidity range of about 40%, 50%, 60%, 70%, 80%, 90%, or 100% humidity. In some embodiments, a d-LAMP assay run in a SlipChip can be imaged and analyzed as a robust assay over a range of humidity from about 0% to about 100% atmospheric humidity.

[00190] In some embodiments, the assay provides a quantitative analytical measurement. For instance, the invention can measure and display the amount and/or the concentration of a nucleic acid sequence within a sample as a quantitative amount. This measurement can be robust with respect to the experimental conditions present during the chemical amplification of the nucleic acid sequence, during the measurement of the optical data, and/or during the processing of the data, for instance.

[00191] In some embodiments, small differences in the concentration of a target can be resolved with greater statistical significance by quantifying the response of two or more genes to antibiotic treatment. For example, in a scenario in which two genes experience a 1.2-fold change in concentration when comparing samples treated with a drug versus samples that are untreated. If quantification yields this 1.2-fold difference with a p-value of around 0.10, then the difference will not be statistically significant when analyzed independently. However, using Fisher's method to combine the results from several independent tests that have the same overall null hypothesis (that the treated and untreated bacterial nucleic acids are the same) will result in a lower p-value than each individual test. The test statistic used to combine the p-values from separate tests is χ^2 and the formula is

$$\chi^2_{2k} = -2\sum_{i=1}^{k} \ln(p_i)$$

[00192] For example, if two independent tests with p-values of 0.10 each are combined using this method, the overall p-value will now be 0.05, which is significant at the 95% confidence level. In some embodiments, digital NA (nucleic acid) quantification is more amenable to POC diagnostics

[00193] In some embodiments, a ratio of the RNA from multiple genes is used to determine drug susceptibility. In some embodiments, this approach can include the use of housekeeping genes to measure relative changes in gene expression. In some embodiments, this includes the use of a ratio between genes upregulated and genes downregulated in response to drug treatment. In some embodiments, ratios of genes are used to enable higher sensitivity of quantification of responses when few cells are present in the sample, in some embodiments as few as 1 cells.

Analysis

[00194] It is to be understood that the exemplary methods and systems described herein can be implemented in various forms of hardware, software, firmware, special purpose processors, or a combination thereof. These instructions and programs can be executed by and/or stored on non-transitory computer readable media. Methods herein can be implemented in software as an application program tangibly embodied on one or more program storage devices. The application program can be executed by any machine, device, or platform comprising suitable architecture. It is to be further understood that, because some of the systems and methods described herein are implemented in software, the actual connections between the system components (or the process steps) can differ depending upon the manner in which the present invention is programmed.

[00195] The number or concentration of target analytes in a sample can be calculated based on the signal generated from the reaction volumes. The number, location, type, or a combination thereof of positive reaction volumes can be used to calculate the number or concentration of target analytes in a sample. The number, location, type, or a combination thereof of positive capture regions can be used to calculate the number or concentration of target analytes in a sample.

[00196] Assay results can be determined by comparison of results to theoretical models. For example, Poisson statistical analysis can be applied to quantify the number of fluorescent and non-fluorescent regions. Combining the results from wells of different volumes fully minimizes the standard error and provides high-quality analysis across a very large dynamic range.

[00197] The computer components, software modules, functions, data stores and data structures described herein can be connected directly or indirectly to each other in order to allow the flow of data needed for their operations. It is also noted that the meaning of the term module includes but is not limited to a unit of code that performs a software operation, and can be implemented for example as a subroutine unit of code, or as a software function unit of code, or as an object (as in an object-oriented paradigm), or as an applet, or in a computer script language, or as another type of computer code. The software components and/or functionality can be located on a single computer or distributed across multiple computers depending upon the situation at hand. In yet another aspect, a computer readable medium is provided including computer readable instructions, wherein the computer readable instructions instruct a processor to execute the methods described herein. The instructions can operate in a software runtime environment. In yet another aspect, a data signal is provided that can be transmitted using a network, wherein the data signal includes data calculated in a step of the methods described herein. The data signal can further include packetized data that is transmitted through wired or wireless networks. In an aspect, a computer readable medium comprises computer readable instructions, wherein the instructions when executed carry out a calculation of the probability of a medical condition in a patient based upon data obtained from the sample. The computer readable instructions can operate in a software runtime environment of the processor. In some embodiments, a software runtime environment provides commonly used functions and facilities required by the software package. Examples of a software runtime environment include, but are not limited to, computer operating systems, virtual machines or distributed operating systems although several other examples of runtime environment exist. The computer readable instructions can be packaged and marketed as a software product, app, or part of a software package. For example, the instructions can be packaged with an assay kit.

[00198] The computer readable medium can be a storage unit. Computer readable medium can also be any available media that can be accessed by a server, a processor, or a computer. The computer readable medium can be incorporated as part of the computer-based system, and can be employed for a computer-based assessment of a medical condition.

[00199] In some embodiment, the calculations described herein can be carried out on a computer system. The computer system can comprise any or all of the following: a processor, a storage unit, software, firmware, a network communication device, a display, a data input, and a data output. A computer system can be a server. A server can be a central server that communicates over a network to a plurality of input devices and/or a plurality of output devices. A server can comprise at least one storage unit, such as a hard drive or any other device for storing information to be accessed by a processor or external device, wherein the storage unit can comprise one or more databases. In an embodiment, a database can store hundreds to millions of data points corresponding to a data from hundreds to millions of samples. A storage unit can also store historical data read from an external database or as input by a user. In an embodiment, a storage unit stores data received from an input device that is communicating or has communicated with the server. A storage unit can comprise a plurality of databases. In an embodiment, each of a plurality of databases corresponds to each of a plurality of samples. An individual database can also comprise information for a plurality of possible sample containment units. Further, a computer system can comprise multiple servers. A processor can access data from a storage unit or from an input device to perform a calculation of an output from the data. A processor can execute software or computer readable instructions as provided by a user, or provided by the computer system or server. The processor can have a means for receiving patient data directly from an input device, a means of storing the subject data in a storage unit, and a means for processing data. The processor can also include a means for receiving instructions from a user or a user interface. The processor can have memory, such as random access memory. In one embodiment, an output that is in communication with the processor is provided. After performing a calculation, a processor can provide the output, such as from a calculation, back to, for example, the input device or storage unit, to another storage unit of the same or different computer system, or to an output device. Output from the processor can be displayed by data display. A data display can be a display screen (for example, a monitor or a screen on a digital device), a print-out, a data signal (for example, a packet), an alarm (for example, a flashing light or a sound), a graphical user interface (for example, a webpage), or a combination of any of the above. In an embodiment, an output is transmitted over a network (for example, a wireless network) to an output device. The output device can be used by a user to receive the output from

the data-processing computer system. After an output has been received by a user, the user can determine a course of action, or can carry out a course of action, such as a medical treatment when the user is medical personnel. In some embodiments, an output device is the same device as the input device. Example output devices include, but are not limited to, a telephone, a wireless telephone, a mobile phone, a PDA, a flash memory drive, a light source, a sound generator, a computer, a computer monitor, a printer, and a webpage. The user station can be in communication with a printer or a display monitor to output the information processed by the server.

[00200] A client-server, relational database architecture can be used in embodiments of the invention. A client server architecture is a network architecture in which each computer or process on the network is either a client or a server. Server computers are typically powerful computers dedicated to managing disk drives (file servers), printers (print servers), or network traffic (network servers). Client computers include PCs (personal computers), cell phones, or workstations on which users run applications, as well as example output devices as disclosed herein. Client computers rely on server computers for resources, such as files, devices, and even processing power. In some embodiments of the invention, the server computer handles all of the database functionality. The client computer can have software that handles all the front-end data management and can also receive data input from users.

[00201] Subject data can be stored with a unique identifier for recognition by a processor or a user. In another step, the processor or user can conduct a search of stored data by selecting at least one criterion for particular patient data. The particular patient data can then be retrieved. Processors in the computer systems can perform calculations comparing the input data to historical data from databases available to the computer systems. The computer systems can then store the output from the calculations in a database and/or communicate the output over a network to an output device, such as a webpage, a text, or an email. After a user has received an output from the computer system, the user can take a course of medical action according to the output. For example, if the user is a physician and the output is a probability of cancer above a threshold value, the physician can then perform or order a biopsy of the suspected tissue. A set of users can use a web browser to enter data from a biomarker assay into a graphical user interface of a webpage. The webpage is a graphical user interface associated with a front end server, wherein the front end server can communicate with the user's input device (for example, a computer) and a back end server. The front end server can either comprise or be in communication with a storage device that has a front-end database capable of storing any type of data, for example user account information, user input, and reports to be output to a user. Data

from each user can be then be sent to a back end server capable of manipulating the data to generate a result. For example, the back end server can calculate a corrections for similar cell phones or compile data generated from similar sample collection units. The back end server can then send the result of the manipulation or calculation back to the front end server where it can be stored in a database or can be used to generate a report. The results can be transmitted from the front end server to an output device (for example, a computer with a web browser or a cell phone) to be delivered to a user. A different user can input the data and receive the data. In an embodiment, results are delivered in a report. In another embodiment, results are delivered directly to an output device that can alert a user.

[00202] The information from the assay can be quantitative and sent to a computer system of the invention. The information can also be qualitative, such as observing patterns or fluorescence, which can be translated into a quantitative measure by a user or automatically by a reader or computer system. In an embodiment, the subject can also provide information other than sample assay information to a computer system, such as race, height, weight, age, gender, eye color, hair color, family medical history, identity, location and any other information that can be useful to the user.

[00203] In some embodiments additional information is provided by sensors associated with the device. For example global positioning data, acceleration data, air pressure, or moisture levels can be measured by a device comprising the image sensor. This additional information can be used by the computer systems of the invention.

[00204] Information can be sent to a computer system automatically by a device that reads or provides the data from image sensor. I n another embodiment, information is entered by a user (for example, the subject or medical professional) into a computer system using an input device. The input device can be a personal computer, a mobile phone or other wireless device, or can be the graphical user interface of a webpage. For example, a webpage programmed in JAVA can comprise different input boxes to which text can be added by a user, wherein the string input by the user is then sent to a computer system for processing. The subject can input data in a variety of ways, or using a variety of devices. Data can be automatically obtained and input into a computer from another computer or data entry system. Another method of inputting data to a database is using an input device such as a keyboard, touch screen, trackball, or a mouse for directly entering data into a database.

[00205] In an embodiment, a computer system comprises a storage unit, a processor, and a network communication unit. For example, the computer system can be a personal computer, laptop computer, or a plurality of computers. The computer system can also be a server or a

plurality of servers. Computer readable instructions, such as software or firmware, can be stored on a storage unit of the computer system. A storage unit can also comprise at least one database for storing and organizing information received and generated by the computer system. In an embodiment, a database comprises historical data, wherein the historical data can be automatically populated from another database or entered by a user.

[00206] In an embodiment, a processor of the computer system accesses at least one of the databases or receives information directly from an input device as a source of information to be processed. The processor can perform a calculation on the information source, for example, performing dynamic screening or a probability calculation method. After the calculation the processor can transmit the results to a database or directly to an output device. A database for receiving results can be the same as the input database or the historical database. An output device can communicate over a network with a computer system of the invention. The output device can be any device capable delivering processed results to a user.

[00207] Communication between devices or computer systems of the invention can be any method of digital communication including, for example, over the internet. Network communication can be wireless, ethernet-based, fiber optic, or through fire-wire, USB, or any other connection capable of communication. In an embodiment, information transmitted by a system or method of the invention can be encrypted.

[00208] It is further noted that the systems and methods can include data signals conveyed via networks (for example, local area network, wide area network, internet), fiber optic medium, carrier waves, wireless networks for communication with one or more data processing or storage devices. The data signals can carry any or all of the data disclosed herein that is provided to or from a device.

[00209] Additionally, the methods and systems described herein can be implemented on many different types of processing devices by program code comprising program instructions that are executable by the device processing subsystem. The software program instructions can include source code, object code, machine code, or any other stored data that is operable to cause a processing system to perform methods described herein. Other implementations can also be used, however, such as firmware or even appropriately designed hardware configured to carry out the methods and systems described herein.

[00210] A computer system can be physically separate from the instrument used to obtain values from the subject. In an embodiment, a graphical user interface also can be remote from the computer system, for example, part of a wireless device in communication with the network. In another embodiment, the computer and the instrument are the same device.

[00211] An output device or input device of a computer system can include one or more user devices comprising a graphical user interface comprising interface elements such as buttons, pull down menus, scroll bars, fields for entering text, and the like as are routinely found in graphical user interfaces known in the art. Requests entered on a user interface are transmitted to an application program in the system (such as a Web application). In one embodiment, a user of user device in the system is able to directly access data using an HTML interface provided by Web browsers and Web server of the system.

[00212] A graphical user interface can be generated by a graphical user interface code as part of die operating system or server and can be used to input data and/or to display input data. The result of processed data can be displayed in the interface or a different interface, printed on a printer in communication with the system, saved in a memory device, and/or transmitted over a network. A user interface can refer to graphical, textual, or auditory information presented to a user and can also refer to the control sequences used for controlling a program or device, such as keystrokes, movements, or selections. In another example, a user interface can be a touch screen, monitor, keyboard, mouse, or any other item that allows a user to interact with a system of the invention.

Use of Antibiotic Susceptibility Data

[00213] In yet another aspect, a method of taking a course of medical action by a user is provided including initiating a course of medical action based on sample analysis. The course of medical action can be delivering medical treatment to said subject. The medical treatment can be selected from a group consisting of the following: a pharmaceutical, surgery, organ resection, and radiation therapy. The pharmaceutical can include, for example, a chemotherapeutic compound for cancer therapy. The course of medical action can include, for example, administration of medical tests, medical imaging of said subject, setting a specific time for delivering medical treatment, a biopsy, and a consultation with a medical professional. The course of medical action can include, for example, repeating a method described above. A method can further include diagnosing the medical condition of the subject by said user with said sample. A system or method can involve delivering a medical treatment or initiating a course of medical action. If a disease has been assessed or diagnosed by a method or system of the invention, a medical professional can evaluate the assessment or diagnosis and deliver a medical treatment according to his evaluation. Medical treatments can be any method or product meant to treat a disease or symptoms of the disease. In an embodiment, a system or method initiates a course of medical action. A course of medical action is often determined by a medical professional evaluating the results from a processor of a computer system of the invention. For

example, a medical professional can receive output information that informs him that a subject has a 97% probability of having a particular medical condition. Based on this probability, the medical professional can choose the most appropriate course of medical action, such as biopsy, surgery, medical treatment, or no action. In an embodiment, a computer system of the invention can store a plurality of examples of courses of medical action in a database, wherein processed results can trigger the delivery of one or a plurality of the example courses of action to be output to a user. In an embodiment, a computer system outputs information and an example course of medical action. In another embodiment, the computer system can initiate an appropriate course of medical action. For example, based on the processed results, the computer system can communicate to a device that can deliver a pharmaceutical to a subject. In another example, the computer system can contact emergency personnel or a medical professional based on the results of the processing. Courses of medical action a patient can take include self-administering a drug, applying an ointment, altering work schedule, altering sleep schedule, resting, altering diet, removing a dressing, or scheduling an appointment and/or visiting a medical professional. A medical professional can be for example a physician, emergency medical personnel, a pharmacist, psychiatrist, psychologist, chiropractor, acupuncturist, dermatologist, urologist, proctologist, podiatrist, oncologist, gynecologist, neurologist, pathologist, pediatrician, radiologist, a dentist, endocrinologist, gastroenterologist, hematologist, nephrologist, ophthalmologist, physical therapist, nutritionist, physical therapist, or a surgeon.

[00214] Once the number of positive wells or reaction chambers has been determined, that number is processed using Poisson statistics and prior knowledge about the chip in question to determine the original concentration of sample in the chip. This information is then automatically sent via email to any valid email account and is then received by the original person who took the image regardless of where they are in the world relative to the computer that performs the image analysis. The time that elapses between the taking of the image and the receipt of email confirmation has been performed in well under 1 minute, although actual time is subject to the upload speed on the network of the cell phone and download speed on the network of the computer. This is important, because if an error is detected in the course of an analysis, such as not being able to find all 4 spots, the user needs to be quickly alerted that another image must be taken. The software has been programmed to do such, and the user typically knows in under 1 minute to take another image. Having the ability to notify by email can give the ability to notify via text. Cell phone providers can have a service that will send the body of an email as a text to specific users. Other servers that can be leveraged as SMS messengers. The analysis process can use computer automation to notify a user if the image can be used. The notification

can be an SMS message, email message, phone call, web posting, or electronic message for example. In some embodiments, the amount of time from the uploading of the image until the user is notified can be referred to as the analysis process. The analysis process can take less than 5 min, 4 min, 3 min, 2 min, 1 min, 50 sec, 45 sec, 40 sec, 30 sec, 20 sec, 10 sec, 9 sec, 8 sec, 7 sec, 6 sec, 5 sec, 4 sec, 3 sec, 2 sec, 1 sec, 0.5 sec, 0.4 sec, 0.3 sec, 0.2 sec, or 0.1 sec, for example. In some embodiments, the analysis process takes less than 1 min.

[00215] At least one calibration source for providing a calibration emission, and at least one calibration photodiode for sensing the calibration emission wherein the control circuitry has a differential circuit for subtracting the calibration photodiode output from each of the detection photodiode outputs can be provided in some embodiments.

[00216] A communication interface can be a universal serial bus (USB) connection such that the outer casing is configured as a USB drive.

[00217] In some instances the information is transmitted back to the mobile device which was used for imaging. For example an image can be obtained, send to a separate computer for analysis, and then the image or date related to the image can be transmitted back to the mobile device. In some embodiments an image and/or a processed image and/or resulting data the user is transmitted to a separate device, e.g. a physician's mobile device can receive the information. In some instances two or sets of information are transmitted to two or more devices. The two or more sets of information can be the same information, or in some embodiments, separate data is sent to each user. For example a patient can receive some information related to an image while the patient's doctor receives information more suitable for a physician's analysis.

[00218] While offloading the analysis of images to "the cloud" provides a number of benefits, including traceability and archiving of raw data, global access, and compatibility with virtually all smartphone operating systems, it requires a wireless data connection of sufficiently high bandwidth; thus, direct on-phone analysis could be preferable in some scenarios.

Platforms / Devices

[00219] Provided herein are devices (e.g., microfluidic devices) and methods that can rapidly identify a cell, including a cancer cell, or microorganism, including a pathogen, quantify their load, and diagnose their susceptibility or resistance to drugs, such as antibiotics. In some embodiments the devices can enable phenotypic detection and metabolic profiling of drug susceptibility or drug resistance using individual microorganisms or cells which can originate from various sample types, including clinical or environmental samples. These sample types can include, but are not limited to, blood, cerebral spinal fluid (CSF), saliva and urine and can also include environmental samples, such as from water or a hospital surface. In some embodiments, the devices enable incubation of cells with drugs, such as antibiotics, and then rapidly extract and quantify nucleic acids or other molecules in a contamination-free platform. The devices can use digital single- molecule measurements in microfluidics devices, which provide ultra-sensitive measurements that improve detection limits while providing quantitative data, important for differentiating pathogens from contaminants and enabling earlier differentiation between drug-resistant and susceptible organisms or cells. In some embodiments, these devices can differentiate the state of individual microorganisms or cells from a clinical sample, and understand the timing of their individual responses to drugs, such as for example antibiotics, providing ultra-fast drug- susceptibility measurements.

[00220] In some embodiments the devices and methods can be used in assessing gene duplications measurements for each given cells, as in response to antibiotic stress some microbes can replicate antibiotic resistant genes (like lactamases) to 100s of copies, allowing them to undergo evolution.

[00221] In some embodiments, the devices and methods allow identification of drug-resistant bacteria from a pool of bacteria, such as a clinical sample, that can include drug-susceptible bacteria and/or drug-resistant bacteria and/or contamination with pathogenic and/or nonpathogenic bacteria – or some combination of these types. In some embodiments the devices can be used to incubate cells with drugs and then rapidly extract and quantify nucleic acids, such as for example RNA, in a contamination-free platform to determine drug susceptibility. In some embodiments, the methods and devices provided herein enable microbial and cell identification and drug susceptibility testing outside of CLIA clinical laboratories

[00222] Devices can comprise channels and flowpaths, such as microfluidic channels. Devices can comprise inlets, outlets, or any combination thereof. Devices can comprise wells, reservoirs, or any combination thereof. Devices can comprise reaction volumes. Devices can comprise pre-loaded reagents. In some cases, the microfluidic device comprises a SlipChip device, as

described for example in U.S. Patent Application No. 13/257,811, in PCT Application No. PCT/US2010/028361, in US Patent Publication No. 20120329038 A1, and in International Patent Publication No. WO 2013072069 A1, each of which is hereby incorporated by reference in its entirety. In some embodiments, the samples are contacted with a reagent for performing a reaction. As used herein, "contacting" refers to introducing a substance or maintaining a substance in an area or in a solution comprising the entity the substance is to be contacted to. For example, contacting a region for the amplification of a target nucleic acid with a reagent for performing the reaction can include flowing the reagent into the chamber, either separately or already together with the target nucleic acid, or having the reagent already preloaded into the region for amplification. Contacting can occurs when two substances are interacting under conditions to bind, touch, facilitate a reaction, or otherwise maintain proximity under desired conditions.

[00223] Platforms can comprise fluid handling mechanisms enabling loading, unloading, mixing, and other handling of sample volumes, reagent volumes, and other fluids. For example, a microfluidic device can be used comprising channels for loading fluids into wells or droplets, for mixing contents of wells or droplets, or for off-loading of contents of wells or droplets.

[00224] Some platforms are useful for conducting assays in a digital or quasi-digital format, as described herein. For example, wells, well plates, microwells, microfluidic droplets, emulsions, beads, and microarrays can provide a useful platform for conducting a digital or quasi-digital assay. In such an assay, the compartments can comprise individual wells, droplets, beads, or microarray spots.

[00225] In some embodiments, devices described herein incorporate SlipChip digital amplification technology and SlipChip sample preparation technology (Shen, et al., 2010, Analytical Chemistry, 4606-4612, Shen, et al., 2010, Lab on a Chip, 2666-2672, Shen, et al., 2011, Analytical Chemistry, 3533- 3540, Shen, et al., 2011, J Am Chem Soc, 17705-17712) and can perform rapid single- molecule identification, and quantification of nucleic acids from cells and microorganisms, such as for example *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and extra-intestinal pathogenic *E. coli*. Samples can be obtained from a variety of human bodily fluids, such as for example blood, plasma, saliva, CSF or urine, or a variety of environmental samples, such as for example water or hospital surfaces.. In some embodiments, this can enable clinical validation of workflow that in some embodiments provides a differential diagnosis of an infection, such as for example a UTI. In some embodiments, this device can provide quantification of total cell load and species and/or strain identification. In some embodiments,

the results of the assay can be available in less than 45 minutes, less than 30 minutes, less than 20 minutes, less than 10 minutes, less than 5 minutes, less than 3 minutes, or less than 1 minute.

[00226] In some embodiments, the technology described here enables identification and quantification of nucleic acids, such as for example bacterial DNA and RNA extracted from urine samples spiked with a cell culture of reference strains. Reference strains are those are used routinely in clinical labs as part of their quality control protocol, such as for example *K. pneumoniae* (ATCC 700603), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922).(Institute., 2012, M07-A09) In some embodiments the technology and devices described in this disclosure can be integrated with standard bench methods and kits (Qiagen Qiamp DNA kit) to extract nucleic acids, and previously published universal primers can be used to target genes, such as for example the conserved region of the 16S gene, in order to quantify nucleic acid concentration (Clifford, *et al.*, 2012, PLoS ONE, e48558, Nadkarni, *et al.*, 2002, Microbiology, 257-266),. For example, the technology can be used to target hyper-variable regions of the 16S gene to quantify and identify microorganisms (Baker, *et al.*, 2003, Journal of Microbiological Methods, 541-555, Hansen, *et al.*, 2013, PLoS ONE, e61439, Spilker, *et al.*, 2004, Journal of Clinical Microbiology, 2074-2079).

[00227] In some embodiments, the devices described herein allow identification of drug-resistant bacteria from a pool of bacteria, such as a clinical sample, that can include drug-susceptible bacteria and/or drug-resistant bacteria and/or contamination with pathogenic and/or nonpathogenic bacteria, or any combination thereof.

[00228] In some embodiments, the technology can be used for bacterial DNA and RNA amplification assays using the digital SlipChip over a dynamic range of, for example, 1,000 to $1x10^7$ copies/mL with, for example, three-fold resolution and a 95% confidence interval. This dynamic range is well covered by the digital SlipChip that is commercially manufactured, which contains 10,240 of 0.84 nL wells with a dynamic range of 450 to $9.7x10^7$ copies/mL and a detection limit of 120 copies/mL.

[00229] A workflow of determining the resistance or susceptibility of a cell in a sample of urine to a drug according to an embodiment of the invention is shown in Figure 5. In some embodiments, the device enables assays that perform both sample prep (e.g., exposure of organism in the sample to a drug and extraction of target nucleic acids from the sample) and digital amplification (e.g., distributing and amplifying target nucleic acids to quantify the target nucleic acids present in the sample to determine a susceptibility or resistance of the organism to the drug).

Integrated Devices

[00230] In some embodiments, the devices provided herein are integrated devices comprising one or more modules. These modules include, but are not limited to, an incubation module, a sample preparation module, an amplification module, and a readout module. Each of these modules is described herein. In some embodiments, the integrated device combines 2 or more modules to provide a simplified processing flow for detecting the properties of an organism in a cell in response to a drug. An example of an integrated device comprising an incubation chamber and devices to regulate flow of the sample into the incubation chamber is depicted in Figures 6A, 6B, and 6C.

Incubation Module

[00231] In some embodiments, the integrated device comprises an incubation module. The incubation module can contain an incubation chamber for, e.g., exposing a microorganism to a drug (see Figures 6A, 6B, and 6C). In some embodiments, the incubation module contains inlets allowing for the inserting of fluids, such as sample, media, reagents, or other solutions. These solutions are pushed into the SlipChip-like device through some means, an example being positive pressure from a pumping lid or plunger as shown in Figure 6C. In some embodiments, the layer adjacent to the SlipChip layer contains channels through which the sample can flow. In some embodiments, the uppermost layer has one or more openings for venting and controlling pressures. In some embodiments, the openings of the SlipChip layer are filled with solution, splitting the sample into different volumes. This layer is then slipped, the openings of the SlipChip layer are moved to form a connection with a separate layer, for example an opening to incubation chambers. The samples can be transported from the central SlipChip layer and mixed with solution, such as media or antibiotics, contained within the incubation chambers. In some embodiments, the incubation chambers can be sitting in a heated bath or otherwise in contact with heating elements for incubation. In some embodiments, membranes such as hydrophobic films are used for venting. In some embodiments, one or more openings are present in one or more layers of the device for controlling pressures. In some embodiments, the layer containing the incubation chamber can also be slipped. In some embodiments, fluids from the incubation chambers are transported to a separate module, such as the amplification module. In some embodiments, one or more SlipChip layers are used, for example, below the incubation chambers with openings to the sample prep or other module. In some embodiments, the integrated device has additional incubation modules.

[00232] In some embodiments, the incubation chamber in the incubation module is used for antibiotic susceptibility testing. In some embodiments, cells are pre-sorted through a filter. In

some embodiments, a filter is used to capture bacterial cells. In some embodiments, mammalian cells are lysed selectively without lysing bacterial cells of interest. In some embodiments, selective lysis and filtration steps are combined. In some embodiments, the incubation chamber comprises an array of sub-chambers for confinement of cells.

[00233] In some embodiments, the incubation chamber is a module configured to perform one or more of the following: (i) partitioning the sample, (ii) partitioning the drug/antibiotic, (iii) combining the sample with the drug/antibiotic, (iv) mixing, (v) incubating the sample, or (vi) transporting the fluids to a separate chamber.

[00234] In some embodiments, digital assays can use the confinement of cells from a sample into an incubation chamber or a clonal isolation regions. In some embodiments, the isolated cells are cultured in individual incubation chamber to generate a plurality of clonal populations from the sample. In some embodiments, the isolated cells are treated with a drug before or after expansion. In some embodiments, after culture and expansion from an isolated cell, the clonal population is divided into two or more treatment regions. As such, at least one portion of the clonal population can be treated with a drug, while another portion of the clonal population is not treated with a drug. Then, the assays described herein can be performed to determine whether the cells in the clonal population are susceptible or resistant to one or more drugs.

[00235] In some embodiments, splitting is performed through a SlipChip design utilizing dead-end filling. In some embodiments, to achieve dead-end channel filling with an aqueous solution, a hydrophobic membrane can be used at the end of the channel which blocks the flow of aqueous solution but allows non-aqueous liquid and gas to flow through.

[00236] In some embodiments, slipping (i.e., movement of the layers) can be actuated automatically using a rotating shaft. In some embodiments, this shaft can be controlled by a motor, optionally encoded or programmed to specific speeds, directions, and/or rotation angles.

[00237] In some embodiments, combination and mixing of the sample with the drug/antibiotic is performed by pressurization of a chamber which transports one or more of the solutions into the other solution. In some embodiments, pressurization of chambers is generated by a pumping lid which creates a seal and changes the volume in that compartment; this can be actuated manually or through a rotating shaft, among other actuations. In some embodiments, transport of fluids into or out of the growth chamber module can be performed by pressurization of the chamber, such as for example by a pumping lid (either manually or automatically), the opening of a valve, or by other methods, such as e.g. pipetting.

[00238] In some embodiments, the incubation chamber module contains a heating element. Some examples of heating element control include a circuit board with a means to control the temperature and timing or phase change material

[00239] In some embodiments, the incubation chamber is designed to process between 1 mL and 10 mL. In some embodiments, the incubation chamber is designed to process between 100 μL and 3 mL. In some embodiments, the incubation chamber is designed to process between 10 μL and 100 mL. In some embodiments, the incubation chamber is designed to process samples likely containing single cells.

Sample Preparation Module

[00240] In some embodiments, the device comprises a sample preparation module. In some embodiments, samples can be split into different compartments that may or may not contain a drug of interest (such as an antibiotic). In some embodiments, after incubation of the sample with/without antibiotic drug, the cells of interest (such as for example those of a bacterial pathogen) are lysed (such as by lysis buffer) in order to extract RNA and/or DNA. In some embodiments, the sample-preparation module can be used to automatically extract nucleic acids from urine. In some embodiments, for sample preparation, a filter column can be used to capture RNA/DNA from a lysed sample and wash away cell debris or other components present in the lysed sample.

[00241] In some embodiments, an automated or semi-automated sample preparation module is provided. In some embodiments, the automated or semi-automated sample preparation module generates pressure to the lysed sample in a sealed environment, forcing the lysed sample through a filter column (e.g., a nucleic acid-binding column, such as a glass fiber column).

[00242] In some embodiments, the sample prep module is designed to process between 1mL and 10 mL. In some embodiments, the sample prep module is designed to process between 100 μL and 100 mL. In some embodiments, the sample prep module is designed to process between 10 μL and 1 L. In some embodiments, the sample prep module is designed to process between 1 μL and 1 mL. In some embodiments, the sample preparation module can be made of plastic and in some embodiments does not require external power or active user intervention.

Digital Quantification Module

[00243] In some embodiments, devices contain digital (or binary) quantification modules, such as modules containing multiple detection elements in which 0, 1, or more target (e.g. RNA/DNA) molecules are captured per each detection element and are suitable for integration.

[00244] In some embodiments, enriched target (e.g. DNA/RNA) are recovered from the device for use in subsequent or parallel experiments. In some embodiments, the integrated device has a plurality of quantification modules.

[00245] In some embodiments, the quantification module is designed to process between 30 μL and 60 μL. In some embodiments, the quantification module is designed to process between 1 μL and 100 μL. In some embodiments, the quantification module is designed to process between 100nL and 1mL. In some embodiments, the quantification module is designed to process between 10nL and 10mL.

Reagent Storage

[00246] In some embodiments, the integrated device comprises stored reagents. In some embodiments, the liquids in the devices (including those described above) can be stored, such as in blister packs, reagent packs, or other types of containers in which they are sealed (Figure 7).

[00247] In some embodiments, the device comprises a reagent pack suitable for preparing nucleic acid of the cell or microorganism for nucleic acid quantification reaction. In some embodiments, the device comprises a reagent pack suitable for nucleic acid quantification reaction. In some embodiments, the reagent pack comprises enzymes for performing a nucleic acid quantification reaction. In some embodiments, the reagent pack comprises primers for performing a nucleic acid quantification (e.g., for PCR or isothermal amplification)

[00248] Exemplary reagents in the reagent pack can include, but are not limited to, lysis solutions, wash solutions, elution solutions, rehydration solutions, enzyme solutions (e.g., nucleic acid amplification enzymes, polymerase enzymes, restriction enzymes), buffers, liquid, powder, pellets, a gel, microbeads, probes, primers, nucleic acids, DNA, RNA, polypeptides, nucleoside triphosphates (NTPs), antibodies, a sacrificial reagent or any combination thereof. A sacrificial reagent can comprise an aqueous solution, a lubricant, an oil, an aqueous-immiscible liquid, a gel, a gas, a fluorocarbon oil, a surfactant, gas, air, or any combination thereof. For example, the air can be used to generate air bubble for mixing. As another example, air and immiscible liquid can be used to remove leftover solution (dead volume) in the matrix. Reagents can be mixed to change their composition. For example, one type of buffer can be mixed with another buffer or a dry reagent to change its composition to another buffer.

[00249] In some embodiments, the device comprises a reagent pack for assaying enzyme presence and/or activity, such as, for example, monobromobimane, 7-Diethylamino-3-(4'-Maleimidylphenyl)-4-Methylcoumarin, N-(7-Dimethylamino-4-Methylcoumarin-3-yl))Maleimide), NiWa blue (1-Benzyl 4-methyl 5-(tert-butoxycarbonylamino)-2-(2,5- dioxo-2,5-dihydropyrrol-1-yl)terephthalate), NiWa Blue II (Dimethyl 5-acetamido-2-(2,5-dioxo-2,5-

dihydropyrrol-1- yl)terephthalate), NiWa orange (Dimethyl 2-amino-5-((2,5-dioxo-2,5-dihydro-1 H-prrol-`- yl)ethyl)amino)terephthalate), Ellman's reagent/DTNB (5,5'-dithiobis-(2-nitrobenzoic acid)), Umbelliferone-derived cephalosporins, Fluorescein-derived cephalosporins, Resorufin-derived cephalosporins, Rhodamine-derived cephalosporins, Imipenem, and p-nitrophenol releasing substrates

[00250] In some embodiments, the device comprises a reagent pack containing one or more drugs at one or more concentration. In some embodiments, the device comprises a reagent pack containing one or more antibiotics at one or more concentration. In some embodiments, the device comprises a reagent pack containing components suitable for accelerating response of a cell or microorganism to a drug (e.g. quorum sensing molecules, etc.). In some embodiments, the device comprises a reagent pack containing culture media to enhance cell growth.

[00251] In some embodiments, the device comprises a reagent pack containing gases or gas mixtures, containing H_2S, CO, and NO. Such gases are known, for example, to affect susceptibility of microorganisms to antibiotics. Such gases can be diluted with a gas mixture which could be anaerobic, aerobic, or microoxic. Such gas mixture can contain CO_2. In some embodiments, the device comprises a reagent pack containing lysis reagents to expose intercellular components.

[00252] In some embodiments, these containers avoid evaporation of solutions. In some embodiments, reagents can be released from one or more container by piercing certain regions of the containers, or by selectively opening parts of the containers at the appropriate times. In some embodiments, the generated pressure in the device can release the contents of the blisters/containers; in some embodiments these fluids are transported to other parts of the device. In some embodiments, reagents (such as e.g. amplification reagents) are stored as dried reagents, on device, in blister packs, or in containers (or some combination thereof). The reagents can be lyophilized, stored with sugars (e.g. sucrose, trehalose, among others), and/or stored with beads.

Operation Module

[00253] In some embodiments, the device contains an operation module, such as for example a module to operate devices (including integrated devices). In some embodiments, an operation module can perform one or more of the following functions: motor control, shaft rotation, heating, image capture, and image processing. In some embodiments, an operation module can use one or more motors and shafts. In some embodiments, an operation module can use one or more rotary valves.

[00254] In some embodiments, an operation module consists of one or multiple components, of which one or multiple components can be driven/rotated by a spring or motor (Figure 8). In some

embodiments, a spring or motor can be used to rotate a threaded part, which can be connected to a separate lid/plunger with the inverse thread of the former part (as described in pumping lid patent application). In some embodiments, as the spring or motor rotates the first threaded part, the plunger/lid can move and change the volume and pressure present in one or more cavities. In some embodiments, the lid/plunger forms a hermetic seal within one or more cavities. In some embodiments, the generated pressure can be used to flow a liquid sample that is present in the cavity through a filter column.

Fluid Management

[00255] In some embodiments, the device is configured to transport a volume of lysed samples out of device for a separate or parallel experiment. In some embodiments, valves for generating pressure to control the flow of solutions through the device (such as in which different solutions are flowed through the column or temporarily prevented from flowing through the column) are incorporated into the device. In some embodiments, negative pressures are utilized during device operation. In some embodiments, chambers within the device can be pressurized or depressurized prior to the initiation of the device.

[00256] In some embodiments, a one-step or multistep process can be controlled, such as by placing a filter column in the rotating layer of the device. In some embodiments, as the filter column is rotated in the device, the sample can be moved to different chambers in the device that can contain different reagents; these fluids can flow sequentially or nonsequentially through the filter column. In some embodiments, the liquid present in one or more chambers will be moved (such as via the generated pressure of a pumping lid) and a solution will flow through the filter column. In some embodiments, when a filter column in the device is in the first position, a solution can be pumped out of a first cavity; in some embodiments when the device is moved into a second position, a second solution is pumped. Devices can contain one or more overlaps of the filter column and one or more chambers containing liquid. In some embodiments, the distance and/or angle among different overlap positions can be programmed by the device design.

[00257] In some embodiments, a lysed sample can be flowed through a filter column to capture a target (e.g. DNA or RNA) on a filter column. In some embodiments, subsequent steps involve washing— e.g. the captured DNA or RNA specific to resistant or susceptible cells can be washed, such as with a washing buffer, and eluted off the column with elution buffer to be released from the column.

[00258] In some embodiments, after the target (e.g. DNA/RNA) is enriched and eluted off the column, the eluted DNA/RNA can be transported from the column into the next chamber or

module, also called quantification module or device, that in some embodiments is suitable for quantification of target (e.g. DNA/RNA) molecules.

Other Modules and Elements

[00259] In some embodiments, the device contains an operation module, such as for example a module to operate devices (including integrated devices). In some embodiments, an operation module is configured to perform one or more of the following functions: motor control, shaft rotation, heating, image capture, and image processing. In some embodiments, an operation module comprises one or more motors and shafts. In some embodiments, an operation module comprises one or more rotary valves. In some embodiments, one or more layers are connected to a shaft and are capable of rotation. In some embodiments, one or more layers are configured to disconnect from the shaft. In some embodiments, one or more layers comprise a detent ball and remain connected to the shaft at low torques but disconnect at high torques (i.e. when the layer is physically stopped).

[00260] In some embodiments, one or more layers are preprogrammed to rotate a specified degree. In some embodiments, the operation module comprises a stopping component to stop one or more layers physically.

[00261] In some embodiments, the device contains an enrichment or pre-concentration module. In some embodiments, the enrichment module concentrates the number of organisms. In some embodiments, these devices contain a threshold module. In some embodiments, the cutoff module limits the number of organisms to a threshold value. In some embodiments, the enrichment and threshold module are combined. In some embodiments, the enrichment module is designed to process between 1 mL and 10 mL. In some embodiments, the enrichment module is designed to process between 100 μL and 100 mL. In some embodiments, the enrichment module is designed to process between 100 μL and 1 L.

[00262] In some embodiments, the device contains an incubation chamber and at least one detection chamber in proximity, such as within 10 cm of each other. In some embodiments, the device contains an incubation chamber and at least one detection chamber in proximity, such as within 20 cm of each other. In some embodiments, the device contains an incubation chamber and at least one detection chamber in proximity, such as within 1 m of each other.

[00263] In some embodiments, the integrated device integrates one or more of the following: enrichment module, cutoff module, incubation module, sample prep module, quantification module, and operation module. In some embodiments, the integrated device integrates the incubation module, with the autonomous device and digital SlipChip. In some embodiments, modules have interlocking features. In some embodiments, modules are interchangeable. In

some embodiments, modules useful for sample preparation and handling are included in the device.

[00264] In some embodiments, an integrated device has dimensions smaller than 10x10x10 cm, or integrated device has dimensions smaller than 20x20x20 cm.

[00265] In some embodiments, the device comprises flexible materials such as, but not limited to PDMS, Tango+ (a 3D-printable soft material), or rubber. The flexible materials enable the device to perform leak-proof liquid movement of a sample among modules (e.g., between the sample preparation module to the amplification module). In some embodiments, the device comprises channels/microchannels to interface modules. In some embodiments, the device comprises syringe pumps/teflon tubing. In some embodiments, the device comprises embedded channels in 3D-printed parts. In some embodiments, the device comprises 3D-printed valves. These components can also enable the device to perform leak-proof liquid movement of a sample among modules.

[00266] In some embodiments, a quantification device has a series of compartments with varying volumes. In some embodiments, a quantification device incorporates a series of on-chip dilutions before loading a series of single volume compartments. In some embodiments, these devices enable a large dynamic range without abnormally large compartment sizes

[00267] As an example, a sample can be diluted 1:10 four consecutive times. Each dilution is loaded into a series of single volume digital compartments. 400 compartments can quantify and resolve 1.5-fold differences in concentration with a 1-log dynamic range. Thus, the example device consists of four sets of 400 compartments each. The four 10x dilutions can be loaded into these four sets of single volume compartments and will enable 1.5-fold resolution with 4-log dynamic range and antibiotic susceptibility testing.

[00268] In some embodiments, the devices comprise a readout module or mechanism to generate a readout. Platforms can be compatible with one or more readout or detection mechanisms. For example, a platform can be transparent or translucent in part or in total, allowing fluorescent measurement, detection of precipitate or gas bubble, or other visual observation. A platform can comprise visual detectors, such as CCDs, CMOS sensors, cameras, photon detectors, and other sensors. In another example, a platform can comprise electrical sensors, such as electrodes positioned within microwells. Platforms can be compatible with off-loading of samples for analysis. For example, a platform can permit unloading of droplets or contents of wells for mass spectrometry, sequencing, or electrophoresis.

[00269] In some embodiments, the method of reading the quantification module include, but are not limited to, fluorescence microscopy, brightfield microscopy, a camera, a digital camera, or a cell phone camera, among others. In some embodiments, a base station with an camera is used to capture the image. Image processing can be integrated in the base station or the image file can be transferred to a computer. In some embodiments, a cell-phone picture is taken. Phone software can be utilized to process the image or the image can be uploaded to a server for image processing.

[00270] In some embodiments, heating elements are embedded in the device, such as those into which an incubation module can be inserted, such as for example a bath containing high thermal conductivity materials. In some embodiments, amplification and detection of a target (e.g. RNA/DNA) can include heating elements on the device. Examples of heating elements can include, but are not limited to, heating by using electrical power, chemical reaction, or phase change materials. In some embodiments, transparent thermally conductive materials can be used such that images of the amplification region can be captured through the heating unit.

[00271] In some embodiments, these devices incorporate mixing methods. In some embodiments, jet macro-mixing can be used to mix solutions (Nealon, A. J., O'Kennedy, R. D., Titchener-Hooker, N. J., & Lye, G. J. (2006). Quantification and prediction of jet macro-mixing times in static microwell plates. Chemical engineering science, 61(15), 4860-4870). In such an approach, one or more solutions are pressurized and an opening between them allows one solution to flow into the other, or for both solutions to collide. In some embodiments, one or more solutions are gaseous mixtures, and generate bubbles to form a bubble mixer. In some embodiments, mixing is used to mix liquid samples and dry reagents. Alternating positive and negative pressures can be applied to improve mixing. In some embodiments, mixing can be accomplished mechanically, for example by an impeller, magnetic bead agitation, gravity, passive structures, etc., or combination of such methods.

[00272] In some embodiments, these devices contain a chamber or array of chambers for combining or mixing reagents.

[00273] In some embodiments, these devices contain special materials. In some embodiments, one or more materials in the device are compatible with organisms (e.g. materials that do or do not negatively affect viability). In some embodiments, a material or surface coating can be added to materials for one or more of the following purposes: to enhance thermal conductive properties, control surface chemistry/surface tension (hydrophobicity), enhance organism viability, alter diffusive properties, prevent adsorption, sealing, prevent leeching (i.e. plastics), alter rigidity, among others.

[00274] In some embodiments, these devices utilize one or more manual steps —such as but not limited to addition of sample, insertion of reagents, adjusting settings, slipping, rotation, attaching pumping lid, actuating pumping, inserting materials, connecting modules, initiating the device, initiating heating, transferring fluids, removing modules capturing images, etc.

[00275] In some embodiments, devices can be fabricated by a number of methods including 3D printing, injection molding, embossing, wet etching, and other methods known in the art.

Device Uses

[00276] The digital platform in some embodiments of the device allows for detection of lower abundance bacteria than in current nucleic acid-based technologies, which enables more robust analysis of blood and CSF samples. The increased precision of digital technology improves monitoring of patient response to therapy.

[00277] The sample-prep module can provide rapid nucleic acid extraction and purification within 5 min, reducing degradation of unstable RNA.

[00278] The point-of-care module can allow for broad-based testing in clinical populations across a wide range of infections, and in both clinical trials and surveillance.

[00279] The digital amplification module can allow for a clearer understanding of low-level infection in a more proximate timeframe than culture methods and can inform on the heterogeneity of response to antimicrobials.

[00280] In one embodiment, the devices can be used with sample preparation technology to isolate bacterial DNA and RNA rapidly and in high yield from target microorganisms or cells, such as for example *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and extra- intestinal pathogenic *E. coli* and can include clinical samples and can include a variety of bodily fluids, such as for example urine. Sample-prep SlipChip for bacterial DNA and RNA extraction and purification from urine samples containing Chlamydia trachomatis has previously been validated. Viral RNA extraction and purification from plasma samples containing HCV or HIV viral particles has also been validated. In one embodiment, the devices can be used to perform sample preparation in less than 5 min and with samples handling a range of volumes, such as for example up to 0.5 mL. In some embodiments, the device can be used for rapid detection of samples containing low bacterial loads.

[00281] In some embodiments, the devices can be used to extract and purify nucleic acids, such as bacterial DNA and RNA from samples (e.g. a urine sample) spiked with a bacterial target (such as for example *K. pneumoniae*, *P. aeruginosa*, or *E. coli*) in some cases in an extremely short period of time (such as for example less than 5 minutes) and in some cases with a yield

above 80% compared to standard bench methods, and at a quality suitable for both digital and real-time quantification.

[00282] Bacterial RNA is known to be unstable and expression levels can change rapidly. In cases where the quantity and quality of purified RNA can be compromised, the devices described in this disclosure can be used to further shorten the sample preparation protocol, such as for example to less than 3 min. This time frame has previously been demonstrated for isolation of viral RNA from blood plasma. In some embodiments, the lysis step can be modified by adding additional detergents or inhibitors to minimize the activity of RNase.

[00283] In some embodiments, the device can combine SlipChip sample preparation and digital quantification technologies for rapid, reproducible and quantitative measurements of nucleic acids, such as for example bacterial RNA levels in a sample, (e.g. a clinical urine sample) spiked with, for example, *K. pneumoniae*, *P. aeruginosa*, and *E. coli* from 100 CFU/mL to $1x10^6$ CFU/mL with 3-fold resolution. In some embodiments, the devices described herein have a performance greater than 99% specificity.

[00284] In some embodiments, the device or platform combines more than one module to quantify and identify the target microorganisms in for example, clinical urine samples.

[00285] In some embodiments, the devices will enable assays that perform both sample prep and digital amplification. In some embodiments, the total assay time can be less than 60 min, less than 30 minutes, less than 15 minutes. Current PCR protocols can be shortened by optimizing denaturation time, denaturing temperature, or by combining the annealing and extension steps(Bio-Rad). Using optimized amplification enzymes or fast thermal cyclers, 10 minute amplification can be achieved.(Wheeler, et al., 2011, Analyst, 3707-3712, Neuzil, et al., 2006, Nucleic Acids Research, e77). In some embodiments, these devices can perform sample prep in less than 5 min, a reverse transcription step of less than 5 min, and a digital SlipChip workflow less than 35 min, to achieve for example a less than 45 min workflow. In another embodiment, this RT digital PCR instrument can be used to help optimize the chip thermal cycling protocol.

[00286] In some embodiments, the devices can enable rapid diagnosis of drug resistance using small numbers of cells originating directly from clinical samples. The devices described here can in some embodiments be used to answer the following questions, which are essential to the design of rapid AST assays that don't rely on many rounds of cell division, and essential for doing AST assays when a limited number of cells is available, as is often the case for blood and CSF: (i) What are the single-cell growth, phenotypic, and gene expression profiles of drug-susceptible and drug-resistant microbial populations or cells shortly upon drug exposure? (ii) Are

there "sentinel" cells that can very rapidly (within less than 15 or even 7 minutes) predict the susceptibility or resistance of the population or cells? (iii) How many cells are necessary for reliable prediction of the drug susceptibility or resistance of the clinically relevant bacterial population? (iv) What is the gene expression signature and the shortest drug exposure that reliably predicts antibiotic susceptibility and resistance when the measurement is performed on pooled cells, and how closely can this measurement approximate the performance of the single-cell assays?

[00287] In some embodiments, the devices described herein can be used to monitor single cells from the target organisms or cells in response to drug exposure. In some embodiments, these devices have the capability to monitor growth, metabolic activity, phenotypic variation, and levels of gene expression at the single cell level.

[00288] In some embodiments, the devices enable measurement of the distribution of growth rates and doubling times of susceptible strains or cells in the presence of standard dilutions of a drug, such as for example ciprofloxacin (a fluoroquinolone) or ampicillin (such as a β- Lactam). Ciprofloxacin is regarded by clinicians as having the ability to prevent growth immediately (Barcina, et al., 1995, Journal of Microbiological Methods, 139-150), while ampicillin's mode of action is believed to allow as many as 5 generations of cell division before lysis occurs(Rolinson, 1980, Journal of General Microbiology, 317-323). It is well known that antibiotics can be rapidly lost in plastic devices, such as PDMS used for Fluidigm chips. Therefore, in some embodiments our devices can be composed of glass and in some embodiments they can be used to recover fluids(Ma, et al., 2014, PNAS, Ma, et al., 2014, Integrative Biology) to measure post-assay antibiotic concentrations.

[00289] In some embodiments the devices can measure gene expression by quantifying RNA present in single cells. The SlipChip device can analyze DNA from live bacteria from individual wells (Ma, et al., 2014, PNAS, Ma, et al., 2014, Integrative Biology) and can quantify the number of RNA copies in each well. In some embodiments, the devices can combine these applications to create single-cell gene expression assays.

[00290] In some embodiments, the devices can use genes reported to be differently regulated in response to antibiotic, such as recA and LexA (Barczak, et al., 2012, Proceedings of the National Academy of Sciences, 6217-6222), genes induced by OxyR and SoxS in response to antibiotic-induced oxidative stress(Dwyer, et al., 2014, Proceedings of the National Academy of Sciences, E2100-E2109), and measure genes near the origin of replication (oriC) that become amplified after treatment with antibiotics targeting DNA replication(Burgess, 2014, Nat Rev Genet, 362-362, Slager, et al., 2014, Cell, 395-406).

[00291] In one embodiment, the devices can be used for specific quantification of gene expression markers (such as for example 16S RNA and pre-rRNA), to identify drug susceptibility (Mach, et al., 2011, The Journal of Urology, 148-153, Rolain, et al., 2004, Journal of Antimicrobial Chemotherapy, 538-541). In another embodiment the devices can be used to establish the smallest change detectable in gene expression with at least 20 clinical isolates under a variety of incubation conditions and times (such as for example less than 10 min, or around 10 min, 20 min, or 30 min).

[00292] The technology described herein may, in some embodiments, optimize and/or accelerate these methods for resistance quantification of clinical samples. In some embodiments, the results of assays done on the devices described herein will be available in minutes rather than hours. In some embodiments, the invention enables a diverse set of manipulations on diverse volume scales (such as for example sample preparation to single-cell manipulation, single-molecule quantification, and cell-phone readout).

[00293] In some embodiments, the invention uses digital single molecule measurements to address antimicrobial susceptibility and provide ultra-sensitive measurements that can radically improve detection limits and can provide quantitative data, which is important for differentiation of pathogens from contamination and enabling earlier differentiation between drug-resistant and susceptible organisms. In some embodiments, this invention can be used for phenotypic, metabolic, and gene- expression profiling measurements in response to antibiotics and can use individual bacterial cells and can use samples originating from a clinical setting.

[00294] In some embodiments, this invention uses high-throughput single-bacterial-cell measurements in the clinic to determine antibiotic susceptibility. In some embodiments, this invention allows differentiation of the state of individual microorganisms or cells, such as for example samples obtained from a clinical human sample or from an environmental sample, and can allow temporal quantification of their individual responses to antibiotics and therefore ultra-fast drug-susceptibility measurements.

[00295] In some embodiments, the devices can be used to rapidly identify cells, quantify their load, and provide their susceptibility profile. In some embodiments the devices can be used to incubate cells with drugs and then rapidly extract and quantify nucleic acids, such as for example RNA, in a contamination-free platform to determine drug susceptibility. In some embodiments, this invention enables microbial and cell identification and drug susceptibility testing outside of CLIA clinical laboratories.

[00296] In some embodiments, the devices enable handling of large sample volumes over multiple steps, enabling fast, efficient nucleic acid extraction. Sample preparation SlipChip has

been validated previously for extraction and purification of bacterial RNA from urine spiked with *C. trachomatis* and viral RNA from blood plasma spiked with HCV viral particles. This device can process a 0.5 mL clinical sample, and the entire workflow, from raw sample to purified nucleic acid, can be completed within 5 minutes with no user intervention. This device has been validated for analyzing bacterial DNA from urine samples spiked with C. trachomatis, viral RNA from blood plasma spiked with HIV viral particles, and viral RNA from culture of influenza viruses.

[00297] In some embodiments, this invention includes a device for determining drug resistance of a cell or microorganism that incorporates nucleic acid quantification. In some embodiments this invention includes a device in which a sample derived from an organism pre-incubated with a drug is introduced into the devices described above.

[00298] In some embodiments, the devices measure the change in expression or in copy numbers of genes, such as for example genes close to OriC (such as for example, competence genes), after a sublethal dose of drug exposure. In some embodiments, gene expression, such as expression of the OriC-proximal genes, can be compared to the expression of household genes or to selected genes, such as genes located at the terminator (ter) site.

[00299] In some embodiments, the devices can assess cell competence itself in response to sublethal doses of a drug as an assay for drug susceptibility.

[00300] In some embodiments, the devices can assay bacterial susceptibility, such as for example susceptibility to β-lactam antibiotics, by compartmentalizing cells or microorganisms in small volumes via stochastic confinement (e.g. via droplets or wells of small size, such as picoliter or nanoliter). In some embodiments, these samples are taken from blood or urine or CSF. In some embodiments the cells are in the presence of an antibiotic and a fluorogenic reporter. In some embodiments, the devices can detect persisters and genotypically resistant, but phenotypically silent, cells, such as for example by induction of a resistant phenotype.

[00301] In some embodiments, the devices and methods herein can be used to analyze a time course of gene expression of microorganisms in a sample. For example, this analysis can monitor a change of gene expression in response to a drug. For example, time points of gene expression immediately prior to treatment with a drug and at one or more time intervals after addition of the drug to the microorganisms could be used (such time intervals can include one or more of about 3 minutes, about 5 minutes, about 7 minutes, about 10 minutes, about 15 minutes, about 20 minutes).

[00302] In some embodiments, the devices and methods herein can include incubation of microorganisms at a controlled temperature, such as for example 35°C, 37°C, 38°C, 39°C, or 40°C.

[00303] Using single cell analysis will enable observation of stochastic response times, enabling outlier cells that respond quickly to provide earlier-than-average results.

[00304] In some embodiments, the device provides a mechanism to perform single–molecule and single-cell measurements to detect small changes in relative nucleic acid concentrations and heterogenous cellular response to antibiotic treatment in pathogenic bacteria (Figure 9). First, a group of cells are subjected to antibiotic treatment, then changes in a selected gene's expression are quantified by two methods. For single-molecule digital amplification, cells are lysed together then RNA is quantified with high resolution to detect small overall changes. For single-cell measurements, cells are confined to individual wells in a microfluidic device and qPCR on each cell is used to take advantage of large, early responses in "sentinel" cells.

[00305] In some embodiments, the device can be used to assay bacteria from isolates and clinical samples in our devices and evaluate whether single cell analysis reveals "sentinel cells" that predict susceptibility or resistance earlier than possible in pooled-cell analysis (Figure 9). In some embodiments, the device can be used to optimize the media and growth conditions within a device to determine the minimum amount of time required to ensure that all vigorous cells have shown signs of growth. In some embodiments, the devices can be used to design and optimize rapid assays to determine drug susceptibility and drug resistance, for example antibiotic susceptibility and resistance of *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, or extra-intestinal pathogenic *Escherichia coli*. In some embodiments the devices can detect hetero-resistance (where the tested sample contains both a susceptible strain or cell and a resistance strain or cell). This phenomenon is well documented for Gram-positive bacteria(Musta, et al., 2009, Journal of Clinical Microbiology, 1640-1644, Kim, et al., 2002, Journal of Clinical Microbiology, 1376-1380, Wong, et al., 1999, Clinical Infectious Diseases, 760-767, Ariza, et al., 1999, The Lancet, 1587-1588, Editors, 2001, Journal of Medical Microbiology, 1018-1020) and some Gram negatives (Pournaras, et al., 2007, Journal of Medical Microbiology, 66-70) and is relevant to patient treatment and outcome, although detection of heteroresistance is not viewed as a frequent clinical problem for Gram-negative bacteria. In some embodiments, when heteroresistance is observed, the devices disclosed herein can be used to identify heteroresistance by sample types so that they can be targeted for analysis, such as for example by the single-cell platform.

[00306] In some embodiments, the devices can be used to incubate a microorganism or cell, such as for example incubating isolates of *K. pneumoniae* with antibiotic samples of, for example, Cefazolin, Ceftriaxone, Ciprofloxacin, Piperacillin-Tazobactam, Trimethoprim-Sulfamethoxazole, and Meropenem and *P. aeruginosa* with Ciprofloxacin, Piperacillin-Tazobactam, and Meropenem.

[00307] In some embodiments the devices can be integrated, so that for example the sample-prep SlipChip and the digital SlipChip are integrated to improve workflow/efficiency and avoid contamination. Integration of the two chips can prevent RNA degradation and eliminate contamination and simplify workflow. In some embodiments the devices can be integrated, so that for example the devices combine the identification device and the AST device to identify the pathogen, its load, and its antibiotic susceptibility (Figure 10).

[00308] In some embodiments, the invention identifies and quantifies bacteria and assesses antimicrobial susceptibility. In some embodiments, the platform leverages existing thermocyclers found in most laboratories, enabling a low-cost digital PCR capability to even the smallest of clinical laboratories in an "open" format that would allow for expansion of the test menu. In some embodiments of the device, sample preparation can be performed on a macro-fluidic, disposable chip that does not require external power.

[00309] In some embodiments, the invention incorporates the capability to quantify and assess antimicrobial susceptibility into a CLIA-waived structure in order to provide access to the technology in primary care and global health settings. In some embodiments, the invention uses isothermal enzymes, which have been well studied on the SlipChip platform, and can allow for equipment-free testing under a range of environmental conditions. In addition, in some embodiments the results are cell phone readable and can be obtained by a minimally trained user.

[00310] In some embodiments, the device can be modified for use with blood and can also include *Acinetobacter baumannii* in the test suite. In some embodiments the device can be used with low-abundance specimens and has RT PCR capability, which allows for more accurate testing of antimicrobial susceptibility even in low-abundance specimens. These capabilities offer significant clinical value in hospital settings.

[00311] In some embodiments, the device can rapidly diagnose an infection, such as for example a urinary tract infection (UTI), through species identification and quantification, and in some embodiments the device can also characterize the drug resistance of the species causing the infection. The device can thus significantly enhance clinicians' ability to provide antibiotic stewardship, and also to avoid inappropriate treatment when a UTI is not present or when bacterial counts do not warrant therapy.

[00312] In some embodiments, the device can quantify the number of bacteria that exist at the time of sample collection to determine if the bacterial load warrants therapy, as physicians typically do not treat at lower bacterial concentrations. In some embodiments, validation can also be done on the device. The bacterial concentration from the device can be correlated with the colony counts obtained using the current "gold standard" culture method for bacterial detection and characterization. In some embodiments, the device can be used to identify which bacteria are present using nucleic acid detection on a digital chip format.

[00313] In some embodiments, the device can be used to assess antimicrobial resistance of the identified organism to help guide choice of therapy. In some embodiments, samples, such as urine samples, can be incubated with a range of drugs, such as antibiotics, and gene expression tests can determine which drug regimen is best suited to treat the infection (for example by identifying to which drugs the bacteria are susceptible).

[00314] In some embodiments, the device can identify bacterial species with greater than 99% specificity and quantify bacterial load in urine over a dynamic range of 100 CFU/mL to 1,000,000 CFU/mL with better than 3-fold resolution.

[00315] In some embodiments of the device and methodology, the workflow allows rapid AST (anti- microbial susceptibility testing) for one or more species or strains or cell types against a panel of one or more drugs in less than 95 minutes, less than 30 minutes, less than 15 minutes, less than 5 minutes.

[00316] In some embodiments, the device is an integrated device capable of performing sample preparation and digital quantification so that it can be used for both quantification of bacterial load and quantification of gene expression for AST with at least 80% recovery of nucleic acids.

[00317] In some embodiments, the device enables a workflow that combines an identification device and an AST device to identify the organisms, quantify their load, and determine their antibiotic susceptibility.

[00318] A device for sample preparation from urine that has at least 80% recovery of nucleic acids in comparison to standard nucleic acids in comparison to the existing Qiagen sample preparation kit. In some embodiments, sample preparation can be performed automatically, without centrifugation, in less than 5 min, whereas the Qiagen method takes 30 min.

[00319] In some embodiments, bacterial species identification can be performed with greater than 99% specificity for organisms in comparison to culture.

[00320] In some embodiments, quantification of live bacterial load, such as in urine, can be performed over a dynamic range of 100 CFU/mL to 1,000,000 CFU/mL with better than 3-fold resolution compared to common culture methods.

[00321] In some embodiments, the invention enables a workflow that allows rapid AST (anti-microbial susceptibility testing) for one or multiple species against a drug or a panel of two or more antibiotics in less than 95 min.

[00322] In some embodiments, the invention enables a workflow that combines the preparation module or device, identification and quantification module or device and the AST module or device to identify the organisms, quantify their load, and/or determine their antibiotic susceptibility, in less than 2.5 hours, less than 2 hours, less than 1 hour, less than 30 minutes.

[00323] In some embodiments, the device provides multiplexed and quantitative diagnostic measurements of nucleic acids or proteins in CLIA-based and Limited Resource Settings (LRS) at a quality that is on par with the equipment typically used in reference laboratories.

[00324] In some embodiments, the platform is "open" so that reagents for other/additional bacteria can be added and the scope of measurements expanded.

[00325] In some embodiments, the device enables multiplexed and digital nucleic acid-based quantitative measurement of HCV and HIV viral loads with a dynamic range of 10^5. In some embodiments, the device enables multiplexed detection of pathogens (including bacteria and fungi).

[00326] In some embodiments, the device is a microfluidic device, such as a SlipChip, that enables sample preparation with one or more of the following characteristics: a device that meets or exceeds commercial standards for low cost fabrication, a device that ultimately will not require user intervention, a device that ultimately will not require electricity to operate, a sample-prep module or device that enables sample prep for HCV, chlamydia and gonorrhea, and influenza in less than 5 min, a device that uses inexpensive plastics and is amenable to mass-production technologies, a device in which operation and imaging can be performed by an untrained child, and a device in which operation and imaging can be performed using automatic cloud-based analysis and data transmission.

[00327] In some embodiments, the device can identify bacterial species with greater than 99% specificity and quantification of bacterial load in urine over a dynamic range of 100 CFU/mL to 1,000,000 CFU/mL with better than 3-fold resolution.

[00328] In some embodiments, the device can identify and quantitatively measure bacterial DNA and RNA from organisms, such as for example *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and extra-intestinal pathogenic *Escherichia coli* from clinical samples of urine, and can be adapted to also provide a quantitative measure of bacterial load.

[00329] In some embodiments, the device is a digital microfluidic device, such as a digital SlipChip, for identification and quantification of bacterial DNA and RNA extracted from a urine

sample spiked with *K. pneumoniae*, *P. aeruginosa* and *E. coli*. In some embodiments, the device can have above 99% specificity for DNA/RNA amplification for organisms or cells, and provide quantification of DNA/RNA extracted over the dynamic range of 1,000 to 1x107 copies/mL with 3-fold resolution.

[00330] In some embodiments, the device is a digital microfluidic device, such as a digital SlipChip, can be used for quantification of live bacterial load in urine sample by quantification of 16S rRNA. In some embodiments, the device can incorporate a rapid incubation step (less than 10 min) and can evaluate renormalization of gene expression for different sample types under various storage conditions.

[00331] In some embodiments, the device is a digital microfluidic device, such as a digital SlipChip, capable of quantification of RNA markers purified using standard bench method to quantify live bacterial load. In some embodiments, the device is a sample-prep device, such as a sample-prep SlipChip that can have higher than 80% of recovery of both DNA and RNA with reference strains and clinical isolates of *K. pneumoniae*, *P. aeruginosa* and *E. coli*. In some embodiments, the device can recover a quality of purified DNA and RNA suitable for both digital device applications and real time qPCR

[00332] In some embodiments, the device can extract and purify bacterial DNA and RNA from urine sample spiked with reference strains of *K. pneumoniae*, *P. aeruginosa* and *E. coli* with higher than 80% recovery compared to the standard bench method in less than 5 minutes.

[00333] In some embodiments, the methods employ an integrated device with multiple functionalities and in other embodiments the methods employ non-integrated devices, such as separate sample- prep devices and digital devices for identification and quantification. In some embodiments, the combination of the integrated sample-prep and digital devices for identification perform with greater than 99% specificity and are capable of quantification of a bacterial load with 3-fold resolution over the dynamic range of 100 CFU/mL to 1,000,000 CFU/mL.

[00334] In some embodiments, the device can perform a rapid Antimicrobial Susceptibility Test (AST) for organisms, such as *K. pneumoniae*, *P. aeruginosa* and *E. coli* by incubating samples with drugs for a short period of time, then quantifying RNA expression markers. In some embodiments, the device can provide a sample to answer turnaround of less than 2.5 hours or less than 2 hours or less than 1 hour. In some embodiments, the device can be used with clinical isolates or clinical samples.

[00335] In some embodiments, the device can quantify gene expression markers over a dynamic range of 100 copies/mL to 10,000,000 copies/mL. In some embodiments, the device can be used

to identify the detectable minimal difference over the same dynamic range, and this will provide guideline for incubation time and antimicrobial susceptibility test for a particular cell or microorganism.

[00336] In some embodiments, the device can be used to incubate samples with at least 20 clinical isolates of *E. coli* in the presence or absence of Ampicillin. In some embodiments of the device, bacterial RNA can be prepared and one or more RNA markers can be quantified. In some embodiments of the device the results will have at least 95% in agreement with standard clinical methods.

[00337] In some embodiments, the device can be used to incubate *E. coli* with a drug, such as an antibiotic, including Cefazolin, Ceftriaxone, Ciprofloxacin, Piperacillin-Tazobactam, Trimethoprim-Sulfamethoxazole, and Meropenem. In some embodiments of the device, bacterial RNA can be prepared and expression level of RNA markers quantified using digital methods and in a time frame of less than 95 min, less than 60 minutes, less than 30 minutes, less than 15 minutes. In some embodiments, the device can be used to incubate negative urine spiked with at least 20 clinical isolates of *E. coli* in the presence and absence of Cefazolin, Ceftriaxone, Ciprofloxacin, Piperacillin-Tazobactam, Trimethoprim-Sulfamethoxazole, and Meropenem. In some embodiments, the device can quantify expression level of RNA markers with a workflow performance of less than 5% major errors, and no very major errors.

[00338] In some embodiments, the device can incubate samples of at least one clinical isolate in the presence and absence of a drug of a panel of clinically relevant drugs. In some embodiments, the device can quantify expression level of RNA markers in bacterial DNA to perform AST and has less than 5% major errors, and no very major errors.

[00339] In some embodiments, the device integrates sample preparation and digital capabilities. The integration of the devices can prevent degradation and eliminate contamination and enable nucleic acid extraction, purification, and digital quantification with above 99% specificity and in less than 55 minutes, less than 30 minutes, less than 15 minutes.

[00340] In some embodiments, the device can provide quantitative measurement of bacterial DNA and RNA, such as from urine samples spiked with *K. pneumoniae* (ATCC 700603), *P. aeruginosa* (ATCC 27853), and *E. coli* (ATCC 25922) with above 99% specificity and in less than 55 minutes. In some embodiments, the device or integrated device can provide quantification of bacterial load at dynamic range of 100 CFU/mL to 1,000,000 CFU/mL with greater than 3-fold resolution.

[00341] In some embodiments, the device can be used for quantification of gene expression level for at least 20, at least 50 or at least 100 clinical isolates with incubation in the presence

and absence of selected drugs In some embodiments, the device performance, including bacterial load and AST, is comparable to CLSI reference methods. In some embodiments, the error of performance is less than 5% minor errors, less than 2.5% minor errors, less than 1% minor errors.

[00342] In some embodiments, the performance has, less than 1% major errors, less than 0.5% major errors, less than 0.1% major errors. In some embodiments, the device has no very major errors.

Kits

[00343] A kit can include a SlipChip device, and a supply of a reagent selected to participate in nucleic acid amplification. In some embodiments, the reagent can be disposed in a container adapted to engage with a conduit of the first component, the conduit of the second component, or both. Such a container can be a pipette, a syringe, and the like. In some embodiments, the kit includes a heater.

[00344] Some embodiments of the device could be used to detect different biological targets such as, for example, proteins, bacteria, viruses, infectious agents etc., using nucleic acid labels. In some embodiments the target is tagged with an oligonucleotide which can be used for detection. The oligonucleotide tag can be further amplified using any one of a number of different nucleic acid amplification strategies, such as for example, PCR, LAMP, RPA, NASBA, RCA, etc. The oligonucleotide tag could also be visualized using fluorescent probes for example as shown by Chen (Huang, Suxian, and Yong Chen.

[00345] "Polymeric Sequence Probe for Single DNA Detection." *Analytical chemistry* 83.19 (2011): 7250-7254.)

EXAMPLES

[00346] These examples are provided for illustrative purposes only and not to limit the scope of the claims provided herein.

Example 1: Formation of a SlipChip

[00347] The procedure of fabricating desired glass SlipChips using soda lime glass was based on previous work. The two-step exposing-etching protocol was adapted to create wells of two different depths (5 μm for thermal expansion wells, 55 μm for all the other wells). After etching, the glass plates were thoroughly cleaned with piranha acid and DI water, and dried with nitrogen gas. The glass plates were then oxidized in a plasma cleaner for 10 minutes and immediately transferred into a desiccator for 1 hour of silanization. They were rinsed thoroughly with chloroform, acetone, and ethanol, and dried with nitrogen gas before use.

[00348] Plastic polycarbonate SlipChip devices were directly oxidized in a plasma cleaner for 15 minutes after they were received from microfluidic ChipShop GmbH, and then transferred into a desiccator for 90 minutes of silanization. They were soaked in tetradecane for 15 minutes at 65 °C and then rinsed thoroughly with ethanol, then dried with nitrogen gas before use. Plastic SlipChip devices were not reused.

[00349] The SlipChips were assembled under de-gassed oil (mineral oil: tetradecane 1:4 v/v; Fisher Scientific). Both top and bottom plates were immersed into the oil phase and placed face to face. The two plates were aligned under a stereoscope (Leica, Germany) and fixed using binder clips. Two through-holes were drilled in the top plate to serve as fluid inlets. The reagent solution was loaded through the inlet by pipetting.

Example 2: Digital Amplification in a SlipChip.

[00350] Digital PCR was performed on a SlipChip according to the protocols described in Shen et al. 2010). Feng Shen, Wenbin Du, Jason E. Kreutz, Alice Fok, and Rustem F. Ismagilov, "Digital PCR on a SlipChip," Lab Chip **2010** 10: 2666-2672.

[00351] Briefly, in this published method, top and bottom SlipChip plates were etched with microfluidic channels and wells via photolithography and HF etching. The top and bottom plates were aligned in a mixture of 20% mineral oil/80% tetradecane and then clamped to hold in place. DNA from the extractions described above was added to PCR master mix. This solution was loaded into the SlipChip device with a pipette. After loading, a slip broke the channels into 1280 individual 3nL compartments. Next, the SlipChip was clamped, sealed, and placed on a thermocycler for the following temperatures: 92°C for 3 minutes, 40 cycles of: 92°C for 20 seconds, 62°C for 20 seconds, and 72°C for 25 seconds. The SlipChip was imaged, positive and negative wells were counted, and the concentration of target DNA was calculated using Poisson statistics.

Example 3: Detection of differences in rDNA between treated/untreated susceptible *E. coli* over time

[00352] In one example, a ciprofloxacin-susceptible *E. coli* isolate from a patient with a urinary tract infection was pre-cultured in Bacto Brain Heart Infusion (BHI) media to high cell density prior to dilution and treatment with and without 2.5 ug/mL ciprofloxacin for 10, 20, and 30 minutes. DNA from treated and non-treated samples was extracted with Epicentre QuickExtract DNA extraction buffer. Quantitative PCR was performed with primers specific to the target rDNA gene for 23S. The following primers specific for 23S rDNA were used:

5′-TGCCGTAACTTCGGGAGAAGGCA-3′ (SEQ ID NO: 1)

5′-TCAAGGACCAGTGTTCAGTGTC-3′ (SEQ ID NO: 2)

[00353] A 1.37, 1.58, and 1.70 fold change in rDNA copy number between the treated (right bar at each timepoint) and non-treated (left bar at each timepoint) samples was measured at 10, 20, and 30 minutes (Figure 11).

Example 4: Detection of differences in rDNA between treated/untreated susceptible and resistant *E. coli* over time by qPCR and digital PCR

[00354] In one experiment, a ciprofloxacin-susceptible *E. coli* isolate from a patient with a urinary tract infection was pre-cultured in Bacto Brain Heart Infusion media to high cell density prior to dilution and treatment with and without 2.5 ug/mL ciprofloxacin for 30 minutes. DNA from treated and non-treated samples was extracted with Epicentre QuickExtract DNA extraction buffer at 0, 15, and 30 minutes. Quantitative PCR with primers specific to the target 23S rDNA gene as provided in Example 3 was performed on the extracted DNA. At 15 minutes, a 1.27 fold change in rDNA copy number between the treated (right bar at each timepoint) and non-treated (left bar at each timepoint) samples from the susceptible isolate was measured (Figure 12A).

[00355] Digital with primers specific to the target 23S rDNA gene as provided in Example 3 was performed on the extracted DNA in a SlipChip. One chip contained DNA from the untreated bacteria while the other chip contained DNA from the bacteria treated with antibiotic. The concentrations were compared and a fold-difference was calculated. Digital PCR detected a 1.5 fold change in rDNA copy number. No significant change in rDNA copy number was observed for the resistant isolate (Figure 12B).

[00356] This experiment was repeated for a different *E. coli* isolate from a patient with a urinary tract infection which was also susceptible to ciprofloxacin. At 15 minutes, qPCR measured a 1.8 fold change in rDNA copy number and digital PCR detected a 1.5 fold change in rDNA copy number.

Example 5: Detection of differences in rDNA between treated/untreated susceptible *E. coli* over time by digital PCR

[00357] In one experiment, a ciprofloxacin-susceptible *E. coli* isolate from a patient with a urinary tract infection was pre-cultured in pooled human urine prior to dilution and treatment in 1:1 BHI:urine with and without 0.75 ug/mL ciprofloxacin for 15 minutes. DNA from treated and non-treated samples was extracted with Epicentre QuickExtract DNA extraction buffer. A 2.61 fold change in rDNA copy number between the treated and non-treated samples was measured using quantitative PCR on the extracted DNA with primers specific to the target 23S rDNA gene as provided in Example 3. Digital PCR was also performed using primers specific to the target 23S rDNA gene as provided in Example 3 on the extracted DNA. Digital PCR detected a 2.4 fold change in rDNA copy number. The results of the digital PCR data are shown in Figure 13.

Example 6: Detection of differences in susceptible and resistant *E. coli* treated/untreated with tetracycline and trimethoprim/sulfamethoxazole

[00358] In one experiment, *E. coli* isolates from patients with urinary tract infections and susceptible to various antibiotics were pre-cultured in BHI or urine prior to dilution and treatment in 1:1 BHI:urine with and without antibiotics for various treatment times. Treated and non-treated samples were extracted with Epicentre QuickExtract DNA extraction buffer. A detectable fold change in rDNA copy number between the treated and non-treated samples was measured using quantitative PCR with primers specific to the target 23S rDNA gene as provided in Example 3. Under tetracycline treatment a 1.55 fold change in rDNA copy number was observed at 30 minutes (Figure 14A), under nitrofurantoin treatment a 1.62 fold change in rDNA copy number was observed at 15 minutes, and under trimethoprim/sulfamethoxazole treatment a 1.35 fold change in rDNA copy number was observed at 45 minutes (Figure 14B).

Example 7: Comparison of qPCR and digital PCR for detection of differences between resistant and susceptible cells

[00359] In this example, the enhanced resolution of digital amplification over qPCR is shown. Specifically, differences in nucleic acids found in bacteria that have been treated with an antibiotic as compared to untreated can be resolved with digital nucleic acid quantification (including PCR or LAMP) whereas qPCR methods (or qLAMP) may not reliably resolve the difference and/or may not reliably resolve the difference without using many more replicates.

[00360] A ciprofloxacin-susceptible *E. coli* isolate from a patient with a urinary tract infection was pre-cultured in pooled human urine prior to dilution and treatment in 1:1 BHI:urine with and without 0.75 ug/mL ciprofloxacin for 30 minutes. DNA from treated and non-treated samples were extracted with Epicentre QuickExtract DNA extraction buffer.

[00361] Quantitative PCR was performed on each sample with primers specific to the target 32S rDNA gene (3 replicates per sample) with primers as provided in Example 3 and the following Cq's were obtained: treated--22.84 +/- 0.53; untreated--22.13 +/- 0.07. Using the method described in Weaver, et. al. "Taking qPCR to a higher level: Analysis of CNV reveals the power of high throughput qPCR to enhance quantitative resolution," Methods 2010 50: 271-276, this 1.6-fold difference in rDNA copy number is not statistically significant unless there had been 10 qPCR replicates for each sample instead of 3.

[00362] The same samples were diluted 1:10 and loaded into a SlipChip for digital amplification. One chip contained DNA from the untreated bacteria while the other chip contained DNA from the bacteria treated with antibiotic. Digital PCR was performed on the extracted DNA to amplify 23S rDNA using primers provided in Example 3.

[00363] The results were measured and the following concentrations were calculated using Poisson statistics: treated--148,031 +/- 7,103 copies/mL; untreated---84,964 +/- 5,171 copies/mL. Using the statistical methods described in Kreutz et al., "Theoretical Design and Analysis of Multivolume Digital Assays with Wide Dynamic Range Validated Experimentally with Microfluidic Digital PCR," Analytical Chemistry 2011 83: 8158-8168, a statistically significant difference with a p-value of 2e-13 was calculated.

[00364] Thus, digital PCR successfully resolved the treated vs. untreated bacterial DNA while qPCR was unable to resolve the difference in concentration with a typical number of replicates (three).

[00365] The statistical approach described with this experiment is applicable more generally. From a qPCR experiment, a standard deviation of 0.16 or greater in Cq is typical. In order to achieve a 1.25-fold resolution between two samples with this standard deviation, qPCR can require 18 replicates (Weaver et al. (2010)). For the same resolution (1.25), one digital PCR experiment can be performed, as long as the number of compartments in the digital PCR experiment is greater than 1200 (*Id.*).

Example 8: Target mapping of genes relative to origin of replication

[00366] The position of feoB, recA, and DnaAInhibitor protein genes was mapped relative to the origin of replication in a subset of 16 available genome-sequenced *E. coli* strains linked to UTI patients. The results showed a trend for FeoB gene to be located in proximity to origin of replication, while recA and DnaAInhibitor protein genes are positioned more distantly from the origin of replication (Figure 15). As a result of a replication initiation event, feoB gene is expected to replicate early, together with all other genes positioned close to origin of replication positioned at the similar distances from origin of replication as feoB gene (e.g., malB gene (GDB J01648)).

Example 9: DNA markers in different time points of chromosomal replication data

[00367] In this experiment, one susceptible UTI *E. coli* isolate and one resistant UTI *E. coli* were treated with 2.5 ug/mL ciprofloxacin for 15 minutes. Their nucleic acids were extracted with QuickExtra DNA extraction kit (Epicenter) and DNA was quantified via qPCR with rDNA (23S), FeoB gene, DnaA inhibiting protein gene, and RecA gene specific primers.

[00368] Figure 16 shows Cycle threshold (Ct) for quantification of DNA fragments copies number in UTI clinical isolates *E. coli* genomes, in susceptible and resistant of bacteria after exposure ("treated") to a 2.5 ug/ml of ciprofloxacin for 15 minutes in BHI medium. Table 2 provides the cycle threshold and fold change for each target between susceptible and resistant treated / untreated samples.

Table 2

	23S CT Untreated /Treated	23S Fold Change	FeoB Untreated /Treated	FeoB Fold Change	dnaAinh CT Untreated /Treated	dnaAinh Fold Change	recA CT Untreated /Treated	recA Fold Change
Susceptible	20.19/ 20.84	1.57	23.3/ 23.71	1.33	23.93/ 23.97	1.03	24.81/ 24.65	0.89 (1.13 up)
Resistant	20.22/ 20.13	0.94 (1.06 up)	23.19/ 23.1	0.94 (1.06up)	23.68/ 23.89	1.16	27.19/ 27.05	0.91 (1.10 up)

[00369] In a susceptible strain, cells treated with ciprofloxacin showed a 1.57 fold decrease in 23S DNA, a 1.33 fold decrease in FeoB DNA, no change in dnaAinh DNA, and a 1.13 fold increase in recA compared to cells untreated with the antibiotic.

[00370] In a resistant strain, cells treated with ciprofloxacin showed no change in 23S DNA and FeoB DNA, a 1.16 fold decrease in dnaAinh DNA, and a 1.10 fold increase in recA compared to cells untreated with the antibiotic.

[00371] In this example, less rDNA (23S DNA) copies and less FeoB genes copies are observed in the genomes of the susceptible isolates as compared to the resistant isolates; while for other genes fragments - dnaA inhibitor genes and recA genes positioned more distantly from origin of replication, there was no statistically rigorous difference between drug treated and no treated bacteria.

Example 10 – Target mRNA for determining drug resistance or susceptibility

[00372] Our drug susceptibility assay is validated using several targets of interest. These include the targets FeoB and RecA RNA in isolates resistant to ciprofloxacin

[00373] Feo B expression is down-regulated in a presence of ciprofloxacin. Despite being downregulated at a low level it is down regulated in the bacteria which are susceptible (non-dividing, or dying) so it will ensure a good resolution compared to untreated or resistant cells.

[00374] In this example, FeoB and RecA RNA levels were used to determine cells that were susceptible or resistant to ciprofloxacin clinical isolates after 20 min of treatment with 2.5ug/mL cipro in BHI medium. In this example, RNA quantification was done by qRTPCR with FeoB and RecA gene specific primers. In this example, FeoB expression was downregulated in susceptible isolates after antibiotic treatment. RecA expression in the same experiment was not significantly downregulated in susceptible isolates.

[00375] RNA ratios for feoB and recA allow to differentiate between susceptible and resistant UTI *E. coli* isolates after 20 minutes of antibiotic treatment.

[00376] In one example, one susceptible UTI *E. coli* isolate and one resistant UTI *E. coli* from overnight cultures were diluted in warm BHI medium 1:100 (10 μL in 1 mL), and grown for~3-4 hours at 37C. Two samples of each well mixed culture were taken for antibiotic exposure assay. They were added to pre-warmed 1.7mL VWR micro centrifuge tubes containing A) warm (37C) BHI medium (control) or B) warm (37C) BHI medium with added ciprofloxacin at concentration 2.5 μg/mL; the rack for all the tubes has being also pre-warmed to 37°C to minimize the temperature variations. Both control and antibiotic containing samples were incubated for 20 minutes at 37°C. After that the samples A and B were immediately placed on ice, and were diluted 1/5 in RLT buffer (Qiagen). Their total nucleic acids were extracted using Qiagen RNAeasy Mini kit columns and buffers, and analyzed via one-step RT qPCR to quantify FeoB and Rec A RNA, using Maxima H- RT and SSO fast EvaGreen PCR mixture in a presence of 0.3U/μL RNases inhibitor Superase In (Ambion) and 1 μg/μL BSA (Roche). Samples taken from resistant isolates are marked UTI R and samples taken from susceptible samples are marked UTI S in the plots below. Samples incubated in a presence of ciprofloxacin have "cipro" added to their names.

[00377] Figure 17A and Figure 17B show RNA quantification of FeoB and RecA genes expression in UTI clinical isolates susceptible (S) and resistant (R) to ciprofloxacin after 20 minutes of treatment with 2.5ug/mL of ciprofloxacin in BHI medium, versus 20 minutes of being in BHI medium without antibiotics. Relative to susceptible cells exposed to ciprofloxacin, untreated susceptible cells showed a 4.86 fold increase in the expression of the FeoB target, observed as a 2.28 cycle difference. There was no significant change observed the gene expression in resistant cells. Both resistant and susceptible cells showed no significant changes in the expression of recA.

Example 11 – Effects of Ciproflaxin on RNA expression of FeoB and RecA

[00378] Clinical isolates from UTI patients were obtained and recovered on TSA blood agar medium. Colonies from the blood agar plates were inoculated into 3 mL of BHI medium, grown overnight. One susceptible UTI *E. coli* isolate and one resistant UTI *E. coli* from overnight cultures were diluted in warm BHI medium 1:100 (10 μL in 1 mL), grown for about 3 hours at 37C. Two samples of each well mixed culture were taken for antibiotic exposure assay. They were added to pre-warmed 1.7mL VWR micro centrifuge tubes containing A) warm (37°C) BHI medium (control) or B) warm (37°C) BHI medium with added ciprofloxacin at concentration 10ug/mL; the rack for all the tubes has being also pre-warmed to 37°C to minimize

the temperature variations. Both control and antibiotic-containing samples were incubated for 20 minutes at 37°C. After that samples A and B were immediately placed on ice, and 30 µL of each sample were added to 130 µL of cold RLT buffer (Qiagen). Their total nucleic acids were extracted using Qiagen RNAeasy Mini kit columns and buffers, and analyzed via one-step RT qPCR to quantify FeoB and RecA RNA, using Maxima H-RT (and SSO fast EvaGreen PCR mixture in a presence of RNases inhibitors and BSA.

[00379] Figure 18 shows RNA quantification of FeoB and RecA genes expression in UTI clinical isolates susceptible and resistant to ciprofloxacin after 20 minutes of treatment with 10 ug/mL of ciprofloxacin in BHI medium, versus 20 minutes of being in BHI medium without antibiotics. Results are provided in Table 3.

Table 3

	FeoB		**RecA**		
	CT Untreated /Treated	Fold Change Downregulated	CT Untreated /Treated	Fold Change Upregulated	Fold Change Ratio
Susceptible 1	20.88/24.45	11.88	20.87/18.11	6.77	80.45
Susceptible 2	23.99/26.21	4.66	23.00/20.21	6.91	32.22
Susceptible 3	22.28/24.73	5.46	22.08/19.42	6.32	34.54
Resistant	20.45/20.53	1.06	22.54/22.57	0.98	1.04

[00380] Relative to susceptible cells untreated with ciprofloxacin, susceptible cells treated showed significant downregulation of the FeoB gene. Three different susceptible isolates showed 11.88, 4.66, and 5.46 fold decrease in the presence of this RNA in cells. A resistant isolate showed no significant change. Relative to susceptible cells untreated with ciprofloxacin, susceptible cells treated showed significant upregulation of the RecA gene. Three different susceptible isolates showed 6.77, 6.92, and 6.32 fold increase in the presence of this RNA in cells. A resistant isolate showed no significant change. Individually either of these changes can be used to distinguish susceptible from resistant cells, however, taking a ratio of the change in FeoB to RecA provides even greater resolution between the resistant and susceptible cells. The susceptible strains tested showed an 80.45, 32.22, and 34.54 fold change ratio, whereas in resistant cells, this ratio was 1.04.

[00381] While preferred embodiments of the present invention have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way

of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the invention. It should be understood that various alternatives to the embodiments of the invention described herein can be employed in practicing the invention. It is intended that the following claims define the scope of the invention and that methods and structures within the scope of these claims and their equivalents be covered thereby.

WHAT IS CLAIMED IS:

1. A method, comprising:

providing a first sample comprising a target molecule from a first portion of a population of bacteria and a second sample comprising a target molecule from a second portion of said population of bacteria, wherein the first portion has been treated with an antibiotic, and wherein the second portion has not been treated with the antibiotic;

distributing said first sample among a plurality of first analysis regions;

distributing said second sample among a plurality of second analysis regions;

contacting each of the first and second analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions; and

detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said reaction.

2. The method of claim 1, wherein the threshold number is greater than zero, one, two, three, four, or five.

3. The method of claim 1, wherein the distribution of each sample among the analysis regions is effected such that at least some of the analysis regions do not have the target molecule, and at least some of the analysis regions have only one target molecule.

4. The method of claim 1, wherein the distribution of each sample among the analysis regions is effected such that at least one of the analysis regions contains only one target molecule.

5. The method of claim 1, wherein the first sample comprises said reagent, and wherein contacting the plurality of first analysis regions with the reagent comprises said step of distributing said first sample among said plurality of first analysis regions.

6. The method of claim 1, wherein the second sample comprises said reagent, and wherein contacting the plurality of second analysis regions with the reagent comprises said step of distributing said second sample among said plurality of second analysis regions.

7. The method of claim 1, wherein said reaction comprises nucleic acid amplification.

8. The method of claim 7, wherein said nucleic acid amplification is essentially isothermal.
9. The method of claim 7, wherein said nucleic acid amplification is a polymerase chain reaction.
10. The method of claim 7, wherein said nucleic acid amplification is selected from the group consisting of: nucleic acid sequence based amplification (NASBA), self-sustained sequence replication (3SR), loop mediated isothermal amplification (LAMP), strand displacement amplification (SDA), whole genome amplification, multiple displacement amplification, strand displacement amplification, helicase dependent amplification, nicking enzyme amplification reaction, recombinant polymerase amplification, reverse transcription PCR. and ligation mediated PCR.
11. The method of claim 1, wherein said analysis regions comprise wells.
12. The method of claim 1, wherein the target molecule is a nucleic acid.
13. The method of claim 12, wherein the reagent is a nucleic acid amplification reagent.
14. The method of claim 13, further comprising exposing each of the analysis regions to conditions effective for amplification of the nucleic acid.
15. The method of claim 1, wherein the target molecule comprises a segment of a bacterial chromosome or plasmid.
16. The method of claim 15, wherein the target molecule is less than 50 kDa, less than 100 kDa, less than 200 kDa, or less than 400 kDa from the origin of replication.
17. The method of claim 15, wherein the target molecule comprises a sequence of a gene on said bacterial chromosome or plasmid.
18. The method of claim 1, wherein the target molecule comprises mRNA.
19. The method of claim 1, wherein the target molecule is amplified by an amplification reaction, said amplification reaction comprising primers complementary to a sequence of the target molecule.
20. The method of claim 18, wherein the mRNA encodes recA or lexA.
21. The method of claim 1, wherein the reagent is disposed in a plurality of reagent regions.
22. The method of claim 18, wherein contacting is effected by placing the plurality of reagent regions in fluid communication with the first or second plurality of analysis regions.
23. The method of claim 22, wherein contacting comprises effecting relative motion between a substrate comprising the reagent regions with a substrate comprising the first and second plurality of analysis regions.
24. The method of claim 1, further comprising analyzing the detection of the presence or the absence of the threshold number of target molecules in each plurality of analysis regions

to determine a resistance or susceptibility to an antibiotic in the population of bacteria.

25. The method of claim 1, wherein the target molecule has been removed from the bacteria in the first sample, and wherein the time between initial exposure of the bacteria to antibiotic and the removal of the target molecules is less than the mean doubling time of the bacteria during a growth phase.

26. The method of claim 1, wherein said detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 3 hours, 2 hours, or one hour after the end of exposure of the first sample to the antibiotic.

27. The method of claim 26, wherein said detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 45 minutes, 30 minutes, 15 minutes, or 10 minutes after the end of exposure of the first sample to the antibiotic.

28. The method of claim 1, wherein said detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 3 hours, less than 2 hours, or less than 1 hour after the end of exposure of the first sample to the antibiotic.

29. The method of claim 28, wherein said detection of the presence or absence of the threshold number of target molecules in each of the first and second analysis regions is performed less than 45 minutes, less than 30 minutes, less than 15 minutes, or less than 10 minutes after the end of exposure of the first sample to the antibiotic.

30. The method of claim 1, wherein said bacteria from said first sample have been treated with antibiotic for a period of no more than 2 hours, no more than 1 hour, no more than 45 minutes, no more than 30 minutes, no more than 15 minutes, or no more than 10 minutes.

31. The method of claim 1, wherein the time from the first exposure of the bacteria from the first sample to an antibiotic to the detection step is less than 3 hours, less than 2 hours, less than 1 hour after, less than 45 minutes, or less than 30 minutes.

32. The method of claim 1, wherein the first plurality of analysis regions comprises at least 10, 20, 30, 40, or 50 analysis regions.

33. The method of claim 1, wherein the second plurality of analysis regions comprises at least 10, 20, 30, 40, or 50 analysis regions.

34. The method of claim 1, wherein the distribution of the first and second sample into the first and second plurality of analysis regions is performed in parallel.

35. The method of claim 1, wherein the first sample or second sample comprises less than 10,000, 5,000, 1,000, 500, 200, 100, 50, 20, or 10 target molecules.

36. A method of determining a resistance or susceptibility to an antibiotic in a population of bacteria, comprising:

providing a first sample comprising a target molecule from a first portion of a population of bacteria and a second sample comprising a target molecule from a second portion of said population of bacteria, wherein the first portion has been treated with an antibiotic, and wherein the second portion has not been treated with the antibiotic;

distributing said first sample among a plurality of first analysis regions;

distributing said second sample among a plurality of second analysis regions;

contacting each of the first and second analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions;

detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said reaction; and

analyzing the results of the detection to determine a resistance or susceptibility to said antibiotic in the population of bacteria.

37. A method of determining a resistance or susceptibility to an antibiotic in a population of bacteria, comprising:

distributing a population of bacteria into a plurality of clonal isolation regions, the distribution being effected such that at least some of the clonal isolation regions are statistically estimated to each contain a single isolated bacterium;

expanding each of the single isolated bacteria to generate a plurality of clonal populations;

distributing each of said plurality of clonal populations into at least one treatment region from a plurality of treatment regions and into at least one control region from a plurality of control regions;

contacting the first plurality of treatment regions with an antibiotic, the plurality of control regions not contacted with the antibiotic;

for each of the first plurality of treatment regions and each of the plurality of control regions, distributing one or more target molecules from each clonal population into a distinct plurality of analysis regions;

contacting each of the analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target molecules in each of the first and second analysis regions; and

detecting the presence or absence of the threshold number of target molecules in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target molecules is detected in some of the distinct plurality of analysis regions and is not detected in some of the distinct plurality of analysis regions after said reaction.

38. The method of claim 37, further comprising analyzing the results of the detection to determine a resistance or susceptibility to said antibiotic in at least some of said plurality of clonal populations.

39. A method, comprising:

providing a first sample comprising a target analyte from a first portion of a population of cells and a second sample comprising a target analyte from a second portion of said population of cells, wherein the first portion has been treated with a drug, and wherein the second portion has not been treated with the drug;

distributing said first sample among a plurality of first analysis regions;

distributing said second sample among a plurality of second analysis regions;

contacting each of the first and second analysis regions with a reagent for performing a reaction to detect the presence or absence of a threshold number of target analytes in each of the first and second analysis regions; and

detecting the presence or absence of the threshold number of target analytes in each of the first and second analysis regions, wherein the distribution of each sample among the analysis regions is effected such that the threshold number of target analytes is detected in some of the first and second analysis regions and is not detected in some of the first and second analysis regions after said reaction.

40. A kit comprising:

a container comprising

a plurality of analysis regions,

a plurality of reagent regions comprising a component of a nucleic acid

amplification, wherein the container comprises a first layer and a second layer configured to move relative to the other between a first position, where the plurality of analysis regions and the plurality of reagent regions are isolated from each other, and a second position, wherein at least some of the plurality of analysis regions is in fluid communication with at least some of the plurality of reagent regions; and

instructions for use for determining a resistance or susceptibility to an antibiotic in a population of bacteria.

41. The kit of claim 39, wherein said component of a nucleic acid amplification comprises primers for amplifying recA or lexA mRNA.

42. The kit of claim 39, wherein said component of a nucleic acid amplification comprises primers for amplifying a nucleic acid target on a bacterial chromosome or plasmid less than 50kDa, less than 100 kDa, less than 200 kDa, or less than 400 kDa from the origin of replication.

43. The kit of claim 39, wherein the container comprises at least 10, 20, 30, 40, or 50 analysis regions.

44. The kit of claim 39, wherein the container comprises at least 10, 20, 30, 40, or 50 reagent regions.

45. The kit of claim 39, wherein said antibiotic is selected from the group consisting of: aminoglycosides, cephalosporins, tetracyclines, sulfonamides, macrolides, vancomycin, and β-lactams.

46. The kit of claim 45, wherein said instructions indicate that a decrease in nucleic acid target detection in a portion of the population of bacteria treated with said antibiotic as compared to a portion of the population of bacteria not treated with said antibiotic indicates that the population of bacteria is susceptible to said antibiotic.

47. The kit of claim 39, wherein said antibiotic is selected from the group consisting of: HPUra, hydroxyurea, trimethoprim, ciprofloxacin, and MMC.

48. The kit of claim 47, wherein said instructions indicate that an increase in nucleic acid target detection in a portion of the population of bacteria treated with said antibiotic as compared to a portion of the population of bacteria not treated with said antibiotic indicates that the population of bacteria is susceptible to said antibiotic.

49. A device for processing a sample, comprising an incubation module, a sample preparation module, and a digital quantification module, wherein said device is configurable to place each module in fluid communication with the other modules;

wherein said incubation module comprises an incubation chamber configured to incubate an organism with a drug, wherein said sample preparation module is configured to extract a target analyte from said organism; and wherein said digital quantification module comprises a plurality of reaction areas configured to perform digital detection of the presence or absence of said target analytes in said reaction area.

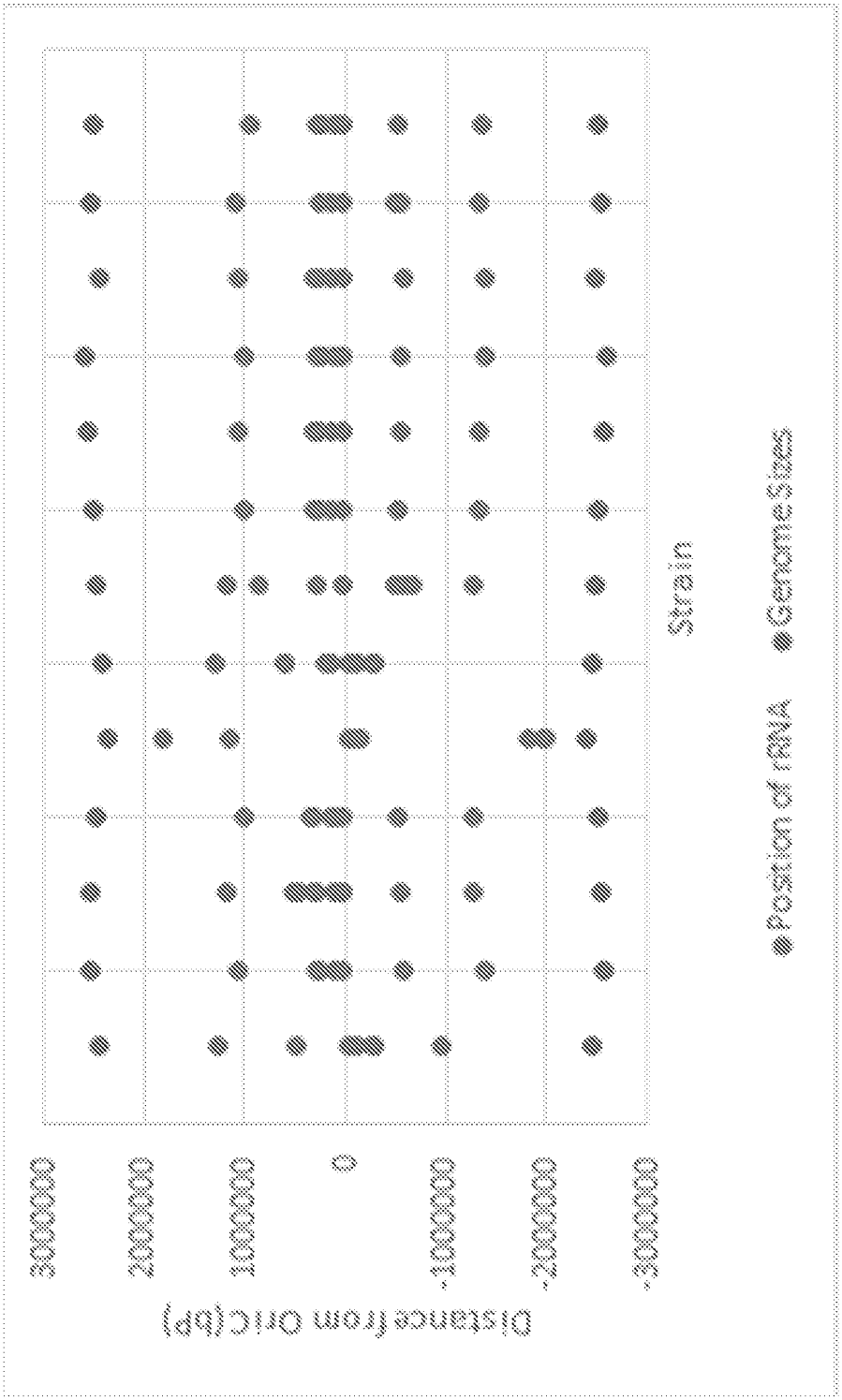
Distance from oriC (bp)
3000000
2000000
1000000
0
-1000000
-2000000
-3000000
Strain
Position of rRNA
Genome Sites

FIG. 1

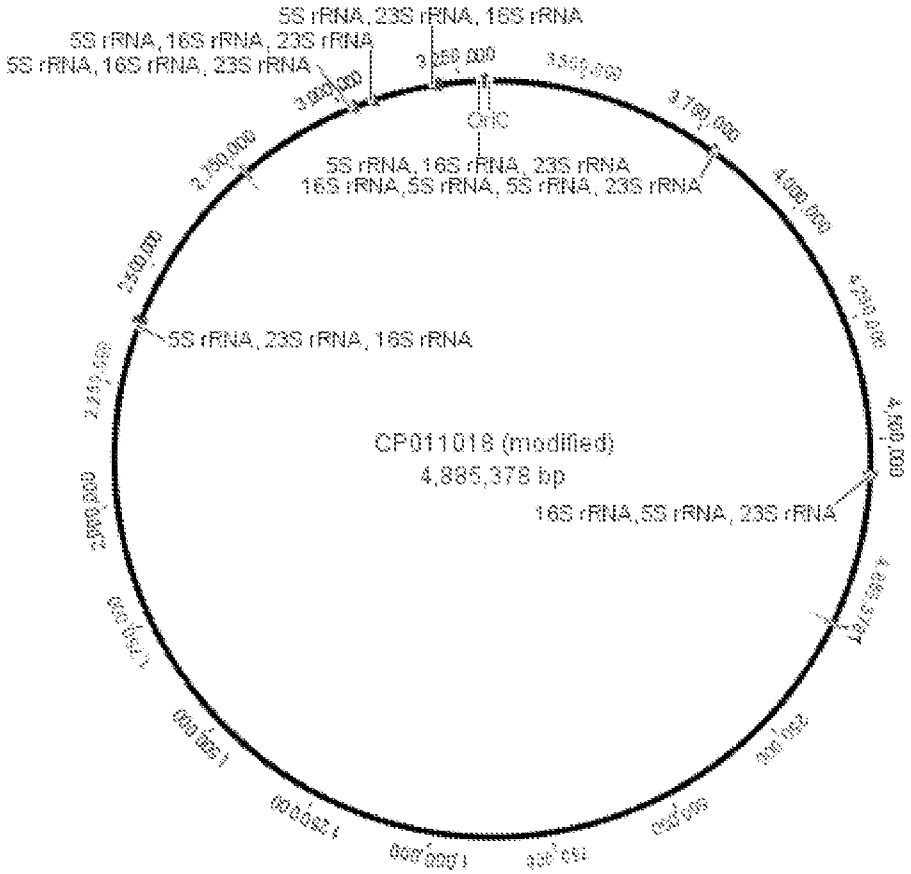
5S rRNA, 23S rRNA, 16S rRNA
5S rRNA, 16S rRNA, 23S rRNA
5S rRNA, 16S rRNA, 23S rRNA
3,250,000
3,500,000
3,750,000
4,000,000
4,250,000
4,500,000
4,885,378
3,000,000
2,750,000
2,500,000
2,250,000
2,000,000
1,750,000
1,500,000
1,250,000
1,000,000
750,000
500,000
250,000
OriC
5S rRNA, 16S rRNA, 23S rRNA
16S rRNA, 5S rRNA, 5S rRNA, 23S rRNA
5S rRNA, 23S rRNA, 16S rRNA
CP011018 (modified)
4,885,378 bp
16S rRNA, 5S rRNA, 23S rRNA

FIG. 2

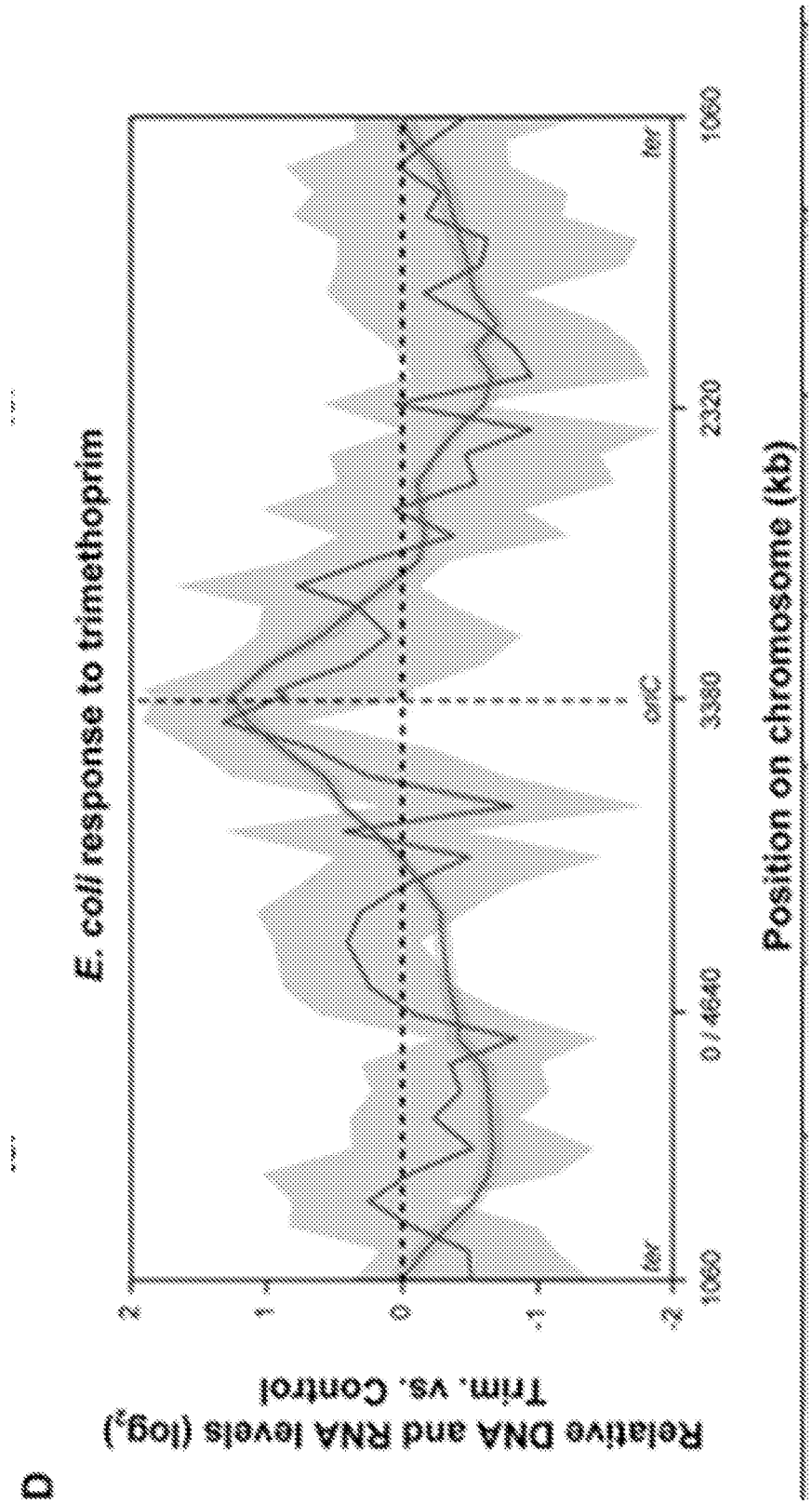
D
E. coli response to trimethoprim
Relative DNA and RNA levels (log2)
Trim. vs. Control
2
1
0
-1
-2
1060
0 / 4640
3380
2320
1060
oriC
ter
ter
Position on chromosome (kb)

FIG. 3

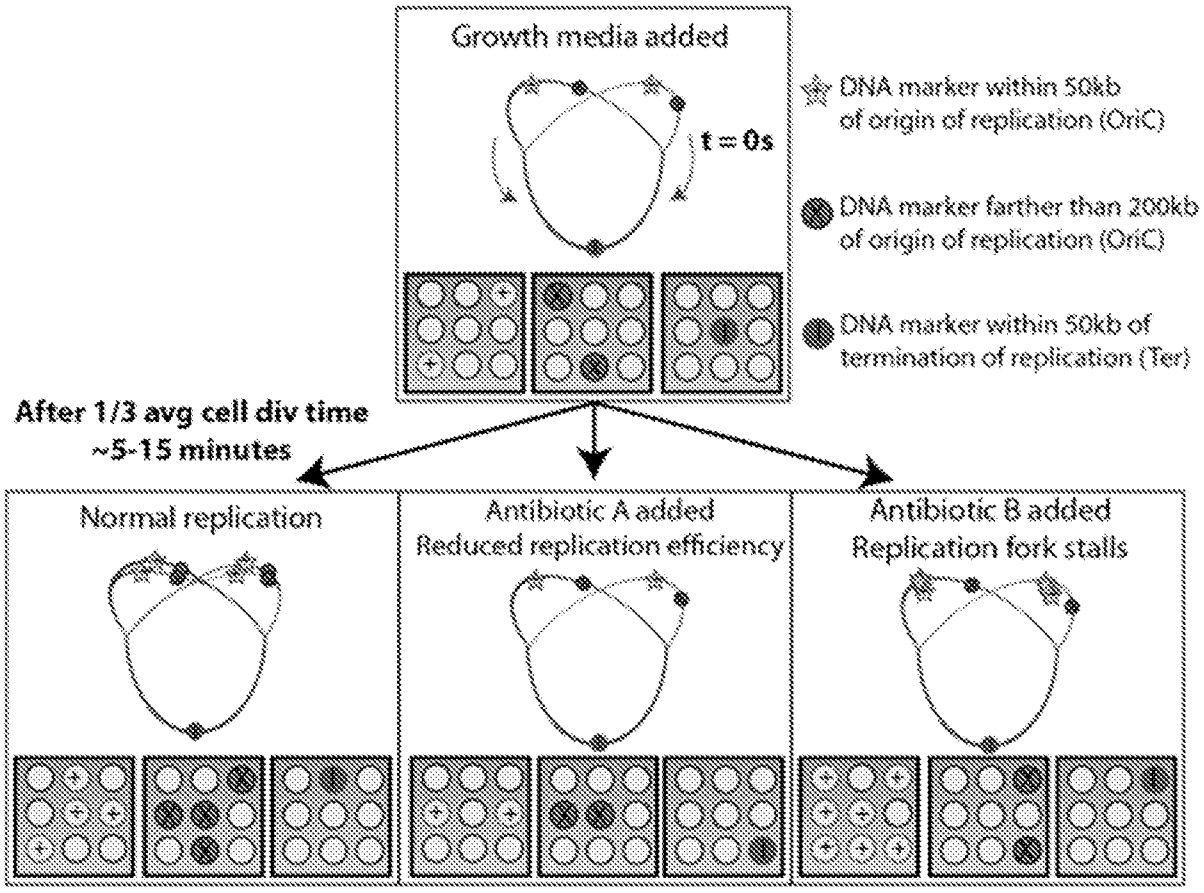
Growth media added
t = 0s
DNA marker within 50kb of origin of replication (OriC)
DNA marker farther than 200kb of origin of replication (OriC)
DNA marker within 50kb of termination of replication (Ter)
After 1/3 avg cell div time ~5-15 minutes
Normal replication
Antibiotic A added
Reduced replication efficiency
Antibiotic B added
Replication fork stalls

FIG. 4

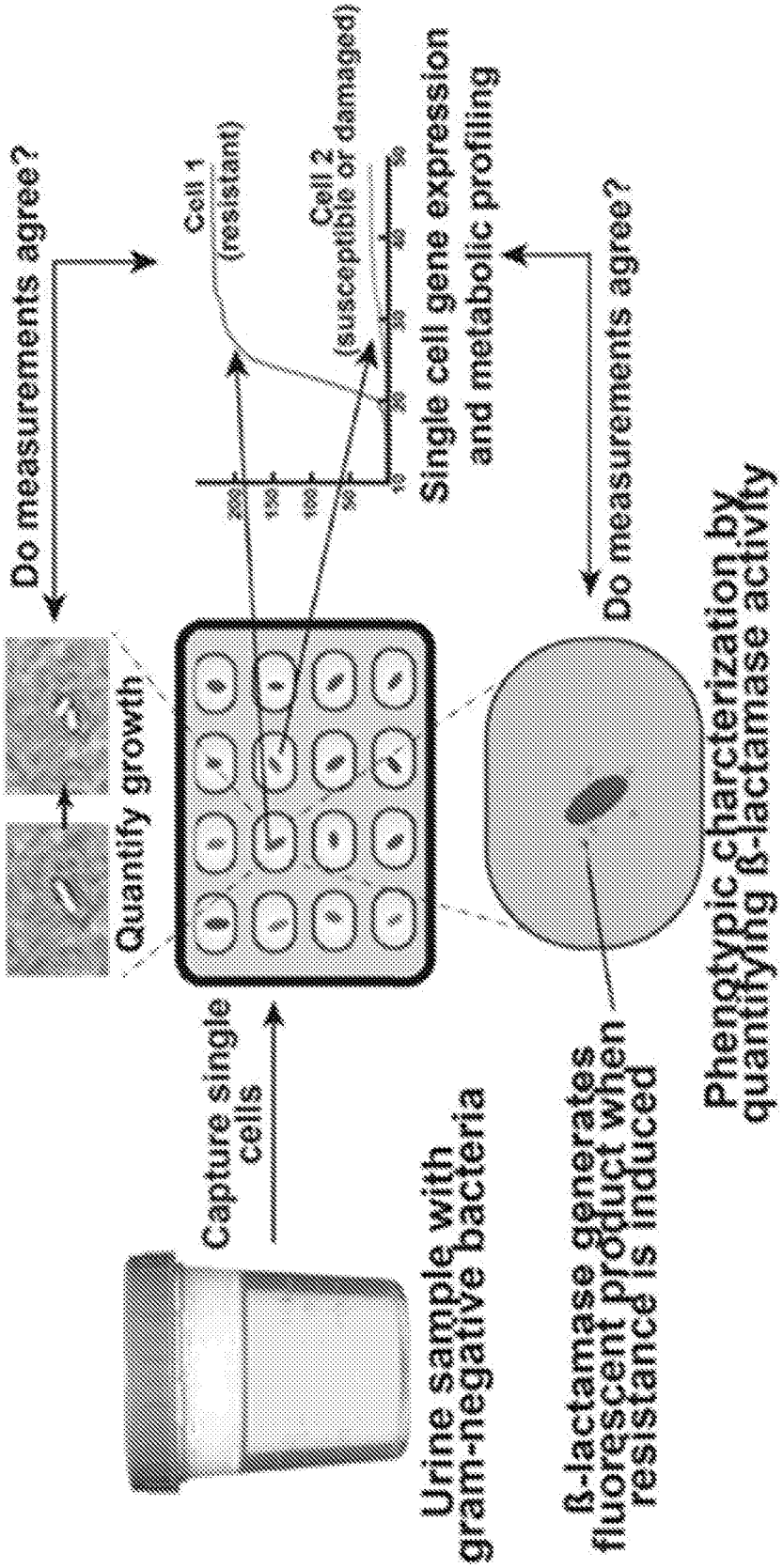
Urine sample with gram-negative bacteria
Capture single cells
Quantify growth
Do measurements agree?
Cell 1 (resistant)
Cell 2 (susceptible or damaged)
Single cell gene expression and metabolic profiling
Do measurements agree?
Phenotypic charcterization by quantifying ß-lactamase activity
ß-lactamase generates fluorescent product when resistance is induced

FIG. 5

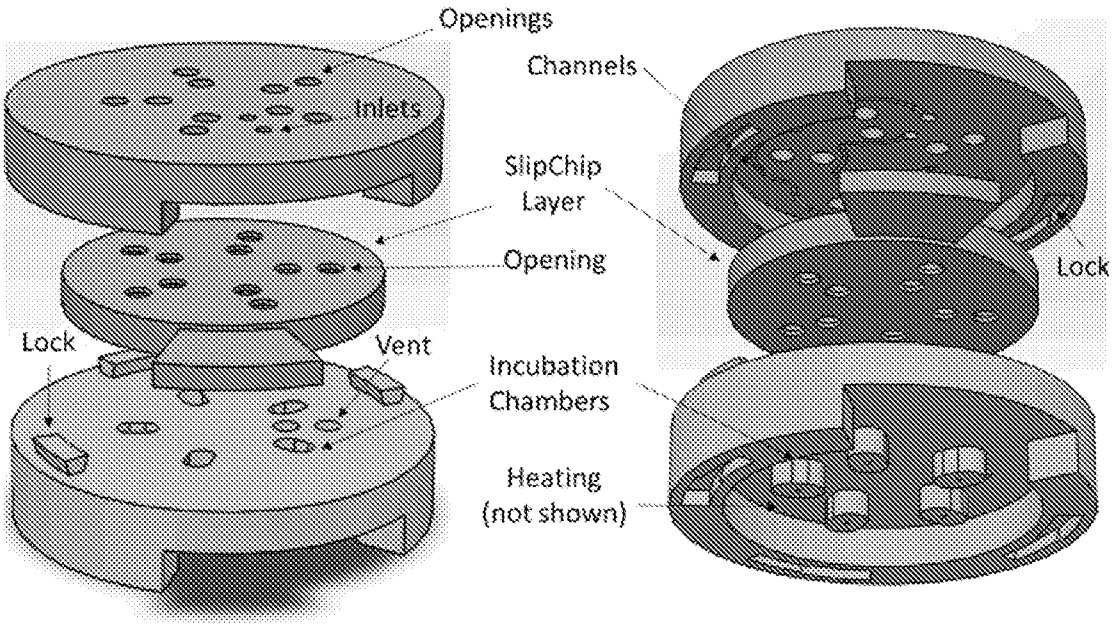
Openings
Channels
Inlets
SlipChip Layer
Opening
Lock
Lock
Vent
Incubation Chambers
Heating (not shown)

FIG. 6A

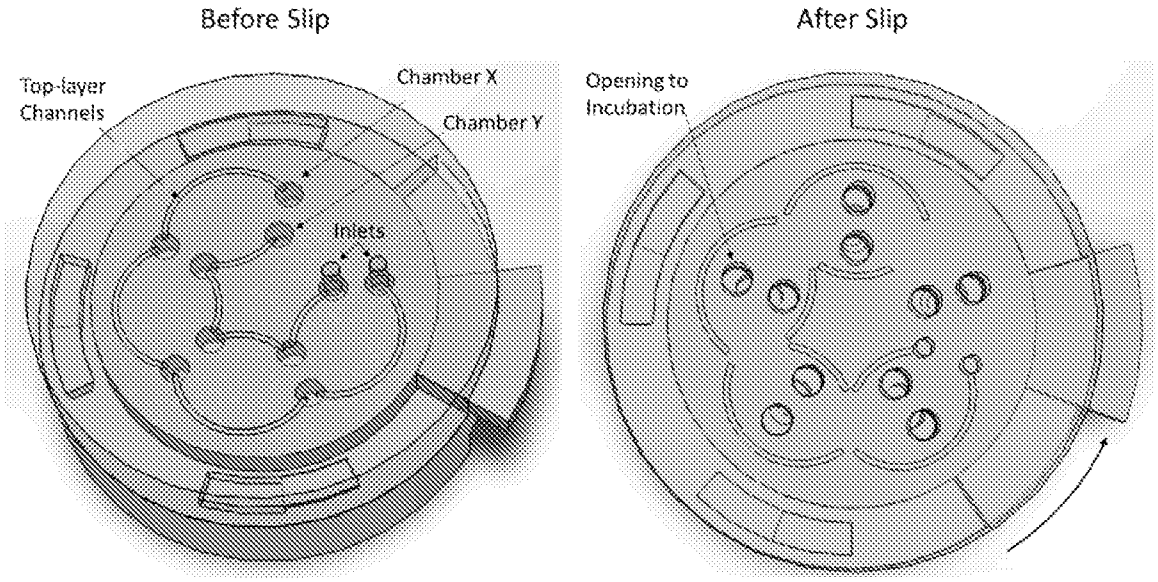
Before Slip
After Slip
Top-layer Channels
Chamber X
Chamber Y
Inlets
Opening to Incubation

FIG. 6B

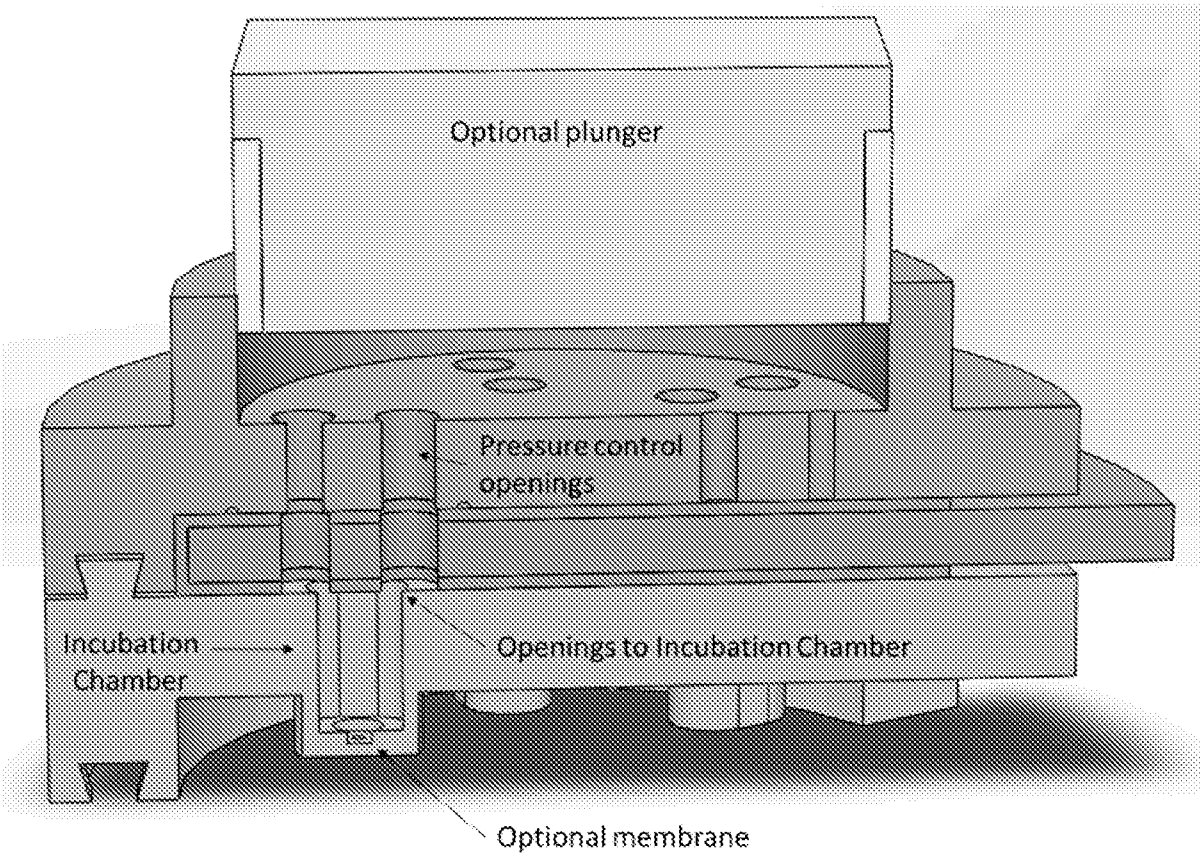
Optional plunger
Pressure control openings
Incubation Chamber
Openings to Incubation Chamber
Optional membrane

FIG. 6C

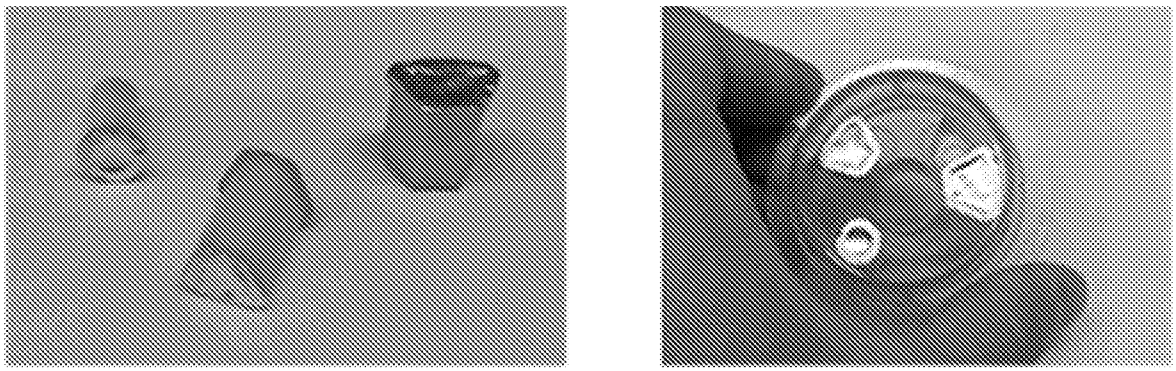

FIG. 7

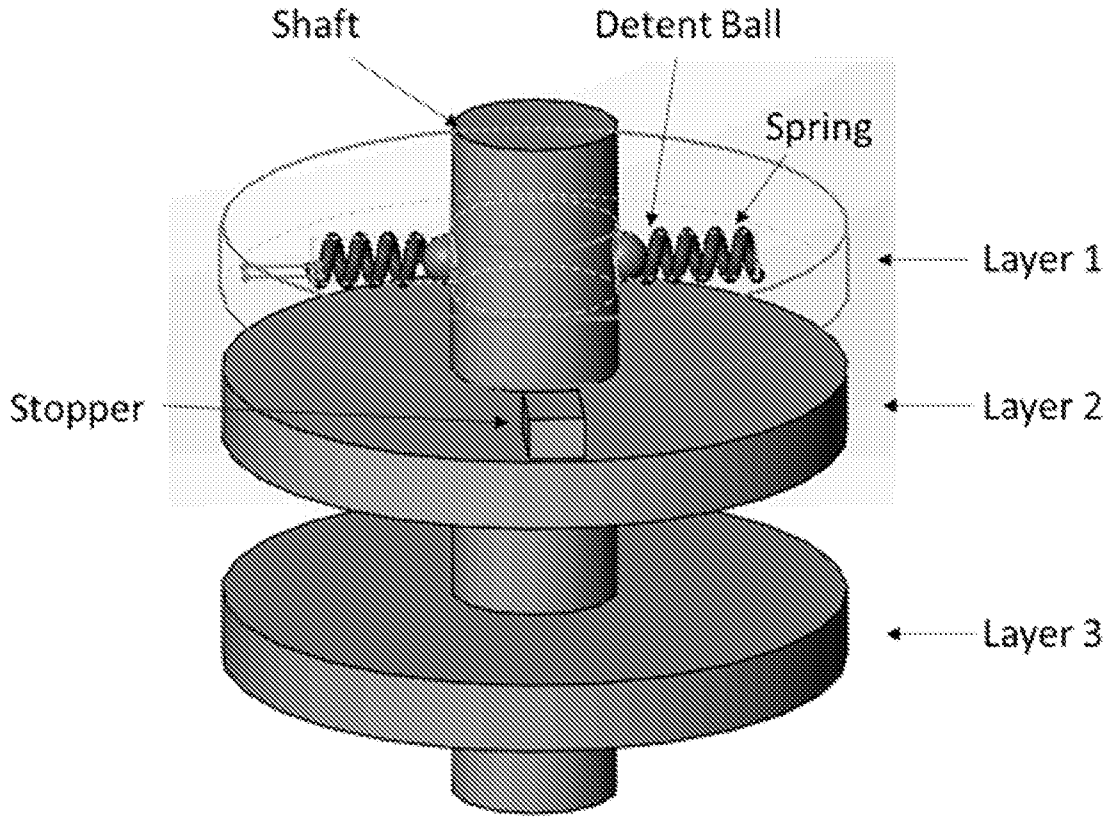

FIG. 8

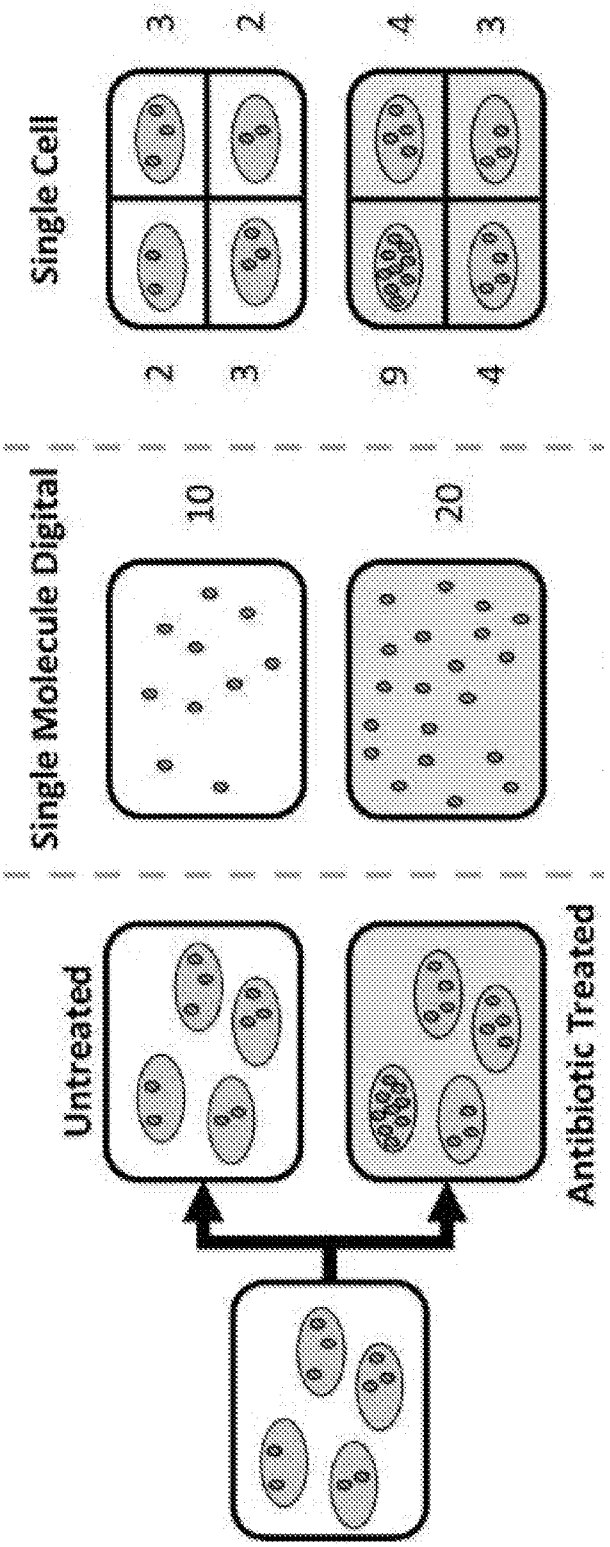
Untreated
Antibiotic Treated
Single Molecule Digital
10
20
Single Cell
2
3
9
4
3
2
4
3

FIG. 9

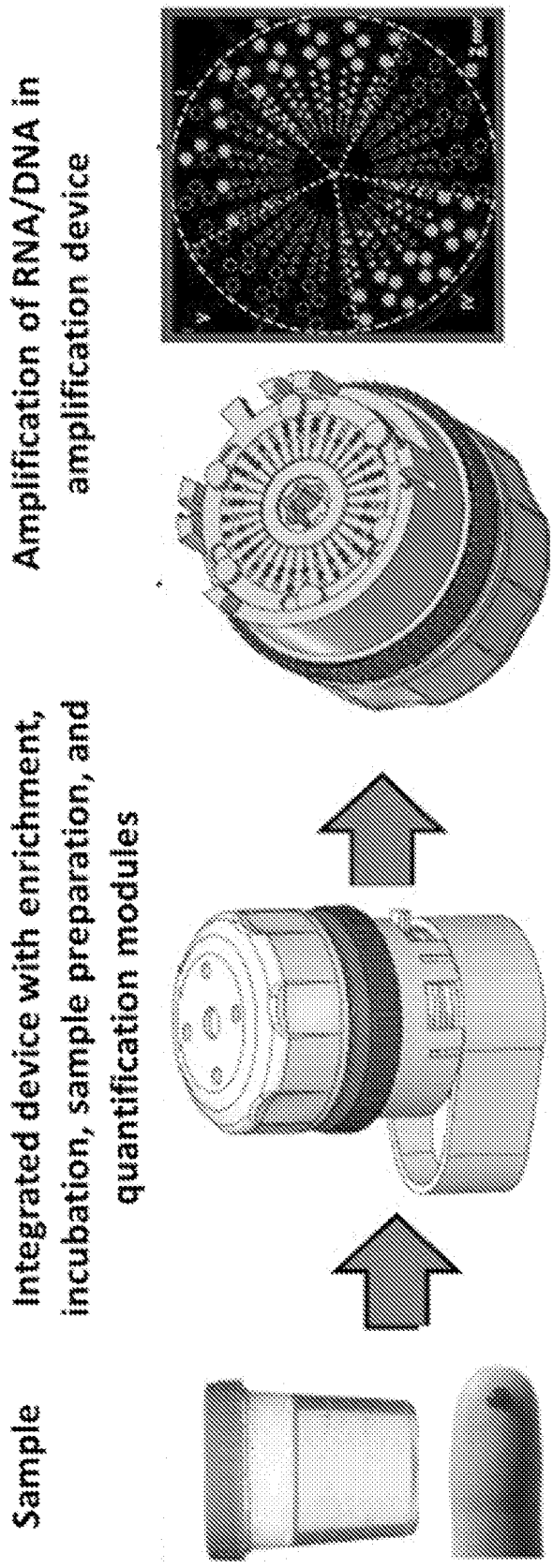
Sample
Integrated device with enrichment, incubation, sample preparation, and quantification modules
Amplification of RNA/DNA in amplification device

FIG. 10

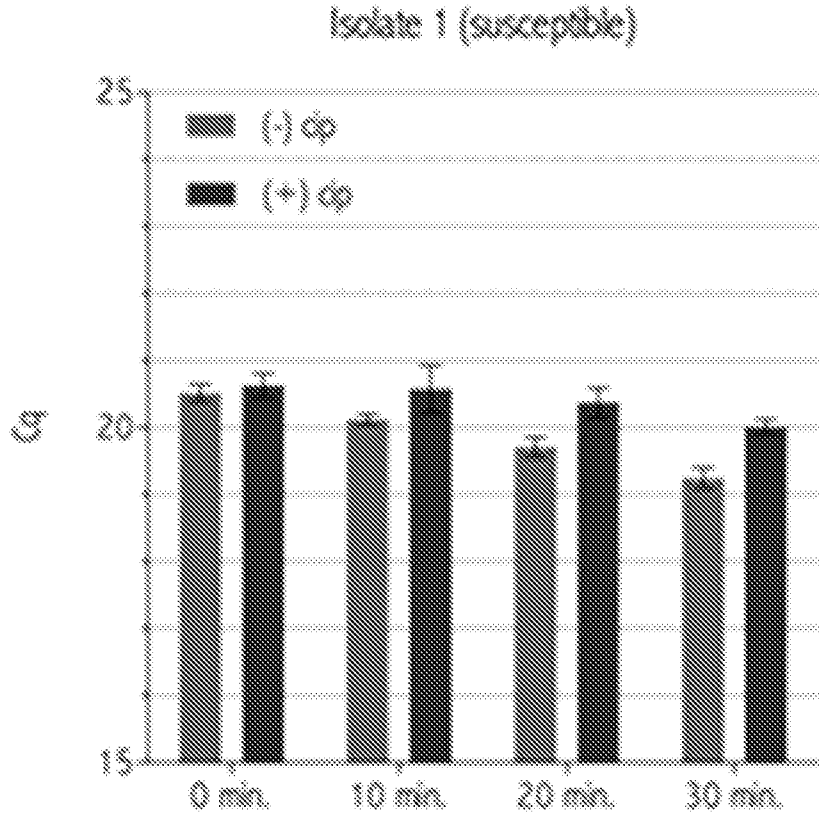

FIG. 11

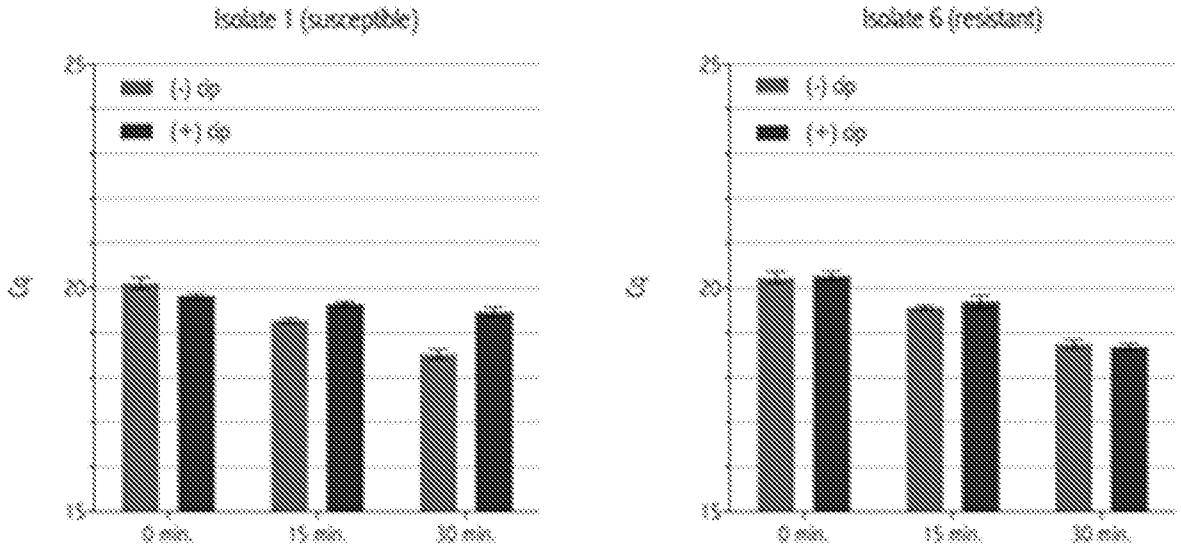

Figure 12A

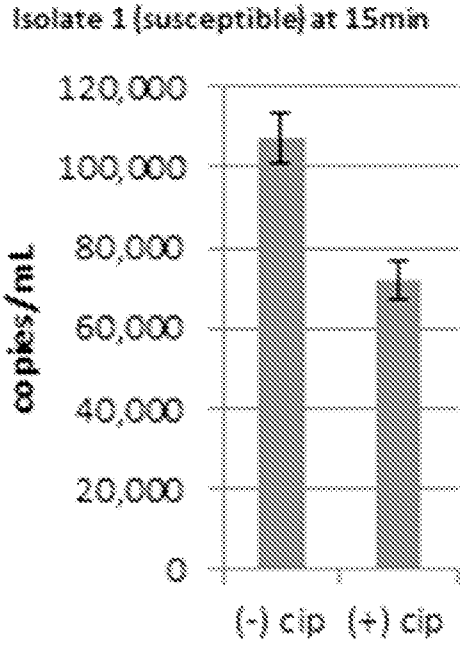

FIG. 12B

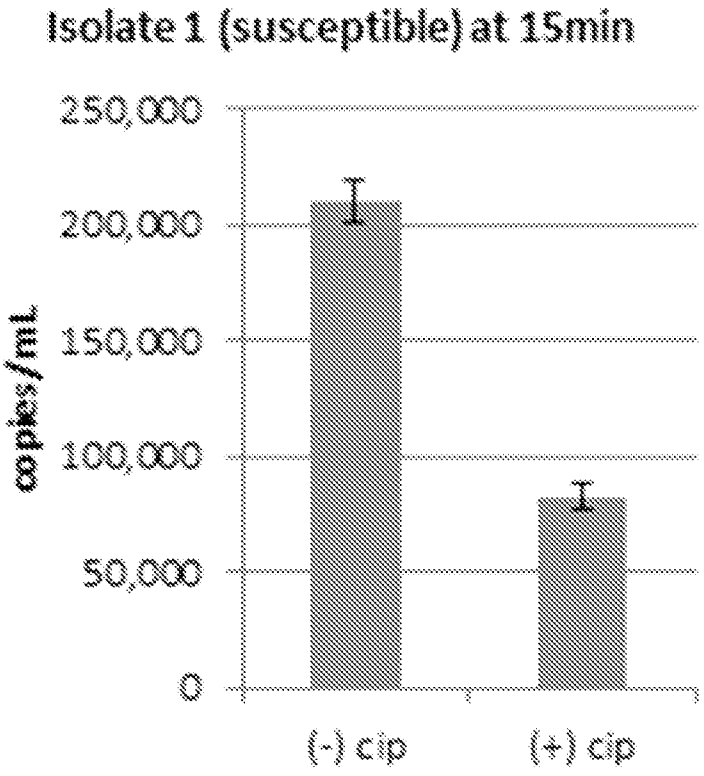
Isolate 1 (susceptible) at 15min
250,000
200,000
150,000
100,000
50,000
0
copies/mL
(-) cip
(+) cip

FIG. 13

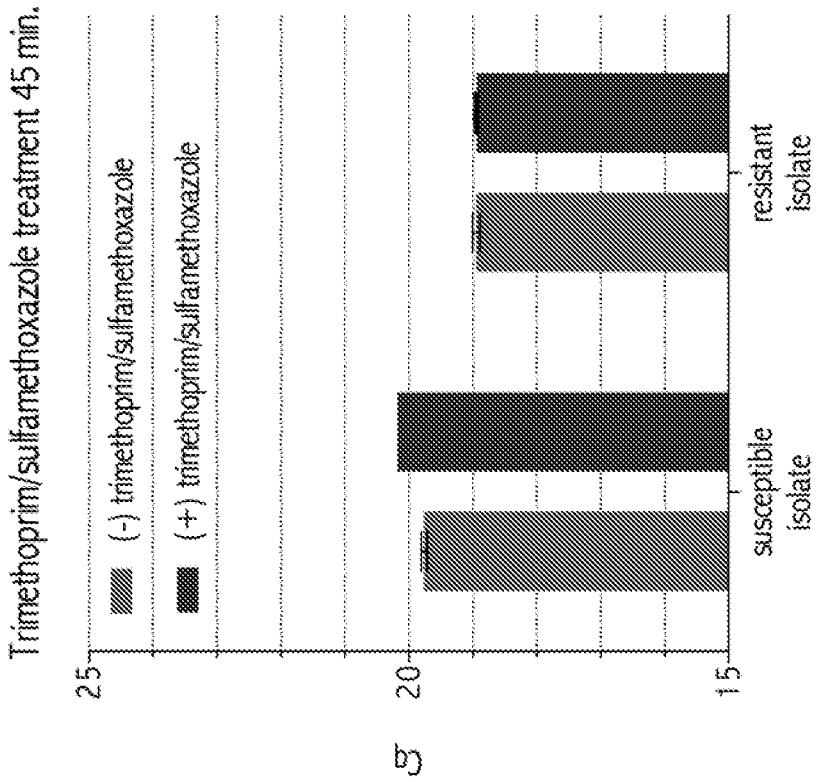

FIG. 14B

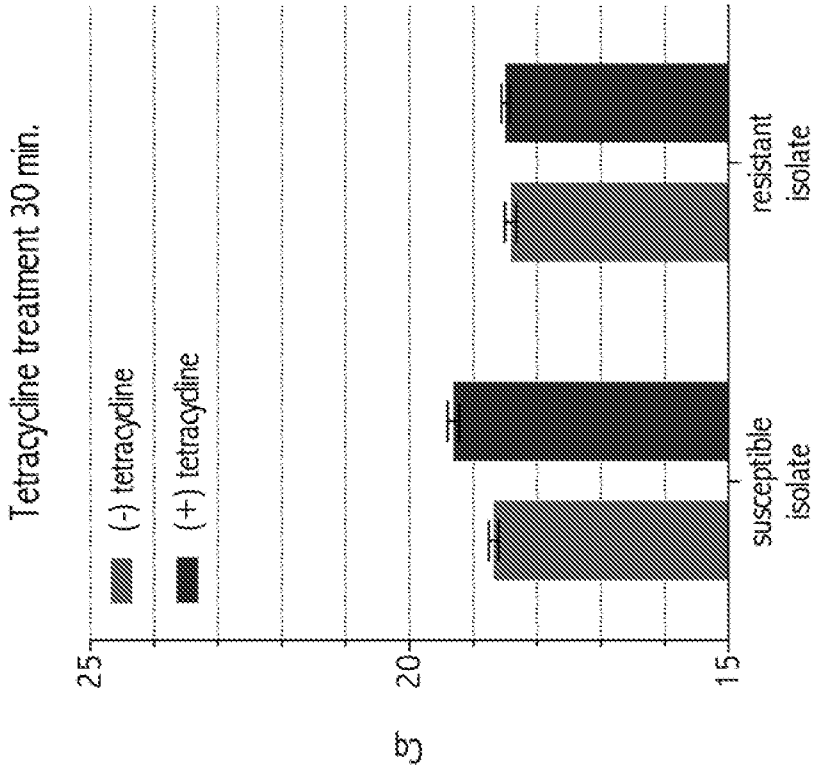

FIG. 14A

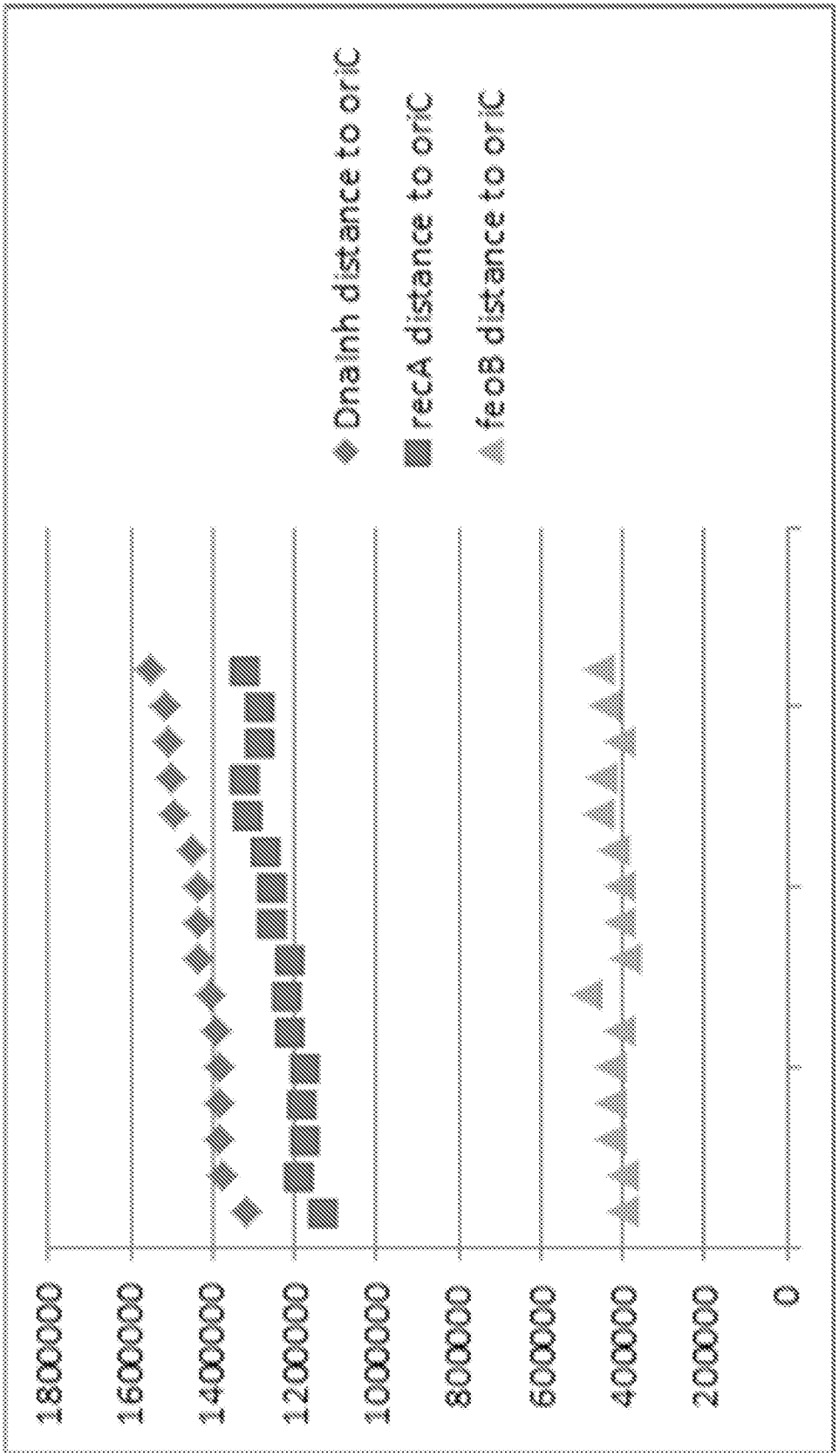
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0
Onainh distance to oriC
recA distance to oriC
feoB distance to oriC

FIG. 15

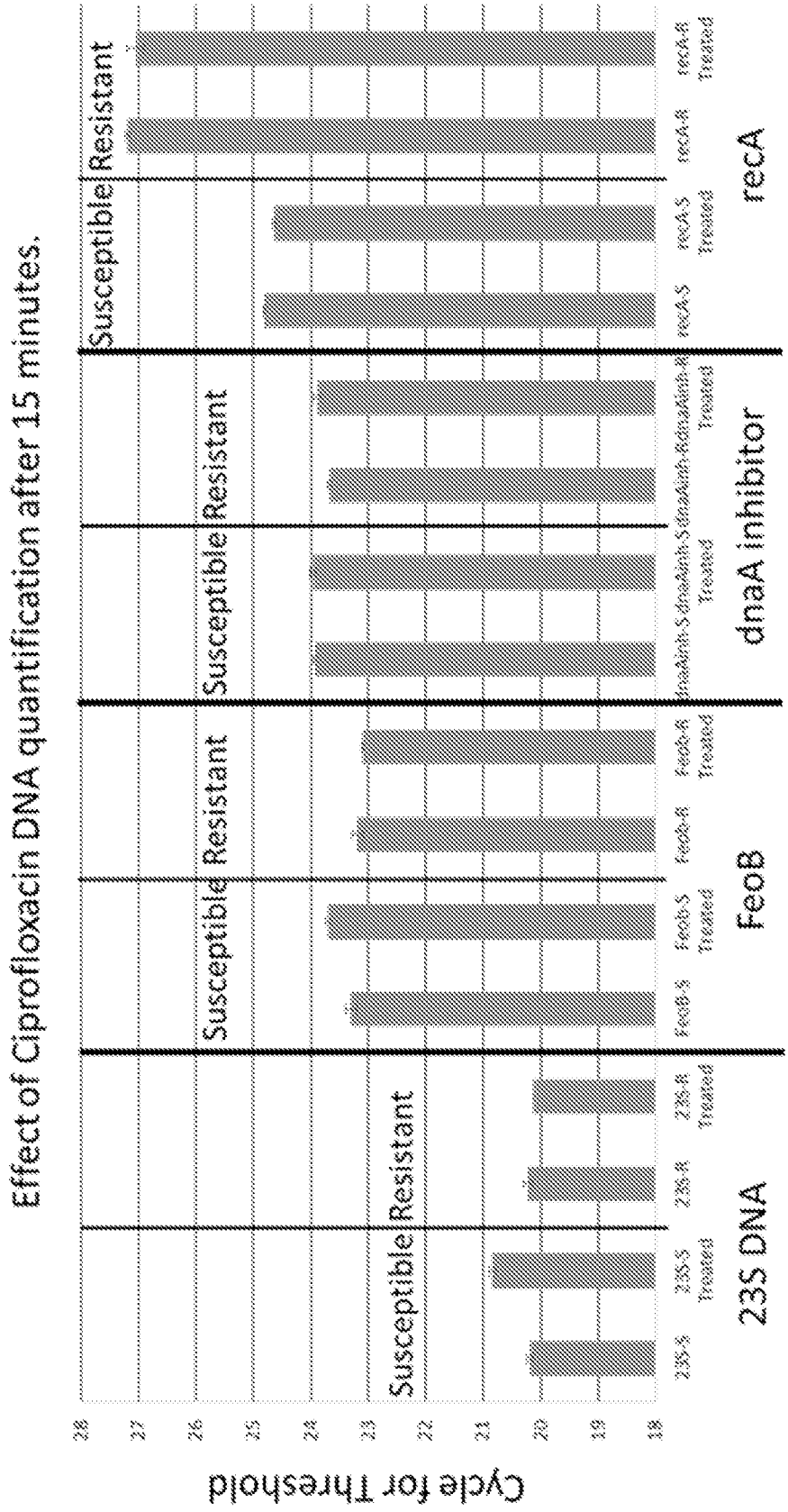
Effect of Ciprofloxacin DNA quantification after 15 minutes.
Cycle for Threshold
28
27
26
25
24
23
22
21
20
19
18
23S DNA
FeoB
dnaA inhibitor
recA
Susceptible
Resistant
23S-S
23S-S Treated
23S-R
23S-R Treated
FeoB-S
FeoB-S Treated
FeoA-R
FeoA-R Treated
dnaAInh-S
dnaAInh-S Treated
dnaAInh-R
dnaAInh-R Treated
recA-S
recA-S Treated
recA-R
recA-R Treated

FIG. 16

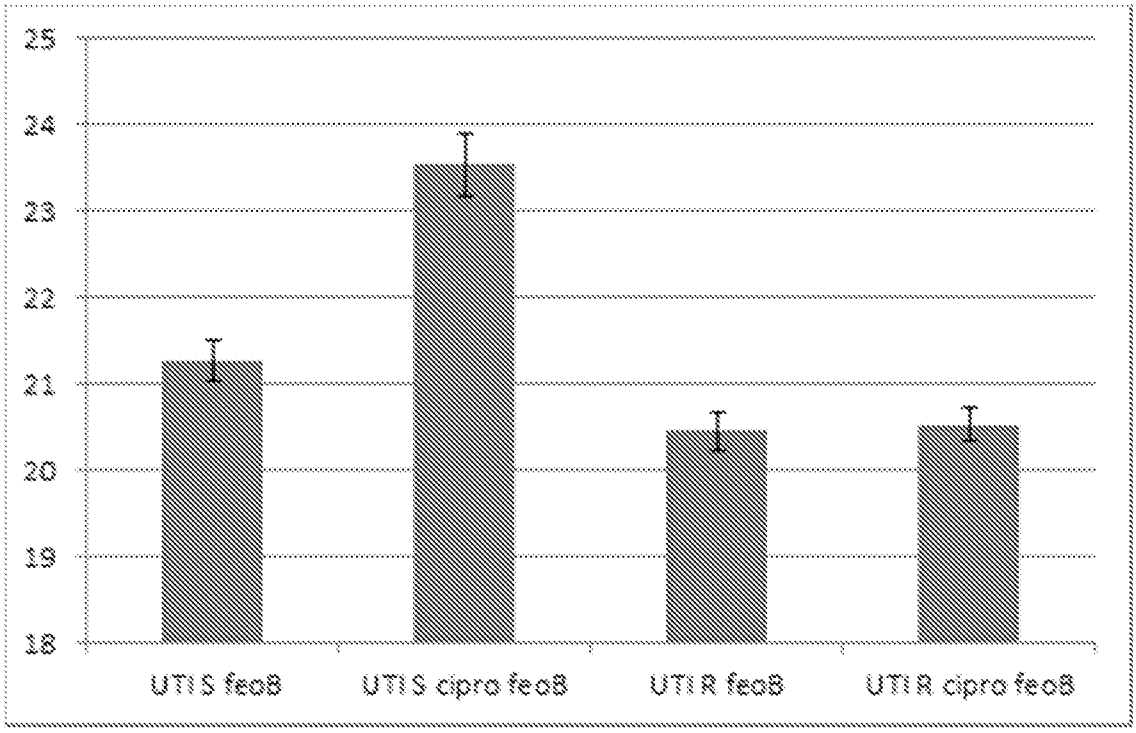

FIG. 17A

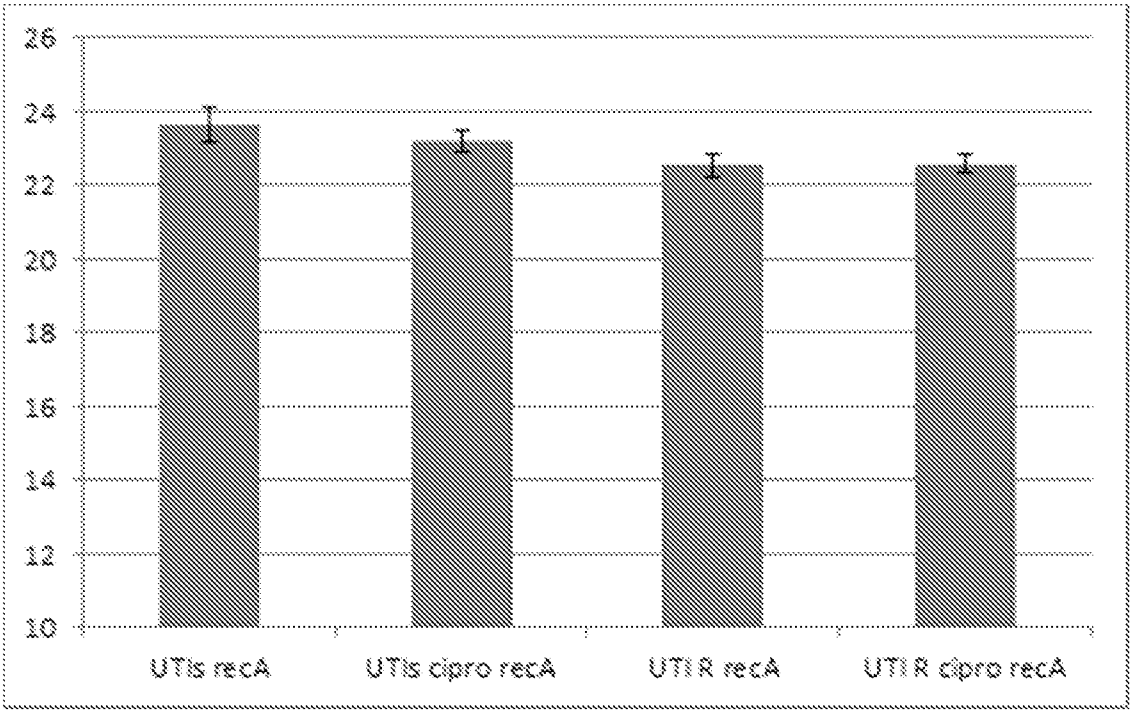

FIG. 17B

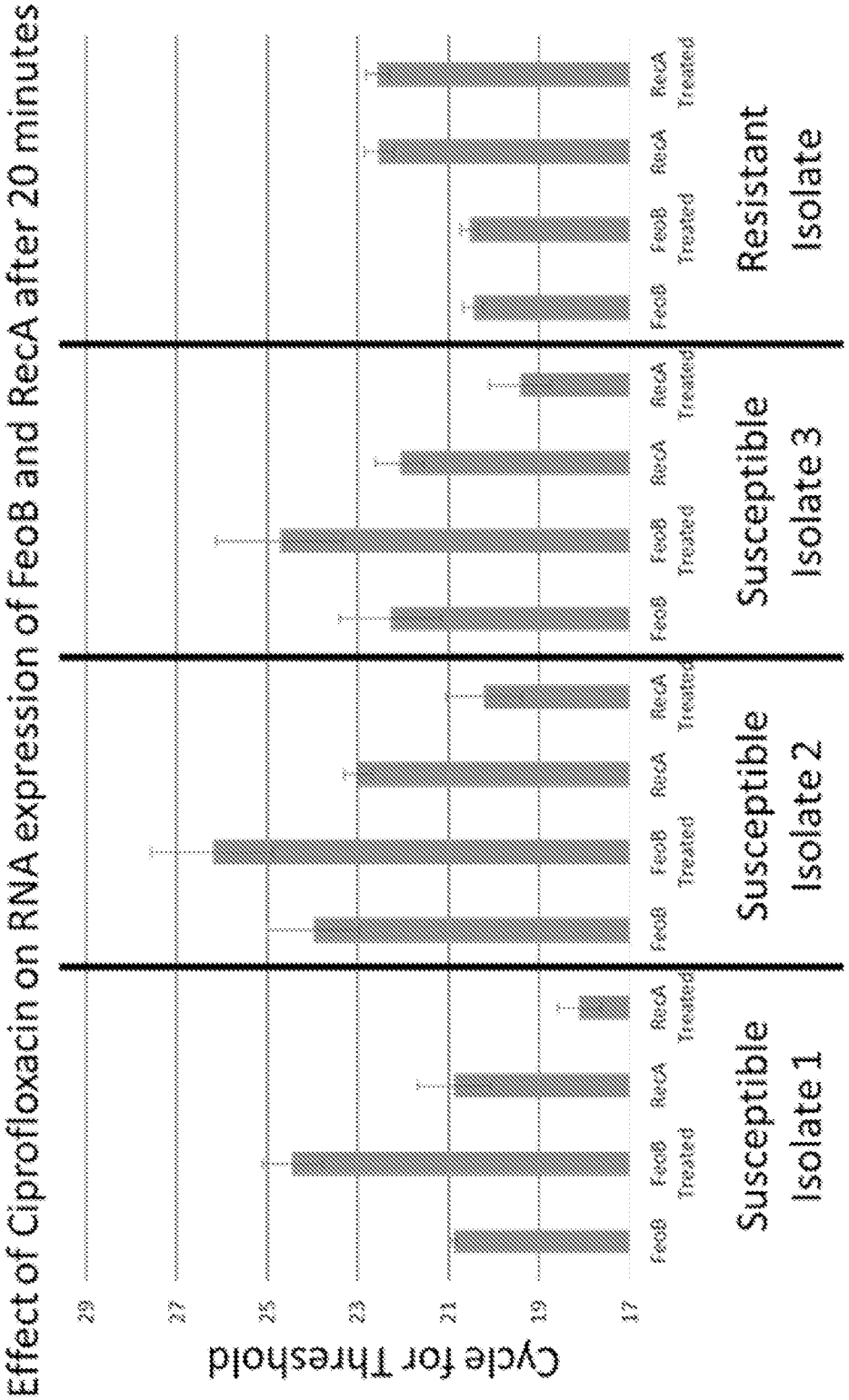
Effect of Ciprofloxacin on RNA expression of FeoB and RecA after 20 minutes
Cycle for Threshold
29
27
25
23
21
19
17
FeoB
FeoB Treated
RecA
RecA Treated
Susceptible Isolate 1
Susceptible Isolate 2
Susceptible Isolate 3
Resistant Isolate

FIG. 18

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WIPO | PCT

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C12Q 1/68 (2006.01)

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5 November 2015 (05.11.2015)

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(74) Agents: **SHUSTER, Michael, J.** et al.; Fenwick & West LLP, Silicon Valley Center, 801 California Street, Mountain View, CA 94041 (US).

(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT,

[Continued on next page]

(54) Title: MICROFLUIDIC MEASUREMENTS OF THE RESPONSE OF AN ORGANISM TO A DRUG

(57) Abstract: Disclosed herein are methods and devices for rapid assessment of whether a microorganism present in a sample is susceptible or resistant to a treatment.

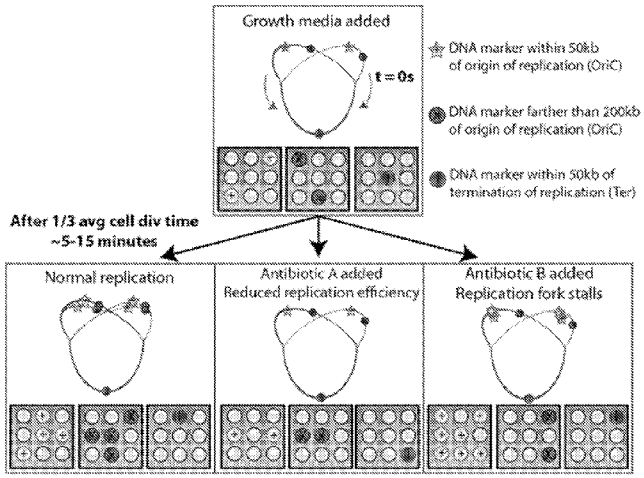

FIG. 4

WO 2016/085632 A3

HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

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INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/59344

A. CLASSIFICATION OF SUBJECT MATTER
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CPC - C12Q 1/02, 1/68, 1/70, 1/025, 1/689, 1/6888, 1/6809, 1/6895
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC(8): C12Q 1/02, 1/68, 1/70 (2016.01)
CPC: C12Q 1/02, 1/68, 1/70, 1/025, 1/689, 1/6888, 1/6809, 1/6895

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatSeer (US, EP, WO, JP, DE, GB, CN, FR, KR, ES, AU, IN, CA, INPADOC Data); EBSCO Discovery; PubMed; Google; Google Scholar; Google Patents; KEYWORDS: first sample, second sample, antibiotic, detect, lexA, recA

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ----- Y	US 2013/0190196 A1 (ONDERDONK, A et al.) July 25, 2013; abstract; figure 23; paragraphs [0007], [0011], [0048], [0071], [0082], [0090], [0098], [0165], [0180], [0184]-[0186], [0193], [0194], [0200], [0202] column 31; claims 1, 9	1-7, 9, 12-18, 20, 24, 30-31, 35-39 --- 8, 10-11, 19, 21-23, 25-29, 32-34, 40-49
Y	US 2009/0181395 A1 (BECKER, M et al.) July 16, 2009; abstract; paragraphs [0025], [0073]	8, 10, 19, 41-42
Y	US 2013/0052653 A1 (STEIN, E et al.) February 28, 2013; abstract; paragraphs [0241], [0304], [0307], [0315]	11, 21-23
Y	US 2011/0269130 A1 (SHI, S et al.) November 3, 2011; abstract; paragraphs [0009], [0039], [0059], [0061]	32-34, 43-44
Y	US 2013/0309679 A1 (SLIPCHIP, LLC, et al.) November 21, 2013; figure 1; paragraphs [0036], [0061], [0207]	40-48
Y	US 8,476,063 B2 (INTEGENX, INC.) July 2, 2013; abstract; claim 1	49

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
6 June 2016 (06.06.2016)	12 JUL 2016
Name and mailing address of the ISA/ Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300	Authorized officer Shane Thomas PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US15/59344

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:

   a. ☐ forming part of the international application as filed:

      ☐ in the form of an Annex C/ST.25 text file.

      ☐ on paper or in the form of an image file.

   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.

   c. ☒ furnished subsequent to the international filing date for the purposes of international search only:

      ☒ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).

      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☒ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

(19)中华人民共和国国家知识产权局

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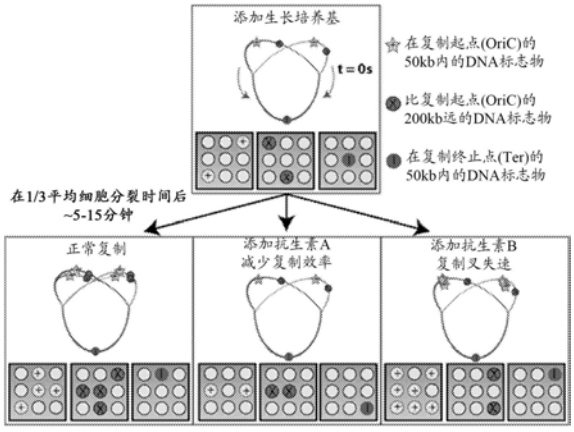
权利要求书4页 说明书58页 附图17页

(54)发明名称

生物体对药物的响应的微流体测量

(57)摘要

本文公开了用于快速评价样品中存在的微生物是否对处理易感或耐受的方法和装置。



CN 107636173 A

1.一种方法,所述方法包括:

提供第一样品和第二样品,所述第一样品包含来自细菌群体的第一部分的靶分子,所述第二样品包含来自所述细菌群体的第二部分的靶分子,其中所述第一部分已用抗生素处理,并且其中所述第二部分未用所述抗生素处理;

在多个第一分析区域中分配所述第一样品;

在多个第二分析区域中分配所述第二样品;

使所述第一分析区域和所述第二分析区域的每一个与用于进行反应的试剂接触以检测所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在;以及

检测所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在,其中每一个样品在所述分析区域中的分配被实现为使得在所述反应之后在所述第一分析区域和所述第二分析区域的一些中检测到阈值数目的靶分子,并且在所述第一分析区域和所述第二分析区域的一些中未检测到阈值数目的靶分子。

2.如权利要求1所述的方法,其中所述阈值数目大于零、一、二、三、四或五。

3.如权利要求1所述的方法,其中每一个样品在所述分析区域中的分配被实现为使得所述分析区域的至少一些不具有所述靶分子,并且所述分析区域的至少一些仅具有一个靶分子。

4.如权利要求1所述的方法,其中每一个样品在所述分析区域中的分配被实现为使得所述分析区域的至少一个仅包含一个靶分子。

5.如权利要求1所述的方法,其中所述第一样品包含所述试剂,并且其中使所述多个第一分析区域与所述试剂接触包括在所述多个第一分析区域中分配所述第一样品的所述步骤。

6.如权利要求1所述的方法,其中所述第二样品包含所述试剂,并且其中使所述多个第二分析区域与所述试剂接触包括在所述多个第二分析区域中分配所述第二样品的所述步骤。

7.如权利要求1所述的方法,其中所述反应包括核酸扩增。

8.如权利要求7所述的方法,其中所述核酸扩增是基本上等温的。

9.如权利要求7所述的方法,其中所述核酸扩增是聚合酶链式反应。

10.如权利要求7所述的方法,其中所述核酸扩增选自由以下组成的组:基于核酸序列的扩增(NASBA)、自主序列复制(3SR)、环介导的等温扩增(LAMP)、链置换扩增(SDA)、全基因组扩增、多重置换扩增、链置换扩增、解旋酶依赖性扩增、切口酶扩增反应、重组聚合酶扩增、逆转录PCR和连接介导的PCR。

11.如权利要求1所述的方法,其中所述分析区域包括孔。

12.如权利要求1所述的方法,其中所述靶分子是核酸。

13.如权利要求12所述的方法,其中所述试剂是核酸扩增试剂。

14.如权利要求13所述的方法,所述方法还包括将所述分析区域的每一个暴露于有效扩增所述核酸的条件。

15.如权利要求1所述的方法,其中所述靶分子包括细菌染色体或质粒的区段。

16.如权利要求15所述的方法,其中所述靶分子距离复制起点小于50kDa、小于100kDa、

小于200kDa、或小于400kDa。

17.如权利要求15所述的方法，其中所述靶分子包括在所述细菌染色体或质粒上的基因的序列。

18.如权利要求1所述的方法，其中所述靶分子包括mRNA。

19.如权利要求1所述的方法，其中所述靶分子通过扩增反应扩增，所述扩增反应包含与所述靶分子的序列互补的引物。

20.如权利要求18所述的方法，其中所述mRNA编码recA或lexA。

21.如权利要求1所述的方法，其中所述试剂被布置在多个试剂区域中。

22.如权利要求18所述的方法，其中通过使所述多个试剂区域与所述第一多个分析区域或所述第二多个分析区域流体连通来实现接触。

23.如权利要求22所述的方法，其中接触包括在包含所述试剂区域的基底与包含所述第一多个分析区域和所述第二多个分析区域的基底之间实现相对移动。

24.如权利要求1所述的方法，所述方法还包括分析检测每一多个分析区域中阈值数目的靶分子的存在或不存在，以确定所述细菌群体对抗生素的耐受性或易感性。

25.如权利要求1所述的方法，其中所述靶分子已从所述第一样品中的细菌中取出，并且其中所述细菌最初暴露于抗生素和取出所述靶分子之间的时间小于生长期中所述细菌的平均倍增时间。

26.如权利要求1所述的方法，其中在所述第一样品暴露于所述抗生素结束之后小于3小时、2小时或一小时进行所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在的所述检测。

27.如权利要求26所述的方法，其中在所述第一样品暴露于所述抗生素结束之后小于45分钟、30分钟、15分钟或10分钟进行所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在的所述检测。

28.如权利要求1所述的方法，其中在所述第一样品暴露于所述抗生素结束之后小于3小时、小于2小时或小于1小时进行所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在的所述检测。

29.如权利要求28所述的方法，其中在所述第一样品暴露于所述抗生素结束之后小于45分钟、小于30分钟、小于15分钟或小于10分钟进行所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在的所述检测。

30.如权利要求1所述的方法，其中来自所述第一样品的所述细菌已用抗生素处理持续以下的时间段：不超过2小时、不超过1小时、不超过45分钟、不超过30分钟、不超过15分钟、或不超过10分钟。

31.如权利要求1所述的方法，其中从来自所述第一样品的细菌首次暴露于抗生素至检测步骤的时间小于3小时、小于2小时、小于1小时、小于45分钟或小于30分钟。

32.如权利要求1所述的方法，其中所述第一多个分析区域包括至少10个、20个、30个、40个或50个分析区域。

33.如权利要求1所述的方法，其中所述第二多个分析区域包括至少10个、20个、30个、40个或50个分析区域。

34.如权利要求1所述的方法，其中并行地进行所述第一样品和所述第二样品到所述第

一多个分析区域和所述第二多个分析区域中的分配。

35.如权利要求1所述的方法，其中所述第一样品或所述第二样品包含小于10,000个、5,000个、1,000个、500个、200个、100个、50个、20个或10个靶分子。

36.一种确定细菌群体对抗生素的耐受性或易感性的方法，所述方法包括：

提供第一样品和第二样品，所述第一样品包含来自细菌群体的第一部分的靶分子，所述第二样品包含来自所述细菌群体的第二部分的靶分子，其中所述第一部分已用抗生素处理，并且其中所述第二部分未用所述抗生素处理；

在多个第一分析区域中分配所述第一样品；

在多个第二分析区域中分配所述第二样品；

使所述第一分析区域和所述第二分析区域的每一个与用于进行反应的试剂接触以检测所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在；

检测所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在，其中每一个样品在所述分析区域中的分配被实现为使得在所述反应之后在所述第一分析区域和所述第二分析区域的一些中检测到阈值数目的靶分子，并且在所述第一分析区域和所述第二分析区域的一些中未检测到阈值数目的靶分子；和

分析所述检测结果以确定所述细菌群体对所述抗生素的耐受性或易感性。

37.一种确定细菌群体对抗生素的耐受性或易感性的方法，所述方法包括：

将细菌群体分配到多个克隆分离区域中，所述分配被实现为使得所述克隆分离区域的至少一些被统计地估计为每一个克隆分离区域包含单个分离的细菌；

扩增所述单个分离的细菌的每一个以产生多个克隆群体；

将所述多个克隆群体的每一个分配到来自多个处理区域的至少一个处理区域中和来自多个对照区域的至少一个对照区域中；

使所述第一多个处理区域与抗生素接触，所述多个对照区域不与所述抗生素接触；

对于所述第一多个处理区域的每一个和所述多个对照区域的每一个，将来自每一个克隆群体的一个或更多个靶分子分配到不同的多个分析区域中；

使所述分析区域的每一个与用于进行反应的试剂接触以检测所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在；以及

检测所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分子的存在或不存在，其中每一个样品在所述分析区域中的分配被实现为使得在所述反应之后在所述不同的多个分析区域的一些中检测到阈值数目的靶分子，并且在所述不同的多个分析区域的一些中未检测到阈值数目的靶分子。

38.如权利要求37所述的方法，所述方法还包括分析所述检测的结果以确定所述多个克隆群体的至少一些对所述抗生素的耐受性或易感性。

39.一种方法，所述方法包括：

提供第一样品和第二样品，所述第一样品包含来自细胞群体的第一部分的靶分析物，所述第二样品包含来自所述细胞群体的第二部分的靶分析物，其中所述第一部分已用药物处理，并且其中所述第二部分未用所述药物处理；

在多个第一分析区域中分配所述第一样品；

在多个第二分析区域中分配所述第二样品；

使所述第一分析区域和所述第二分析区域的每一个与用于进行反应的试剂接触以检测所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分析物的存在或不存在;以及

检测所述第一分析区域和所述第二分析区域的每一个中阈值数目的靶分析物的存在或不存在,其中每一个样品在分析区域中的分配被实现为使得在所述反应之后在所述第一分析区域和所述第二分析区域的一些中检测到阈值数目的靶分析物,并且在所述第一分析区域和所述第二分析区域的一些中未检测到阈值数目的靶分析物。

40.一种试剂盒,所述试剂盒包括:

容器,所述容器包括

多个分析区域,

多个试剂区域,所述多个试剂区域包含核酸扩增组分,其中所述容器包含第一层和第二层,所述第一层和所述第二层被配置为相对于另一层在第一位置和第二位置之间移动,在所述第一位置中所述多个分析区域和所述多个试剂区域彼此分离,在所述第二位置中所述多个分析区域的至少一些与所述多个试剂区域的至少一些流体连通;以及

用于确定细菌群体对抗生素的耐受性或易感性的使用说明。

41.如权利要求39所述的试剂盒,其中所述核酸扩增组分包括用于扩增recA或lexA mRNA的引物。

42.如权利要求39所述的试剂盒,其中所述核酸扩增组分包括用于扩增细菌染色体或质粒上距离复制起点小于50kDa、小于100kDa、小于200kDa、或小于400kDa的核酸靶的引物。

43.如权利要求39所述的试剂盒,其中所述容器包含至少10个、20个、30个、40个或50个分析区域。

44.如权利要求39所述的试剂盒,其中所述容器包含至少10个、20个、30个、40个或50个试剂区域。

45.如权利要求39所述的试剂盒,其中所述抗生素选自由以下组成的组:氨基糖苷类、头孢菌素类、四环素类、磺胺类、大环内酯类、万古霉素和β-内酰胺类。

46.如权利要求45所述的试剂盒,其中所述说明指示,与未用所述抗生素处理的所述细菌群体的部分相比,用所述抗生素处理的所述细菌群体的部分中核酸靶检测的减少表明所述细菌群体对所述抗生素易感。

47.如权利要求39所述的试剂盒,其中所述抗生素选自由以下组成的组:HPUra、羟基脲、甲氧苄啶、环丙沙星和MMC。

48.如权利要求47所述的试剂盒,其中所述说明指示,与未用所述抗生素处理的所述细菌群体的部分相比,用所述抗生素处理的所述细菌群体的部分中核酸靶检测的增加表明所述细菌群体对所述抗生素易感。

49.一种用于加工样品的装置,所述装置包括孵育模块、样品制备模块和数字定量模块,其中所述装置可被配置为使每一个模块与其他模块流体连通;其中所述孵育模块包括孵育室,所述孵育室被配置为使生物体与药物孵育,其中所述样品制备模块被配置为提取来自所述生物体的靶分析物;并且其中所述数字定量模块包括多个反应区域,所述多个反应区域被配置为进行所述反应区域中所述靶分析物的存在或不存在的数字检测。

生物体对药物的响应的微流体测量

[0001] 交叉参考

[0002] 本申请要求于2014年11月5日提交的美国临时申请号62/075,648的权益，该申请通过引用以其整体并入本文。

[0003] 关于联邦资助研究的声明

[0004] 本发明根据由美国国立卫生研究院(National Institutes of Health)(NIH)授予的拨款号EB012946、GM074961和DP1OD003584以及根据由美国国防高级研究计划局(Defense Advanced Research Projects Agency)的合作协议号HR-0011-11-2-0006的支持下进行。政府具有本发明中的某些权利。

技术领域

[0005] 本申请涉及微流体领域以及生物实体的检测和扩增领域。

[0006] 发明背景

[0007] 抗生素耐受性(resistance)对全球公共健康构成主要的迫在眉睫的威胁。来自CDC(美国卫生与人类服务部(U.S.Department of Health and Human Services)，2013,114)和WHO(组织(Organization)，2014,257)的统计资料令人震惊，并强调目前的自由和非选择性抗生素使用模式不能继续。对其存在有限治疗选择方案的革兰氏阴性生物体的耐受性的出现尤为令人担忧。诊所(clinics)中广谱抗生素的过度使用是药物耐受病原体进化和传播的主要贡献者;然而，对抗耐受性要求所有环境(settings)中的诊断开发。

[0008] 为了改善护理，临床实验室要求鉴定病原体并确定其抗微生物剂易感性的快速测试。实验室目前经由花费24-48小时的基于培养的方法进行鉴定和抗微生物剂易感性测试(AST)。因为医师在最接近的时间帧内不知道存在什么样的生物体、细菌负荷或生物体对各种治疗方法的易感性，所以临床医师通常选择用广谱(例如第二代和第三代)抗生素治疗，并且甚至可以治疗亚临床感染(sub-clinical infection)或假阳性。在复杂、复发和特殊病例中，抗生素开处方过度(Overprescribing antibiotics)最为突出。这些包括儿童发热(febrile children)、阻塞性患者(obstructed patients)，诸如有BPH、肾结石的男性，特别是用重复仪器的那些男性、有妊娠期间感染的女子、住院患者和免疫受损的患者。在这些情况下，临床医师倾向于更激进地(aggressively)治疗。加速周转(turnaround)将允许临床医师通过无延迟地施用正确的抗微生物剂方案来改善患者的护理。因此，为了改善新兴抗微生物剂耐受性的问题并改善护理，初级护理设施(primary care settings)的医师要求鉴定病原体并确定其抗微生物剂易感性的超快速测试。因此，需要的是用于快速地且准确地诊断感染和抗微生物剂耐受性或易感性的装置和方法。

[0009] 发明概述

[0010] 在一方面，本发明提供可以快速地鉴定细胞(包括癌细胞)或微生物(包括病原体)，定量它们的载量，并诊断它们对药物诸如抗生素的易感性或耐受性的装置和方法。在一些实施方案中，装置可以使用可以起源于多种样品类型(包括临床或环境样品)的单独微生物或细胞来实现药物易感性或药物耐受性的表型检测和代谢分析。这些样品类型可以包

括,但不限于,血液、脑脊髓液(cerebral spinal fluid)(CSF)、唾液和尿液,并且还可以包括诸如来自水或医院表面(hospital surface)的环境样品。在一些实施方案中,装置能够使细胞与药物诸如抗生素孵育,并然后在无污染的平台快速地提取和定量核酸或其他分子。装置可以在微流体装置中使用数字单分子测量,所述单分子测量提供改善检测限值同时提供定量数据的超灵敏的测量,对区分病原体与污染物并且实现较早区分药物耐受与易感生物体或细胞非常重要。在一些实施方案中,这些装置可以区分来自临床样品的单独微生物或细胞的状态,并且了解它们对药物(诸如例如抗生素)的单独响应的时机,提供超快的药物-易感性测量。

[0011] 在一些实施方案中,与样品相关的数据包括来自大于一个空间分离隔室的测量,隔室的每一个包含样品的一部分。

[0012] 在一些实施方案中,样品经历核酸扩增。在一些实施方案中,核酸扩增反应是环介导的扩增(LAMP)反应。在一些实施方案中,核酸扩增反应是PCR反应。在一些实施方案中,方法在约55℃-65℃或在55℃-65℃的温度范围来进行。在一些实施方案中,样品的至少一部分被分区为包含至少2个或更多个的阵列。在一些实施方案中,阵列是滑动芯片(SlipChip)。在一些实施方案中,被扩增的核酸是RNA。

[0013] 本文还提供了用于检测和定量来自样品的靶分子的方法,所述方法包括:提供第一样品和第二样品,所述第一样品包含来自细菌群体的第一部分的靶分子,所述第二样品包含来自所述细菌群体的第二部分的靶分子,其中第一部分已用抗生素处理,并且其中第二部分未用抗生素处理;在多个第一分析区域中分配所述第一样品;在多个第二分析区域中分配所述第二样品;使第一分析区域和第二分析区域的每一个与用于进行反应的试剂接触以检测第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在;以及检测第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在,其中每一个样品在分析区域中的分配被实现为使得在所述反应之后在第一分析区域和第二分析区域的一些中检测到阈值数目的靶分子,并且在第一分析区域和第二分析区域的一些中未检测到阈值数目的靶分子。

[0014] 在一些实施方案中,阈值数目大于零、一、二、三、四或五。在一些实施方案中,每一个样品在分析区域中的分配被实现为使得分析区域的至少一些不具有靶分子,并且分析区域的至少一些仅具有一个靶分子。在一些实施方案中,每一个样品在分析区域中的分配被实现为使得至少一个分析区域仅包含一个靶分子。在一些实施方案中,第一样品包含所述试剂,并且其中使多个第一分析区域与试剂接触包括在所述多个第一分析区域中分配所述第一样品的所述步骤。在一些实施方案中,第二样品包含所述试剂,并且其中使多个第二分析区域与试剂接触包括在所述多个第二分析区域中分配所述第二样品的所述步骤。

[0015] 在一些实施方案中,反应包括核酸扩增。在一些实施方案中,核酸扩增是基本上等温的。在一些实施方案中,核酸扩增是聚合酶链式反应。在一些实施方案中,核酸扩增是基于核酸序列的扩增(NASBA)、自主序列复制(3SR)、环介导的等温扩增(LAMP)、链置换扩增(SDA)、全基因组扩增、多重置换扩增、链置换扩增、解旋酶依赖性扩增、切口酶扩增反应、重组聚合酶扩增、逆转录PCR或连接介导的PCR。

[0016] 在一些实施方案中,所述分析区域包括孔。在一些实施方案中,靶分子是核酸。在一些实施方案中,试剂是核酸扩增试剂。在一些实施方案中,检测和定量来自样品的靶分子

的方法还包括将分析区域的每一个暴露于有效扩增核酸的条件。

[0017] 在一些实施方案中，靶分子包括细菌染色体或质粒的区段。在一些实施方案中，靶分子距离复制起点小于50kDa、小于100kDa、小于200kDa、或小于400kDa。在一些实施方案中，靶分子包括在所述细菌染色体或质粒上的基因的序列。在一些实施方案中，靶分子包括mRNA。在一些实施方案中，靶分子通过扩增反应扩增，所述扩增反应包含与靶分子的序列互补的引物。在一些实施方案中，mRNA编码recA或lexA。

[0018] 在一些实施方案中，试剂被布置在多个试剂区域中。在一些实施方案中，通过放置(placing)多个试剂区域与第一多个分析区域或第二多个分析区域流体连通来实现接触。在一些实施方案中，接触包括在包含试剂区域的基底与包含第一多个分析区域和第二多个分析区域的基底之间实现相对移动。在一些实施方案中，检测和定量来自样品的靶分子的方法还包括分析检测每一多个分析区域中阈值数目的靶分子的存在或不存在，以确定细菌群体对抗生素的耐受性或易感性。

[0019] 在一些实施方案中，靶分子已从第一样品中的细菌中取出，并且其中细菌最初暴露于抗生素和取出靶分子之间的时间小于生长期中细菌的平均倍增时间。在一些实施方案中，在第一样品暴露于抗生素结束之后少于3小时、2小时或一小时进行第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在的检测。在一些实施方案中，在第一样品暴露于抗生素结束之后少于45分钟、30分钟、15分钟或10分钟进行第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在的检测。在一些实施方案中，在第一样品暴露于抗生素结束之后少于3小时、少于2小时或少于1小时进行第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在的检测。在一些实施方案中，在第一样品暴露于抗生素结束之后少于45分钟、少于30分钟、少于15分钟或少于10分钟进行第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在的检测。

[0020] 在一些实施方案中，来自所述第一样品的细菌已用抗生素处理持续以下的时间段：不超过2小时、不超过1小时、不超过45分钟、不超过30分钟、不超过15分钟、或不超过10分钟。在一些实施方案中，从来自第一样品的细菌首次暴露于抗生素至检测步骤的时间少于3小时、少于2小时、少于1小时、少于45分钟或少于30分钟。

[0021] 在一些实施方案中，第一多个分析区域包括至少10个、20个、30个、40个或50个分析区域。在一些实施方案中，第二多个分析区域包括至少10个、20个、30个、40个或50个分析区域。在一些实施方案中，并行地进行第一样品和第二样品到第一和第二多个分析区域中的分配。在一些实施方案中，第一样品或第二样品包含少于10,000个、5,000个、1,000个、500个、200个、100个、50个、20个或10个靶分子。

[0022] 本文还提供了一种确定细菌群体对抗生素的耐受性或易感性的方法，所述方法包括：提供第一样品和第二样品，所述第一样品包含来自细菌群体的第一部分的靶分子，所述第二样品包含来自所述细菌群体的第二部分的靶分子，其中第一部分已用抗生素处理，并且其中第二部分未用抗生素处理；在多个第一分析区域中分配所述第一样品；在多个第二分析区域中分配所述第二样品；使第一分析区域和第二分析区域的每一个与用于进行反应的试剂接触以检测第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在；检测第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在，其中每一个样品在分析区域中的分配被实现为使得在所述反应之后在第一分析区域和

第二分析区域的一些中检测到阈值数目的靶分子，并且在第一分析区域和第二分析区域的一些中未检测到阈值数目的靶分子；以及分析检测结果以确定细菌群体对所述抗生素的耐受性或易感性。

[0023] 本文还提供了一种确定细菌群体对抗生素的耐受性或易感性的方法，所述方法包括：将细菌群体分配到多个克隆分离区域中，所述分配被实现为使得克隆分离区域的至少一些被统计地估计为每一个包含单个分离的细菌；扩增单个分离的细菌的每一个以产生多个克隆群体；将所述多个克隆群体的每一个分配到来自多个处理区域的至少一个处理区域中，并且分配到来自多个对照区域的至少一个对照区域中；使第一多个处理区域与抗生素接触，多个对照区域不与抗生素接触；对于第一多个处理区域的每一个和多个对照区域的每一个，将来自每一个克隆群体的一个或更多个靶分子分配到不同的多个分析区域中；使每一个分析区域与用于进行反应的试剂接触以检测第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在；以及检测第一分析区域和第二分析区域的每一个中阈值数目的靶分子的存在或不存在，其中每一个样品在分析区域中的分配被实现为使得在所述反应之后在不同的多个分析区域的一些中检测到阈值数目的靶分子，并且在不同的多个分析区域的一些中未检测到阈值数目的靶分子。

[0024] 在一些实施方案中，确定细菌群体对抗生素的耐受性或易感性的方法还包括分析检测结果以确定所述多个克隆群体的至少一些对所述抗生素的耐受性或易感性。

[0025] 本文还提供了方法，所述方法包括：提供第一样品和第二样品，所述第一样品包含来自细胞群体的第一部分的靶分析物，所述第二样品包含来自所述细胞群体的第二部分的靶分析物，其中第一部分已用药物处理，并且其中第二部分未用药物处理；在多个第一分析区域中分配所述第一样品；在多个第二分析区域中分配所述第二样品；使第一分析区域和第二分析区域的每一个与用于进行反应的试剂接触以检测第一分析区域和第二分析区域的每一个中阈值数目的靶分析物的存在或不存在；并且检测第一分析区域和第二分析区域的每一个中阈值数目的靶分析物的存在或不存在，其中每一个样品在分析区域中的分配被实现为使得在所述反应之后在第一分析区域和第二分析区域的一些中检测到阈值数目的靶分析物，并且在第一分析区域和第二分析区域的一些中未检测到阈值数目的靶分析物。

[0026] 本文还提供了试剂盒，所述试剂盒包含：容器，所述容器包含多个分析区域和多个试剂区域，所述多个试剂区域包含核酸扩增组分，其中容器包含第一层和第二层，所述第一层和所述第二层被配置为相对于另一层在第一位置和第二位置之间移动，在第一位置中多个分析区域和多个试剂区域彼此分离，在第二位置中多个分析区域的至少一些与多个试剂区域的至少一些流体连通；以及用于确定细菌群体对抗生素的耐受性或易感性的说明。

[0027] 在一些实施方案中，核酸扩增组分包括用于扩增recA或lexA mRNA的引物。在一些实施方案中，核酸扩增组分包括用于扩增细菌染色体或质粒上距离复制起点小于50kDa、小于100kDa、小于200kDa、或小于400kDa的核酸靶的引物。在一些实施方案中，容器包含至少10个、20个、30个、40个或50个分析区域。在一些实施方案中，容器包含至少10个、20个、30个、40个或50个试剂区域。

[0028] 在一些实施方案中，抗生素选自由以下组成的组：氨基糖苷类、头孢菌素类、四环素类、磺胺类、大环内酯类、万古霉素和β-内酰胺类。在这些实施方案的某些实施方案中，说明指示，与未用所述抗生素处理的细菌群体的一部分相比，用所述抗生素处理的细菌群体

的一部分中核酸靶检测的减少表明细菌群体对所述抗生素易感。

[0029] 在一些实施方案中，抗生素选自由以下组成的组：HPUra、羟基脲、甲氧苄啶、环丙沙星和MMC。在这些实施方案的某些实施方案中，说明指示，与未用所述抗生素处理的细菌群体的一部分相比，用所述抗生素处理的细菌群体的一部分中核酸靶检测的增加表明细菌群体对所述抗生素易感。

[0030] 本文还提供了一种用于加工样品的装置，所述装置包括孵育模块、样品制备模块和数字定量模块，其中所述装置可以被配置为使每一个模块与其他模块流体连通；其中所述孵育模块包括孵育室，所述孵育室被配置为使生物体与药物孵育，其中所述样品制备模块被配置为提取来自所述生物体的核酸；并且其中所述数字定量模块包括多个反应区域，所述多个反应区域被配置为进行所述反应区域中所述核酸的存在或不存在的数字检测。

[0031] 通过引用并入

[0032] 本说明书中提及的所有出版物、专利和专利申请通过引用并入本文，其程度如同每一个单独出版物、专利或专利申请被具体和单独地指明通过引用并入的相同程度。

[0033] 本文描述了可以单独地或以用于应用的各种组合被使用的多种装置和方法，包括但不限于本文列出的那些。此外，它们可以以与对于先前描述的申请而被先前公开的装置和方法的各种组合被使用。

[0034] 本申请出于任何和所有目的通过引用以其整体并入以下申请：2011年4月5日提交的美国申请61/516,628，“Digital Isothermal Quantification of Nucleic Acids Via Simultaneous Chemical Initiation of Recombinase Polymerase Amplification(RPA) Reactions on Slip Chip,”；2011年5月9日提交的美国申请61/518,601，“Quantification of Nucleic Acids With Large Dynamic Range Using Multivolume Digital Reverse Transcription PCR(RT-PCR)On A Rotational Slip Chip Tested With Viral Load,”；2011年9月20日提交的美国申请13/257,811，“Slip Chip Device and Methods,”；2010年3月23日提交的国际申请PCT/US2010/028361，“Slip Chip Device and Methods,”；2009年11月18日提交的美国申请61/262,375，“Slip Chip Device and Methods,”；2009年3月24日提交的美国申请61/162,922，“Slip Chip Device and Methods,”；2010年3月22日提交的美国申请61/340,872，“Slip Chip Device and Methods,”；2012年4月5日提交的美国申请13/440,371，“Analysis Devices,Kits,And Related Methods For Digital Quantification Of Nucleic Acids And Other Analytes,”；2012年5月9日提交的美国申请13/467,482，“Multivolume Devices,Kits,Related Methods for Quantification and Detection of Nucleic Acids and Other Analytes,”；2013年4月22日提交的美国申请13/868,028，“Fluidic Devices and Systems for Sample Preparation or Autonomous Analysis,”；2013年4月22日提交的美国申请13/868,009，“Fluidic Devices for Biospecimen Preservation,”；2013年4月22日提交的国际申请PCT/US2013/037658，“Fluidic Devices for Biospecimen Preservation,”；2013年4月22日提交的国际申请PCT/US2013/037660，“Fluidic Devices and Systems for Sample Preparation or Autonomous Analysis,”；2013年4月24日提交的美国申请13/869,856，“Slip-Induced Compartmentalization,”；2013年10月4日提交的国际申请PCT/US2013/063594，“Methods and Systems for Microfluidics Imaging and Analysis,”；2014年4月18日提交的国际

申请PCT/US2014/034728,“Parallelized Sample Handling,”;2014年7月17日提交的国际申请PCT/US2014/047092,“Digital Assay for Quantifying and Concentrating Analytes,”;2014年8月15日提交的美国申请62/038,036,“The Pumping Lid:Devices and Methods for Programmable Generation of Positive and Negative Pressures,”;2014年9月15日提交的美国申请62/050,647,“Digital Microfluidics Methods for Optimizing Isothermal Amplification Reactions,”;2014年9月18日提交的国际申请PCT/US2014/056401,“System and Method for Movement and Timing Control,”;以及2014年10月16日提交的国际申请号PCT/US2014/060977“Enhanced Nucleic Acid Identification and Detection”。

[0035] 附图简述

[0036] 本发明的新颖的特征与所附权利要求书中的特殊性一起被陈述。本发明的特征和优势的更好的理解将通过参考陈述其中利用本发明的原理的说明性实施方案的以下详细说明来获得,并且在附图中:

[0037] 图1示出了尿路感染(UTI)大肠杆菌(Escherichia coli)分离株中的rDNA基因相对于复制起点的映射位置(mapped position)。

[0038] 图2示出了从来自UTI患者的大肠杆菌(E.coli)分离株测序的示例性基因组的环状表示和rRNA基因在基因组上的位置。

[0039] 图3基于易感细胞中与复制起点的邻近度(proximity)说明了药物对DNA和RNA水平的影响。

[0040] 图4描述了无抗生素、抗生素A和抗生素B对来自单个细胞、具有距离复制起点的不同距离的靶的复制效率、复制叉失速(replication fork stalls)和数字检测结果的影响。

[0041] 图5描绘了根据本发明的实施方案确定尿液样品中的细胞对药物的耐受性或易感性的工作流程。

[0042] 图6A、6B和6C示出了根据本发明的实施方案的包括孵育模块的集成装置的3个不同视图。

[0043] 图7示出了根据本发明的实施方案的将与集成装置一起使用的泡罩包装(blister packs)、试剂包装或其他类型的容器的照片。

[0044] 图8是根据本发明的实施方案的具有一个或更多个层的由弹簧或马达驱动的操作模块的图解。

[0045] 图9示出了用于进行单个分子和单个细胞测量以检测来自异质群体的单独细胞响应于药物处理的变化的流程图。

[0046] 图10描绘了用于在单个集成装置中提供测定和来自对样品的测定的结果的现场护理装置的流程图。

[0047] 图11示出了如通过qPCR确定的,在10分钟、20分钟和30分钟测量的用2.5ug/mL环丙沙星处理的(在每一个时间点的右侧柱)和未处理的(在每一个时间点的左侧柱)环丙沙星易感的大肠杆菌样品的rDNA拷贝数的倍数变化。

[0048] 图12A示出了如通过qPCR确定的,在0分钟、15分钟和30分钟测量的用2.5ug/mL环丙沙星处理的(在每一个时间点的右侧柱)和未处理的(在每一个时间点的左侧柱)的环丙沙星易感的大肠杆菌样品的rDNA拷贝数的倍数变化。图12B示出了如通过滑动芯片上的数

字PCR确定的rDNA拷贝数的倍数变化。

[0049] 图13示出了如通过滑动芯片上的数字PCR确定的，在15分钟测量的用0.75ug/mL环丙沙星处理的和未处理的环丙沙星易感的大肠杆菌样品的rDNA拷贝数的倍数变化。

[0050] 图14A示出了如通过定量PCR确定的，在30分钟测量的用四环素处理的和未处理的易感和耐受大肠杆菌样品的rDNA拷贝数的倍数变化。图14B示出了如通过定量PCR确定的，在45分钟测量的用甲氧苄啶/磺胺甲噁唑处理的和未处理的易感和耐受大肠杆菌样品的rDNA拷贝数的倍数变化。

[0051] 图15示出了UTI大肠杆菌分离株基因组中feoB、recA和Dna抑制物蛋白基因与oriC的相对距离。x轴表示每一个单独的基因组(任意地组织的)。

[0052] 图16示出了在BHI培养基中暴露(“处理的”)于2.5ug/ml环丙沙星持续15分钟之后的易感和耐受的细菌(A)中，用于定量UTI临床分离株大肠杆菌基因组中的DNA片段拷贝数的循环阈值(Ct)。

[0053] 图17A和图17B示出了对比于在无抗生素的BHI培养基中20分钟，在BHI培养基中用2.5ug/mL环丙沙星处理20分钟之后对环丙沙星易感(S)和耐受(R)的UTI临床分离株中FeoB和RecA基因表达的RNA定量。

[0054] 图18示出了对比于在无抗生素的BHI培养基中20分钟，在BHI培养基中用10ug/mL环丙沙星处理20分钟之后，对环丙沙星易感和耐受的UTI临床分离株中的FeoB和RecA基因表达的RNA定量。

[0055] 发明详述

[0056] 除非另外定义，本文使用的所有技术术语和科学术语具有与本发明要求保护的主题所属领域的技术人员通常理解的相同的含义。除非另外注明，否则遍及本文整个公开内容提及的所有专利、专利申请、公布的申请和出版物、GENBANK序列、网站和其他出版材料均通过引用以其整体并入。在对于本文的术语存在多个定义的情况下，以本章节的那些为准。当参考URL或其他此类标识符或地址时，应当理解，此类标识符可以改变，并且因特网上的特定信息可以更换，但等效信息是已知的并且可以诸如通过搜索因特网和/或适当的数据库容易地访问。对其的参考证明此类信息的可得性和公共传播。

[0057] 除非上下文另外清楚指明，如本文使用的单数形式″一(a)″、″一(an)″和″该(the)″包括复数指代物。在本申请中，除非另有特别说明，单数的使用包括复数。除非另外说明，如本文使用的，“或”的使用意指“和/或”。此外，术语“包括(including)”以及其他形式(例如，“包括(include)”、“包括(includes)”和“包括(included)”)的使用不是限制性的。

[0058] 如本文使用的，范围和量可以被表示为“约”特定值或范围。约也包括精确的量。因此，“约10度”意指“约10度”，且也意指“10度”。通常，术语“约”可以包括将被预期在实验误差范围内的量。

[0059] 测定

[0060] 本文描述的装置和方法可以适用于检测生物体内药物易感性或耐受性的测定。检测可以是通过测定产生的信号的检测，所述测定例如检测与生物体对药物的耐受性或易感性相关的核酸或定量与生物体对药物的耐受性或易感性相关的核酸的测定。

[0061] 测定可以包括对来自暴露于药物的生物体的核酸进行反应(例如，扩增)，并将该

反应的结果(results of the reaction)(例如,反应结果(reaction outcome),阳性或阴性信号产生)与对来自尚未暴露于药物的生物体的核酸进行的反应进行比较。这可以揭示生物体对药物的易感性或耐受性。

[0062] 在某些实施方案中,方法包括将包含微生物的样品的一部分暴露于药物。在一些实施方案中,方法还包括提取来自微生物的核酸。在一些实施方案中,方法包括对来自微生物的核酸进行序列特异性定量。然后将定量信息用于确定或定量微生物对药物的耐受性或易感性。

[0063] 测定可以以数字格式进行,即,可以对分为分区(即,分析区域)的样品进行测定,使得分区的一些不提供信号,而其他分区提供信号。在一些实施方案中,分区包含一个或零个靶分析物(例如,靶细胞、细胞的一部分、或靶分子,诸如核酸分子或蛋白)。在一些情况下,一些分区可以包含多于一个靶分析物。在一些实施方案中,反应效率使得要求阈值数目的靶分析物以实现阳性信号。在这些情况下,如果分区的一些包含高于阈值的数目的靶分析物,且分区的一些包含低于阈值的数目的靶分析物,则格式是数字的。在一些实施方案中,阈值可以在分区之间变化,使得具有较少靶分析物的一些分区产生信号,而具有更多靶分析物的一些分区不产生信号。在这些情况下,只要一些分区不产生信号且一些分区产生信号,仍然可以基于在多个孔内应用的概率阈值来进行数字检测。

[0064] 在一些情况下,大多数分区包含一个或零个靶分析物。该数字或单个分子格式可以与本文描述的测定结合使用,本文描述的测定包括鉴定、检测、基因分型、SNP检测、稀有等位基因(rare allele)检测和核酸定量。

[0065] 测定可以在小于或等于以下内进行:约600分钟、540分钟、480分钟、420分钟、360分钟、300分钟、240分钟、180分钟、120分钟、110分钟、100分钟、90分钟、80分钟、70分钟、60分钟、50分钟、40分钟、30分钟、20分钟、15分钟、10分钟、9分钟、8分钟、7分钟、6分钟、5分钟、4分钟、3分钟、2分钟、或1分钟。测定可以具有至少约50%、60%、70%、80%、90%、95%、96%、97%、98%、99%、99.9%或99.99%的准确度。假阳性率可以低于10%、低于1%、低于0.1%、低于0.01%、低于0.001%、或低于0.0001%。假阴性率可以低于10%、低于1%、低于0.1%、低于0.01%、低于0.001%、或低于0.0001%。

[0066] 测定可以用于检测拷贝数变化(copy number variations)(CNV)。CNV是结构变异的形式,即改变DNA的一个或更多个段(section)的拷贝数的基因组的DNA的改变。CNV可以相应于某些染色体上已被缺失或重复的相当大的基因组区域。像其他类型的遗传变异一样,一些CNV与对疾病的易感性或耐受性相关。在癌细胞中基因拷贝数可以升高。本文描述的方法还允许鉴定在染色体水平的遗传变化。

[0067] 通过测量DNA复制(例如检测CNV)的抗生素易感性测试方法可适用于评价对抗生素以及损害所有单细胞和多细胞生物体(包括真核生物)中的细胞生长和基因组DNA复制的其他剂的响应。“相对染色体DNA复制率”的测量是有用的可测量参数,区分在药物(例如,抗生素)的存在对比于不存在下易感细胞生长与在药物存在对比于不存在下耐受细胞生长。在一些实施方案中,可以使用本文公开的装置和方法在比平均细胞分裂时间短的时间段内观察到这些标志物的差异,使得能够比依赖于细胞分裂的检测方法更早地检测到该细胞中的细胞复制。因此,本文描述的方法和装置使人们能够快速地区分药物耐受和药物易感的细胞。本文提供的方法和装置也可以应用于任何药物筛选,包括筛选人类细胞(诸如例如在

监测响应于治疗的肿瘤活检中)。

[0068] 在一个实施方案中,在有和没有抗生素的存在下细胞被孵育短时间段之后,测量细胞内rDNA的拷贝数,并且该变化幅度的差异用于确定细胞的药物耐受性和易感性。在一些实施方案中,使用核酸扩增技术(诸如例如qPCR或数字PCR或数字等温扩增)确定rDNA拷贝的变化,并且该结果用于确定对药物的耐受性或易感性。在一些实施方案中,用于确定药物易感性的方法使用数字定量。在一些实施方案中,起源于单独细胞的平均DNA片段拷贝被数字定量以测量邻近起点/远离起点选择的基因比率。

[0069] 在一些实施方案中,药物易感性测试是基于RNA,诸如通过比较转录水平。在一些实施方案中,药物易感性测试是基于RNA,诸如通过使用数字定量比较转录水平。在一些实施方案中,定量策略(诸如例如NASBA、qRT-PCR、测序、纳米串(nanostring),以及其他)可以是适当的。在一些实施方案中,来自从样品获得的细胞的RNA以数字格式进行定量。在一些实施方案中,通过在装置诸如本文描述的装置上的单个细胞测量来定量每一个单独细胞中的基因靶表达水平。

[0070] 测定可以用于定量检测核酸,诸如recA mRNA。例如,可以使用一种方法,所述方法包括以下步骤:获取来自患者的样品,访问样品中的RNA或提取来自样品的RNA,使用至少一种RT-LAMP引物以定性和/或以定量格式逆转录mRNA并扩增,并且测试扩增以确认包括但不限于recA mRNA的核酸的存在。

[0071] 在一些实施方案中,使用相对RNA和/或DNA扩增。在一些实施方案中,使用相对RNA和/或DNA定量。在一些实施方案中,数字检测用于RNA和/或DNA定量。在一些实施方案中,多重可靠的RNA和/或DNA靶将以多重格式同时使用。在一些实施方案中,可以使用牵涉在相同生理过程或抗生素响应机制中的RNA靶和/或其基因。

[0072] 在一些实施方案中,本发明可以应用于肿瘤细胞的药物耐受性测试。在一些实施方案中,本发明可以应用于癌性细胞的药物耐受性测试。本文描述的装置可适用于与多种样品类型一起使用,所述多种样品类型包括临床样品类型(诸如,例如,住院对比于门诊、预治疗(pre-treated)对比于未治疗(treatment-naïve))、感染水平(诸如,例如,阴性对比于阳性对比于污染的)和样品存储/处理(诸如,例如,新鲜对比于硼酸盐保存对比于冷藏)。

[0073] 在一些实施方案中,使用本文描述的装置和方法通过染色和遗传标志物组合来评价慢生长的微生物的药物耐受性是可能的。在一些实施方案中,细胞生长的遗传标志物和抗生素易感性的遗传标志物的组合可用于确定慢生长细胞的遗传抗生素耐受性。

[0074] 本文描述的测定、反应和技术可以在任何合适的平台上进行,任何合适的平台包括但不限于管、毛细管、液滴、微流体装置(例如,滑动芯片装置)、孔、孔板、微板、微流体孔、微流体液滴、乳液、固体支持物(例如,珠或微阵列)、微芯片或凝胶(例如,2D凝胶、3D凝胶)和凝胶内的反应,包括在凝胶表面和凝胶中的群落PCR(polony PCR)中的“群落”。

[0075] 在一个实施方案中,确定细胞的耐受性或易感性的测定如下进行:将细胞在多种基质(例如Bacto脑心浸液肉汤(Bacto Brain-Heart Infusion broth)(BHI)、BHI和混合的人类尿液的混合物和/或全人类尿液)中预培养(例如在37℃)至期望的密度(例如,10^1个细胞/mL、10^2个细胞/mL、10^3个细胞/mL、10^4个细胞/mL、10^5个细胞/mL、10^6个细胞/mL、10^7个细胞/mL、10^8个细胞/mL或10^9个细胞/mL),然后被稀释并与药物孵育或不与药物孵育。在一些实施方案中,孵育在期望的温度(例如37℃)进行,并且然后用期望浓度的抗生素处理。可以

将细胞与抗生素以及不与抗生素孵育一定的时间段(例如,<10分钟、10分钟、15分钟、20分钟、30分钟、45分钟、60分钟、或>60分钟),然后将培养物或原始测试样品的等分试样用于核酸提取。可以使用标准方法(诸如例如一步DNA提取缓冲液或一步RNA提取缓冲液(例如从Epicentre购得),以及其他)提取核酸。在一些实施方案中,在提取后,使用核酸扩增技术,诸如例如,定量PCR或数字PCR定量核酸。为了定量肠杆菌属(Enterobacter)中的23S基因,可以使用以下对肠杆菌属特异性的23S引物:TGCCGTAACTTCGGGAGAAGGCA;TCAAGGACCAGTGTTCAGTGTC;428bp产物。(参见,Matsuda K,Tsuji H,Asahara T,Kado Y,Nomoto K.Sensitive Quantitative Detection of Commensal Bacteria by rRNA-Targeted Reverse Transcription-PCR.Applied and Environmental Microbiology.2007;73(1):32-39.doi:10.1128/AEM.01224-06)。大肠杆菌基因组包含七个具有较小序列差异的不同操纵子,诸如(诸如(rrnA、rrnB、rrnC、rrnD、rrnE、rrnG(aka rrnF)和rrnH。如本文使用的,术语"rDNA"指rRNA操纵子序列。该术语包括编码序列和非编码序列/间隔区两者。在一些实施方案中,本发明利用rDNA提供了最保守的序列和足够可变的序列两者以设计靶向特定物种的引物。编码rRNA的典型操纵子(诸如GenBank:J01695.2)编码5S、16S和23S rRNA以及tRNA,并且在这些序列之间具有间隔区。当使用rDNA基因作为复制标志物时,可以选择用于rRNA操纵子的编码部分或它们之间的间隔区或两者的引物。

[0076] 样品

[0077] 本文公开了与样品分析相关的方法、装置和系统。在一些实施方案中,本发明的方法包括获得来自生物体的样品。样品可以从受试者(例如,患者或宠物)获得,并且可以包括血液、粪便、尿液、唾液或其他体液。样品可以由患者或由医学专业人员获得。医学专业人员的实例包括,但不限于,医师、急救医学技术员、护士、第一响应者(first responder)、心理学家、医用物理学人员(medical physics personnel)、执业护士(nurse practitioner)、外科医师、牙科医师和任何其他医学专业人员。样品可以从任何体液获得,任何体液例如,羊水、房水(aqueous humor)、胆汁、淋巴、乳汁(breast milk)、间质液(interstitial fluid)、血液、血浆(blood plasma)、耵聍(cerumen)(耳垢(earwax))、考珀液(Cowper's fluid)(射精前的流体(pre-ejaculatory fluid))、乳糜(chyle)、食糜(chyme)、女性喷射液(female ejaculate)、月经、粘液、唾液、尿液、呕吐物(vomit)、泪液、阴道润滑性分泌物(vaginal lubrication)、汗液、血清、精液、皮脂、脓液、胸膜液(pleural fluid)、脑脊液(cerebrospinal fluid)、滑液、细胞内液和玻璃状液。在一个实例中,样品通过抽血获得,其中医学专业人员诸如通过注射器从受试者抽取血液。

[0078] 样品可以被收集在样品收集容器中。在一些实施方案中,样品收集容器用可以被检测的信息进行编码。例如,检测器可以识别条形码。条形码可以具有关于在何处收集样品或由哪个个体收集样品的信息。检测器可以获取该信息并使用它来加工或传输关于样品产生的数据。例如,照相手机(camera-phone)可以拍摄样品收集容器的照片。照相手机可以识别容器上标识患者的条形码。然后,照相手机可以将关于样品产生的日期与由其获得样品的患者关联。然后可以将关联的数据传输至患者或患者的医师。在一些实施方案中,产生样品收集容器和样品分析单元的单个图像。

[0079] 还可以分析食品样品。样品可以是潜在地包含靶生物体的任何组分。样品的来源包括,但不限于,地热和水热场地(geothermal and hydrothermal fields)、酸性土壤、其

中酶是中性至碱性的硫质喷气孔(sulfotara)和沸泥塘(boiling mud pots)、池塘、热泉(hot-springs)和间歇泉、海洋放线菌、后生动物、内和外共生体(endo and ectosymbionts)、热带土壤、温带土壤、干旱土壤、堆肥堆(compost piles)、粪堆(manure piles)、海洋沉积物、淡水沉积物、水浓缩物、高盐和超冷却海冰、北极冻原、马尾藻海(Sargasso sea)、开放洋面(open ocean pelagic)、海洋雪、微生物垫(诸如海底鲸类遗体(whale falls)、泉和深海热泉(hydrothermal vents))、昆虫和线虫消化道微生物群落、植物内生菌(plant endophytes)、附生水样品、工业场所和离位富集物(ex situ enrichments)。另外,样品可以从以下分离:真核生物、原核生物、粘细菌(埃博霉素)、空气、水、沉积物、土壤或岩石、植物样品、食品样品、消化道样品、唾液样品、血液样品、汗液样品、尿液样品、脊髓液样品、组织样品、阴道拭子、粪便样品、羊水样品、指纹、气溶胶,包括由咳嗽产生的气溶胶、皮肤样品、组织,包括从活检获得的组织、和/或口腔漱口样品。其他样品类型包括用于以下的样品:临床测试(诸如,例如,住院对比于门诊、预治疗对比于未治疗)、感染水平测试(诸如,例如,阴性对比于阳性对比于污染的)和存储/处理测试(诸如,例如,新鲜对比于硼酸盐保存对比于冷藏)。

[0080] 样品可以包括生物体。样品可以包含微生物。样品中的微生物数目可以小于10、小于100、小于1,000、小于10^4、小于10^5、或小于10^6。在一些实施方案中,样品是经加工的样品(例如,浓缩的、过滤的等等)。

[0081] 样品可以包含来自生物体的靶分析物。靶分析物可以包括,例如,细胞、细胞的部分、多肽或核酸。核酸可以是无细胞的核酸。核酸可以从细胞中分离。核酸可以是单链或双链。靶分析物可以包括DNA或RNA。在一些情况下,RNA是tRNA、mRNA、rRNA、trRNA、snRNA、snoRNA、smY、scaRNA、gRNA、RNA酶P、RNA酶MRP、aRNA、crRNA、incRNA、miRNA、piRNA、siRNA、tasi RNA、rasiRNA、7SK、vRNA或其任何组合。DNA可以是ssDNA、dsDNA、cDNA或其任何组合。在一些情况下,DNA包含基因或基因片段。基因或基因片段可以包含突变。突变可以包括点突变、插入、缺失、扩增、易位、倒位、拷贝数变化和/或其他突变。在一些情况下,DNA包含非编码区。非编码区可以包括功能序列、调控元件、转录子(intrans)、外显子、假基因、重复序列、转座子、病毒元件、端粒、基因开关、转录因子位点、操纵子、增强子、沉默子、启动子、绝缘子和/或其他区域。在一些情况下,DNA包括cDNA。在一些情况下,DNA来自细菌或病毒。在一些情况下,从细胞收集DNA。在一些实例中,DNA是细胞内的。在一些情况下,DNA是细胞外的。

[0082] 靶分析物可以包括RNA。在一些情况下,RNA包括mRNA。在一些情况下,RNA包括非编码RNA(ncRNA)。非编码RNA可以包括转运RNA(tRNA)、核糖体RNA(rRNA)、转运-信使RNA(tmRNA)、核仁小RNA(snoRNA)、微小RNA(miRNA)、干扰小RNA(siRNA)、核小RNA(snRNA)、piwi-相互作用RNA(piwi-interacting RNA)(piRNA)、长ncRNA(lncRNA)、和/或其他类型的ncRNA。在一些情况下,RNA来自细菌或病毒。在一些情况下,从细胞收集RNA。在一些实例中,RNA是细胞内的。在一些情况下,RNA是细胞外的。

[0083] 如本文中可互换使用的术语“核酸”和“核酸分子”指包含核苷酸的分子,所述核苷酸即核糖核苷酸、脱氧核糖核苷酸或两者。该术语包括核糖核苷酸和脱氧核糖核苷酸的单体和多聚体,在多聚体的情况下,核糖核苷酸和/或脱氧核糖核苷酸经由5′至3′键合连接在一起。然而,键合可以包括核酸合成领域中已知的任何键合,包括,例如,包含5′至2′键合的

核酸。核酸分子中使用的核苷酸可以是天然存在的或可以是合成产生的能够与天然存在的碱基对形成碱基对关系的类似物。能够形成碱基配对关系的非天然存在的碱基的实例包括，但不限于，氮杂和脱氮嘧啶类似物、氮杂和脱氮嘌呤类似物以及其他杂环碱基类似物，其中嘌呤和嘧啶环的一个或更多个碳和氮原子已被杂原子，例如，氧、硫、硒、磷等取代。核酸可以从样品中被检测到。

[0084] 如本文使用的术语“寡核苷酸”指包含多个核苷酸的核酸分子。寡核苷酸可以包含约2个至约300个核苷酸。

[0085] 靶分析物可以包括细胞生长的遗传标志物和抗生素易感性的遗传标志物。靶分析物可以包括用于肿瘤细胞的药物耐受性或易感性的标志物。靶分析物可以包括用于氧化应激的标志物，所述标志物可以用例如氧化敏感染料检测以测定响应于用药物治疗的细胞存活力。在一些实施方案中，靶分析物包括多肽或蛋白。在一些实施方案中，靶分析物包括细胞膜或细胞膜缔合的分子，其中细胞膜或细胞膜缔合的分子的变化与生物体对药物的响应相关。

[0086] 生物体

[0087] 术语“生物体”指任何生物体或微生物，包括细菌、酵母、真菌、病毒、原生生物(原生动物、微藻类(micro-algae))、古细菌、植物和真核生物。真核生物可以是单细胞真核细胞。细菌包括革兰氏阳性和革兰氏阴性细菌。术语“生物体”指包含可以通过本发明的方法检测和鉴定的核酸的活体物质(living matter)和病毒。生物体包括，但不限于，细菌、古核生物、原核生物、真核生物、病毒、原生动物、支原体、真菌、植物和线虫。不同的生物体可以是不同的品系(strain)、不同的品种(variety)、不同的种、不同的属、不同的科、不同的目、不同的纲、不同的门和/或不同的界。生物体可以从环境来源分离，所述环境来源包括土壤提取物、海洋沉积物、淡水沉积物、热泉、冰架(ice shelves)、地外样品(extraterrestrial samples)、岩石裂缝、云层、附着于来自水性环境的颗粒，并且可以参与与多细胞生物体的共生关系。此类生物体的实例包括，但不限于链霉菌属(Streptomyces)种和来自天然来源的未表征的/未知物种。生物体可以包括遗传工程化生物体或遗传修饰生物体。生物体可以包括转基因植物。生物体可以包括遗传修饰作物。任何生物体都可以被遗传修饰。可以被遗传修饰的生物体的实例包括大蕉(plantains)、薯蓣(yams)、高粱、甘薯、大豆、木薯、马铃薯、水稻、小麦或玉米。

[0088] 生物体可以包括细菌病原体诸如：嗜水气单胞菌(Aeromonas hydrophila)以及其他物种(种)；炭疽芽孢杆菌(Bacillus anthracis)；蜡状芽孢杆菌(Bacillus cereus)；产肉毒神经毒素的梭菌属种(Botulinum neurotoxin producing species of Clostridium)；流产布氏菌(Brucella abortus)；马耳他布氏菌(Brucella melitensis)；猪种布氏菌(Brucella suis)；鼻疽伯克霍尔德菌(Burkholderia mallei)(原名鼻疽假单胞菌(Pseudomonas mallei))；类鼻疽伯克霍尔德菌(Burkholderia pseudomallei)(原名类鼻疽假单胞菌(Pseudomonas pseudomallei))；空肠弯曲菌(Campylobacter jejuni)；鹦鹉热衣原体(Chlamydia psittaci)；肉毒梭菌(Clostridium botulinum)；肉毒梭菌(Clostridium botulinum)；产气荚膜梭菌(Clostridium perfringens)；粗球孢子菌(Coccidioides immitis)；Coccidioides posadasii；反刍动物考德里体(Cowdria ruminantium)(心水病(Heartwater))；贝纳柯克斯体(Coxiella burnetiid)；致泻性大肠

杆菌组(Enterovirulent Escherichia co//group)(EEC组)诸如肠产毒性大肠杆菌(Escherichia coli—enterotoxigenic)(ETEC)、肠病原性大肠杆菌(Escherichia coli—enteropathogenic)(EPEC)、肠道出血性大肠杆菌O157:H7(Escherichia coli—O157:H7enterohemorrhagic)(EHEC)、和肠道侵袭性大肠杆菌(Escherichia coli—enteroinvasive)(EIEC);埃立克体属种(Ehrlichia spp.)诸如查菲埃立克体(Ehrlichia chaffeensis);土拉热弗朗西丝菌(Francisella tularensis);嗜肺军团菌(Legionella pneumophila);Liberobacter africanus;Liberobacter asiaticus;单核细胞性李斯特菌(Listeria monocytogenes);混杂的肠的诸如克雷伯菌(Klebsiella)、肠杆菌(Enterobacter)、变形杆菌属(Proteus)、枸橼酸克雷伯菌(Citrobacter)、气杆菌属(Aerobacter)、普鲁威登菌(Providencia)、和沙雷菌(Serratia);牛分枝杆菌(Mycobacterium bovis);结核分枝杆菌(Mycobacterium tuberculosis);山羊支原体(Mycoplasma capricolum);丝状支原体丝状亚种(Mycoplasma mycoides ssp mycoides);菲律宾指霜霉(Peronosclerospora philippinensis);Phakopsora pachyrhizi;类志贺毗邻单胞菌(Plesiomonas shigelloides);青枯菌3号小种2型生物变种(Ralstonia solanacearum race 3、biovar 2);普氏立克次体(Rickettsia prowazekii);立氏立克次体(Rickettsia rickettsia);沙门菌属种(Salmonella spp.);Schlerophthora rayssiae varzeae;志贺菌属种(Shigella spp.);金黄色葡萄球菌(Staphylococcus aureus);链球菌(Streptococcus);内生集壶菌(Synchytrium endobioticum);非01群霍乱弧菌(Vibrio cholerae non-01);01群霍乱弧菌(Vibrio cholerae 01);副溶血性弧菌(Vibrio parahaemolyticus)和其他弧菌(Vibrios);创伤弧菌(Vibrio vulnificus);水稻黄单胞菌(Xanthomonas oryzae);苛养木杆菌(Xylella fastidiosa)(柑橘色斑萎黄病菌株(citrus variegated chlorosis strain));小肠结肠炎耶尔森菌(Yersinia enterocolitica)和假结核耶尔森菌(Yersinia pseudotuberculosis);和鼠疫耶尔森菌(Yersinia pestis)。

[0089] 可以使用本文公开的方法和装置检测和分析的具有已知的药物耐受性性能的病原体的非限制性列表包括艰难梭菌(Clostridium difficile)、碳青霉烯类耐受肠杆菌科(Enterobacteriaceae)(CRE)、药物耐受淋病奈瑟菌(Neisseria gonorrhoeae)、多药耐受不动杆菌(Acinetobacter)、药物耐受弯曲菌(Campylobacter)、氟康唑耐受假丝酵母(Candida)(真菌)、产生超广谱β-内酰胺酶(extended spectrumβ-lactamase)的肠杆菌科(Enterobacteriaceae)(ESBL)、万古霉素耐受肠球菌(Enterococcus)(VRE)、多药耐受铜绿假单胞菌(Pseudomonas aeruginosa)、药物耐受非伤寒沙门菌(Salmonella)、药物耐受伤寒沙门菌(Salmonella Typhi)、药物耐受志贺菌(Shigella)、甲氧西林耐受金黄色葡萄球菌(Staphylococcus aureus)(MRSA)、药物耐受肺炎链球菌(Streptococcus pneumoniae)、药物耐受结核分枝杆菌(M.tuberculosis)、万古霉素耐受金黄色葡萄球菌(VRSA)、红霉素耐受A组链球菌属(Streptococcus)和克林霉素耐受B组链球菌属。

[0090] 抗微生物剂/抗生素化合物

[0091] 将生物体与药物孵育以确定指示它们对药物的耐受性或易感性的响应。在一些实施方案中,药物包括用于治疗肿瘤的化合物。在一些实施方案中,药物是抗生素化合物或抗微生物剂化合物。如本文使用的,术语抗微生物剂意指包括用于杀死或抑制微生物生长的天然、半合成或合成来源的任何物质。在优选的实施方案中,抗微生物剂(antimicrobials)

不损害微生物的宿主。如本文使用的，术语“抗微生物剂”和术语“抗生素”是可互换的。抗微生物剂或抗生素化合物的实例包括但不限于：阿米卡星、庆大霉素、卡那霉素、新霉素、奈替米星、妥布霉素、巴龙霉素、链霉素、壮观霉素(Bs)、格尔德霉素、除莠霉素、利福昔明、氯碳头孢(Loracarbef)、厄他培南(Ertapenem)、多尼培南(Doripenem)、亚胺培南/西司他丁、美洛培南、头孢羟氨苄、头孢唑啉、头孢噻吩(Cefalotin/Cefalothin)、头孢氨苄、头孢克洛、头孢孟多、头孢西丁、头孢丙烯(Cefprozil)、头孢呋辛、头孢克肟(Cefixime)、头孢地尼、头孢托仑、头孢哌酮、头孢噻肟、头孢泊肟(Cefpodoxime)、头孢他啶、头孢布烯(Ceftibuten)、头孢唑肟(Ceftizoxime)、头孢曲松、头孢吡肟、头孢洛林酯(Ceftaroline fosamil)、头孢托罗(Ceftobiprole)、替考拉宁、万古霉素、替拉万星(Telavancin)、达巴万星(Dalbavancin)、奥利万星(Oritavancin)、克林霉素、林可霉素、达托霉素(Daptomycin)、阿奇霉素、克拉霉素、地红霉素(Dirithromycin)、红霉素、螺红霉素、醋竹桃霉素(Troleandomycin)、泰利霉素(Telithromycin)、螺旋霉素、氨曲南、呋喃唑酮(Furazolidone)、呋喃妥因(Nitrofurantoin)(Bs)、利奈唑胺(Linezolid)、Posizolid、Radezolid、Torezolid、阿莫西林、氨苄青霉素、阿洛西林、羧苄西林、氯唑西林(Cloxacillin)、双氯西林(Dicloxacillin)、氟氯西林(Flucloxacillin)、美洛西林、甲氧西林、纳夫西林(Nafcillin)、苯唑西林(Oxacillin)、青霉素G、青霉素V、哌拉西林、青霉素G、替莫西林(Temocillin)、替卡西林(Ticarcillin)、阿莫西林/克拉维酸(clavulanate)、氨苄西林/舒巴坦、哌拉西林/三唑巴坦(tazobactam)、替卡西林/克拉维酸、杆菌肽、粘菌素、多粘菌素B、环丙沙星、依诺沙星、加替沙星、吉米沙星、左氧氟沙星、洛美沙星、莫西沙星、萘啶酸(Nalidixic acid)、诺氟沙星、氧氟沙星、曲氟沙星(Trovafloxacin)、格帕沙星(Grepafloxacin)、司氟沙星(Sparfloxacin)、替马沙星(Temafloxacin)、磺胺米隆(Mafenide)、磺胺醋酰(Sulfacetamide)、磺胺嘧啶、磺胺嘧啶银(Silver sulfadiazine)、磺胺地托辛(Sulfadimethoxine)、磺胺甲二唑(Sulfamethizole)、磺胺甲噁唑、磺胺(Sulfanilimide)(古旧用法(archaic))、柳氮磺胺吡啶、磺胺异噁唑(Sulfisoxazole)、甲氧苄啶-磺胺甲噁唑(Trimethoprim-Sulfamethoxazole)(复方新诺明(Co-trimoxazole))(TMP-SMX)、2,4-二氨基偶氮苯-4-磺酰胺(Sulfonamidochrysoidine)(古旧用法)、地美环素(Demeclocycline)、多西环素、米诺环素、土霉素、四环素、氯法齐明(Clofazimine)、氨苯砜(Dapsone)、卷曲霉素、环丝氨酸、乙胺丁醇(Bs)、乙硫异烟胺(Ethionamide)、异烟肼、吡嗪酰胺(Pyrazinamide)、利福平(Rifampicin)(美国称利福平(Rifampin))、利福布汀(Rifabutin)、利福喷丁(Rifapentine)、链霉素、肿凡纳明(Arsphenamine)、氯霉素(Bs)、磷霉素、夫西地酸(Fusidic acid)、甲硝唑、莫匹罗星(Mupirocin)、平板霉素(Platensimycin)、喹奴普丁(Quinupristin)/达福普汀、甲砜霉素、替加环素(Bs)、替硝唑和甲氧苄啶(Bs)。

[0092] 不同的抗生素或抗微生物剂可以具有不同的作用机制，并可以不同地影响复制繁殖和复制重新启动。一些抑菌抗生素(诸如氨基糖苷类、头孢菌素类、四环素类、磺胺类和大环内酯类)抑制蛋白合成，而杀菌抗生素可以作用于细胞壁(例如万古霉素和β-内酰胺类)或细菌DNA(诸如氟喹诺酮类)。一些抗生素，诸如甲氧苄啶和磺胺甲噁唑干扰代谢途径并间接影响DNA复制，且一些(呋喃妥因)影响细胞中的多种过程，包括DNA复制。

[0093] 在一些实施方案中，将针对每种抗生素组调整治疗条件以增强抗生素对DNA复制

的影响。在一些实施方案中，将针对每种抗生素组调整治疗条件以加速观察抗生素对DNA复制的影响。在一些实施方案中，本公开内容中描述的方法通过相对基因定量测试抗生素易感性。

[0094] 靶/靶分析物

[0095] 在一些实施方案中，本文提供的方法和装置靶向指示响应于抗微生物剂的存在或不存在的微生物生长的分析物(例如，标志物)。在一些实施方案中，测量接近OriC的基因(诸如例如感受态基因(competence gene))的表达和拷贝数的变化。我们的方法和装置使得能够快速且灵敏地定量每一个感兴趣的细菌物种的邻近ori的总基因拷贝数。

[0096] 慢生长的细胞可以更慢地复制其染色体，允许受抗生素影响的细胞和被抗生素杀死的细胞之间的区分。另外，具有受损的代谢的细胞可以产生或多或少的特异性RNA靶。本文描述了这些靶的选择和定量。

[0097] 在一些实施方案中，感兴趣的靶分析物是DNA。在一些实施方案中，DNA区域，诸如在公布的细菌基因组中以多个拷贝呈现的DNA区域被选择作为靶(诸如rDNA)。在一些实施方案中，选择多于一种靶(诸如DNA片段或基因)用于同时定量；这些靶可以位于接近复制起点和/或可以牵涉复制的两个方向。在一些实施方案中，选择多于一种靶(诸如DNA片段或基因)用于同时定量；这些靶的一个或更多个位于接近复制起点，并且一个或更多个靶远离复制起点。通过测量这些靶在某些时间(在一些情况下，在复制完成之前)的比率，在一些实施方案中可以快速检测复制，而无需独立地测量细胞数目。当样品中存在非常少的细胞时，以及或者当非常少的细胞被分析时，以及或者当划分的样品的每一部分中存在的细胞数目的准确度受泊松统计限制时，这变得有价值。

[0098] 在一些实施方案中，检测由于抗微生物剂处理引起的基因重复将允许检测易感菌株。在一些实施方案中，用于基因剂量方法的靶可以包括Ori或邻近OriC定位的基因(诸如例如在距离OriC的50kb内、在100kb内、在150kb内、在200Kb内、和/或在300Kb内)。在一些实施方案中，药物易感细胞中的DNA/基因剂量将增加而不是降低。

[0099] 在一些实施方案中，在公布的细菌基因组中以单拷贝或多拷贝呈现的DNA靶被选择作为靶(诸如例如rDNA、feoB基因、rpoB基因、感受态基因以及其他)。在一些实施方案中，选择多于一种靶(诸如DNA片段或基因)用于同时定量；这些可以优先地在两个方向接近复制起点定位。在一些实施方案中，感兴趣的靶是核糖体DNA即rDNA序列。核糖体基因序列虽然在细菌中非常保守，可以诸如通过选择特异性探针和/或引物用作鉴定和/或组鉴定的靶。

[0100] 在一些快速生长的细菌物种中，已显示核糖体基因(和许多与转录和翻译相关的基因)倾向于优先接近复制起点定位，表明存在进化压力，这表明与基因组稳定性相关(Etienne Couturier,Eduardo P.C.Rocha.Replication-associated gene dosage effects shape the genomes of fast-growing bacteria but only for transcription and translation genes,2006,Molecular microbiology,第59卷，第5期，第1506-1518页，DOI:10.1111/j.1365-2958.2006.05046.)。这些基因可以用作定量复制的靶，因此使得能够在短时间段(诸如短于细胞复制周期的时间段)内检测暴露于生长条件的生物体。使用这些基因作为用于数字定量的靶，甚至可以检测到不完整的DNA复制(其发生在细胞分裂之前)。

[0101] 例如,在与UTI感染连锁的11个可用的基因组测序的大肠杆菌菌株的子集中,相对于复制起点映射了rDNA基因的位置。图1示出了在UTI大肠杆菌分离株中rDNA与oriC的相对距离。红色点(顶部行和底部行)通过显示对于两个复制区(replichores),环状染色体中从OriC至复制终止的距离来说明基因组大小。具有不同分布的核糖体基因的稀有实例属于经历实验室条件下的人工选择的细菌基因组,诸如图上左侧第五位的基因组,其不是UTI临床分离株,而是来自UTI患者消化道的对于呋喃妥因耐受性选择的大肠杆菌(GenBank:CP007265.1)。这些表明rDNA基因在复制起点附近聚簇的趋势。

[0102] 图2中示出了在复制起点附近的rDNA聚簇的另一实例,显示了从UTI患者测序的示例基因组的环状表示。基因组的核糖体RNA编码区用红色标记。复制起点(OriC)标记在图2的顶部。rDNA优先在复制起点附近聚簇。

[0103] 在一些实施方案中,本文公开的方法利用以下事实:在至少一些感兴趣的细胞中,可以存在特定时间顺序的染色体分裂。例如,在大肠杆菌中,染色体动力学确定了染色体的某些部分以特定时间顺序分裂。在一些实施方案中,早期被复制的基因靶的定量将允许更高分辨率或更快地确定耐受细菌与易感细菌。另外,复制的起点和终点将基因组分为具有不同的链作为前导链的复制区。在一些实施方案中,可以选择性地靶向前导链和滞后链上的靶基因,以测量当复制受损与未受损时它们的不同的转录水平。

[0104] 在一些实施方案中,进行邻近复制起点定位的DNA片段和基因(例如,起点本身或邻近基因,诸如gln udhA dnaB或一些rDNA)的定量。在一些实施方案中,可以通过本文描述的方法和装置(例如,数字扩增)检测抗生素处理的细胞和未处理的细胞之间每细胞的拷贝数差异。

[0105] 在一些实施方案中,定量邻近复制终止位点(ter)定位的DNA靶。由于它们是基因组复制的最后一部分,所以基于细胞生长状态或抗生素的存在,这些区域倾向于表现出拷贝数的较小变化。在一些实施方案中,在ter附近的DNA靶的复制的测量可以用于标准化从其他靶获得的数据。例如,在一些实施方案中,在不足以复制整个基因组的短的药物暴露时间,这些ter-邻近区域可以用作加载的细胞数目的量度,而更接近ori的区域可以用于访问DNA复制和对药物的耐受性;例如,当加载的细胞数目低(例如,小于100或小于10)并且因此难以测量时,这是有用的。在一些实施方案中,有和/或没有用药物处理的这些区域的拷贝数的变化用于区分耐受细菌与易感细菌。导致复制叉失效的药物,诸如抗微生物剂,可以进一步扩大这种效果。在一些实施方案中,靶核酸序列的复制可以用于确定在药物(诸如抗生素)的存在下孵育的细胞(诸如细菌)中基因的耐受性谱。

[0106] 在一些实施方案中,有和/或没有用抗生素处理的这些靶的相对定量将用于确定抗生素易感性。这可以实现耐受菌株和易感菌株之间非常快速区分。

[0107] 在一些实施方案中,诸如当细胞的新复制叉从复制起点开始时,其他较早的复制叉可以在重新启动事件上独立地继续复制。(Fossum,S.,E.Crooke,等(2007).“Organization of sister origins and replisomes during multifork DNA replication in Escherichia coli.”The EMBO Journal26(21):4514-4522。Mohan C.Joshi,等,2010,“Escherichia coli sister chromosome separation includes an abrupt global transition with concomitant release of late-splitting intersister snaps”,PNAS,第108卷,第7期,2765-2770)。因此,在一些实施方案中,用于药

物耐受性/易感性测试的基因靶可以远离起点定位(例如,oriC仍将被足够快速地复制以用于确定药物耐受性/易感性)。

[0108] 通过使用接近复制起点的靶,可以定量在细胞中总DNA遗传物质中的那些基因的相对拷贝数,以便在DNA复制完成之前或细胞分裂完成之前先鉴定在抗生素处理下的易感细胞和耐受细胞。在一些实施方案中,用药物处理的易感生物体的靶基因的拷贝数小于耐受生物体或未经处理的生物体的靶基因的拷贝数,因为在易感生物体中暴露于药物降低复制。然而,一些抗生素使得染色体DNA的某些区域在易感细胞中拷贝数增加。图3说明了抗生素给药对易感细胞的影响,特别是用甲氧苄啶处理之后的大肠杆菌中与距离复制起点(oriC)的距离相比的相对DNA拷贝数,被显示为在两个ter区域与0交叉的线。(Slager,J.,M.Kjos,等(2014).“Antibiotic-Induced Replication Stress Triggers Bacterial Competence by Increasing Gene Dosage near the Origin.”Cell 157(2):395-406)。图3示出了,在暴露于甲氧苄啶之后,与基线水平零相比,DNA拷贝数在接近复制起点时达到峰值。

[0109] 许多抗生素(诸如喹诺酮类)诱导复制叉失速。作为复制叉失速的直接结果,尽管DNA复制起始继续,但靶向DNA复制的抗生素都上调起点邻近基因的拷贝数并且诱导细菌中转录的全局变化。这产生了与减缓用药物处理的易感生物体中的DNA复制的那些抗生素相反的结果,提供了增加数目的阳性结果。本文描述的用于减缓生长的抗生素以及诱导复制叉失速的那些抗生素的数字测定的预测结果示于图4中。在图4中,“抗生素A”表示导致易感细胞中复制效率降低,因此与耐性细胞相比降低了在复制起点附近的基因拷贝数的抗微生物剂。“抗生素B”表示导致细胞中复制叉失速,导致在复制起点附近的新复制起始的增加,因此与耐性细胞相比在易感细胞中增加了复制起点附近的基因的拷贝数的抗微生物剂。

[0110] 如图4中表示的,“抗生素A”包括抑制蛋白合成的抑菌抗生素(诸如氨基糖苷类、头孢菌素类、四环素类、磺酰胺类和大环内酯类),以及作用于细胞壁的杀菌抗生素(诸如万古霉素和β-内酰胺类)。对于减缓DNA复制的这些抗生素,在一些实施方案中,例如,通过控制亚铁离子浓度、pH和镁离子并确保需氧条件改变抗生素暴露以诱导细胞中的氧化应激。

[0111] 如图4中表示的,“抗生素B”包括使得染色体DNA的某些区域在易感细胞中拷贝数增加的抗生素。例如,已知蜡状芽孢杆菌(B.cereus)、金黄色葡萄球菌(S.aureus)、和大肠杆菌以及肺炎链球菌(S.pneumoniae)中oriC-ter比率受一些抗生素处理(例如,HPUra、羟基脲、甲氧苄啶、环丙沙星和MMC)的影响。已知这些抗生素损害染色体复制,因为甲氧苄啶间接影响DNA复制。呋喃妥因直接损害许多细胞过程,包括复制。6(对羟基苯基偶氮)-尿嘧啶(HPUra)是革兰氏阳性细菌DNA的选择性抑制物。羟基尿素(Hydroxycarbamide)降低了脱氧核糖核苷酸的产生。环丙沙星是一种氟喹诺酮,其减速复制叉并且由于其与DNA促旋酶和拓扑异构酶的相互作用导致DNA片段化。(Slager,J.,M.Kjos,等(2014).“Antibiotic-Induced Replication Stress Triggers Bacterial Competence by Increasing Gene Dosage near the Origin.”Cell 157(2):395-406,和Tamayo M,Santiso R,Gosalvez J,Bou G,Fernández JL.Rapid assessment of the effect of ciprofloxacin on chromosomal DNA from Escherichia coli using an in situ DNA fragmentation assay.BMC Microbiology.2009;9:69)。

[0112] 可以使用本文描述的方法和装置检测细胞对任一种抗生素的耐受性或易感性。此

外,取决于在复制叉失速之前复制的基因是否从染色体或质粒DNA片段化,数字PCR将提供不同的结果。如果它们不片段化,则它们不能分离到不同的孔,因此提供另外的分辨率水平来表征细胞。在不同抗生素处理下从拟核释放的DNA片段被估计为50kb至100kb大小(Tamayo M,Santiso R,Gosalvez J,Bou G,Fernández JL.Rapid assessment of the effect of ciprofloxacin on chromosomal DNA from Escherichia coli using an in situ DNA fragmentation assay.BMC Microbiology.2009;9:69),相似于拟核的DNA环的假定大小和突出的DNA促旋酶介导的切割位点(Snyder M,Drlica K.DNA gyrase on the bacterial chromosome:DNA cleavage induced by oxolinic acid.J Mol Biol.1979;131(2):287-302,Condemine,Smith CL.Transcription regulates oxolinic acid-induced DNA gyrase cleavage at specific sites on the E.coli chromosome.Nucleic Acids Res.1990;18(24):7389-7396,和Hsu Y-H,Chung M-W,Li T-K.Distribution of gyrase and topoisomerase IV on bacterial nucleoid:implications for nucleoid organization.Nucleic Acids Res.2006;34(10):3128-3138.)。

[0113] 在一些实施方案中,本文公开的方法用于检测在短时间帧内局部扩增的基因和基因片段,以区分药物耐受细胞与药物易感细胞。在一些实施方案中,方法利用以下事实:一些抗生素导致在复制起点附近定位的DNA片段(诸如一些感受态基因和复制起点本身)的优先扩增。在一些实施方案中,在两个复制区中距离复制起点50-200kb内定位的DNA片段或基因将以数字形式从少量细胞定量以确定它们的药物易感性。在一些实施方案中,方法利用以下事实:一些抗生素导致DNA片段的积累。这已被证明是由导致复制叉失速的抗生素导致的。这可以通过对积累区域内的基因、累积区域外的基因或两者之间的比率进行定量来分析。

[0114] 在一些实施方案中,在暴露于药物小于20分钟之后,在易感分离株中可以检测到对于染色体上选择的位置的相对复制比率的变化。在一些实施方案中,对于染色体上选择的位置的相对复制比率的差异仅可以在某一时间点之后可被辨别,这对于不同的抗生素类型、生物体、细胞和/或药物可以是不同的。在一些实施方案中,在暴露于药物小于5分钟、10分钟、15分钟、20分钟、25分钟、30分钟、45分钟、60分钟、90分钟、120分钟、150或180分钟之后,在易感分离株中可以检测到对于细菌染色体上选择的位置的相对复制比率的变化。

[0115] 在一些实施方案中,加速方案(shift up protocol)(例如添加营养物质和信号转导分子以刺激生长和基因表达和DNA复制的方案)可以用于确保对复制重新启动的时间的一些控制。

[0116] 在一些实施方案中,基因表达的变化用作生物体中抗微生物剂易感性或耐受性的标志物。在一些实施方案中,药物易感性测试是基于RNA,诸如通过比较转录水平。在一些实施方案中,药物易感性测试是基于RNA,诸如通过使用数字定量比较转录水平。在一些实施方案中,可以测量与响应于抗微生物剂的耐受性或易感性相关的标志物。

[0117] 在一些实施方案中,可以使用响应于药物或抗生素而不同地被调控的基因。这些基因可以包括例如由OxyR和SoxS诱导的响应于抗生素诱导的氧化应激的recA和LexA(Barczak,等,2012,Proceedings of the National Academy of Sciences,6217-6222)基因(Dwyer,等,2014,Proceedings of the National Academy of Sciences,E2100-

E2109)。

[0118] 在易感细菌中,已显示许多RNA靶在短时间内响应于药物暴露而被上调或下调。作为对培养条件的响应以及从分离株至分离株的变化,许多此类基因也显示出表达水平的显著变化。响应于多种抗生素,一些基因表达(例如,参与SOS响应中的recA)在几种物种中被上调,而其他转录变化是生物体和/或机制特异性的。

[0119] 由于来自宿主细胞或来自其他微生物的非特异性核酸的存在、以及由于来自临床样品的抑制物的存在以及由于处理的时间短,RNA靶RNA水平的变化(通过qPCR、微阵列、RNAseq)可难以在短时间的药物/抗生素处理之后使用现有的方法(诸如通过常规的逆转录和等温或PCR扩增)批量检测。在一些实施方案中,二进制、数字和多体积数字格式可以用于分离局部浓度高的体积中的靶,使得甚至在此类样品中能够快速且可靠的定量。

[0120] 在一些实施方案中,以较高拷贝数存在于细胞中的DNA靶将用于甚至在非常低数目的细胞(诸如在血液或脑脊髓液)中能够检测耐受性谱。例如,虽然在大部分大肠杆菌菌株和分离株的基因组中,由于至少两个但潜在地许多个复制叉的存在,存在～7个拷贝的rDNA,在一些情况下,取决于其生长速率,人们可以预期具有每细胞12个至35个rDNA拷贝(E.coli and Salmonella,cellular and molecular biology.Frederic C.Neidhardt主编,v2.ASM press,Washington DC.1996)。在一些实施方案中,当存在非常低数目的细胞时,使用细胞中具有较高拷贝数的基因将能够增加定量的统计分辨率以确定抗生素耐受性。在一些实施方案中,这种定量通过扩增进行。在一些实施方案中,这种定量利用数字扩增方法。

[0121] 在一些实施方案中,评价来自公众可用的研究诸如由Broad Institute出版的那些研究的另外的靶。在一些实施方案中,用于分析的靶从DNA/RNA-seq数据的分析产生。在一些实施方案中,可以使用定量策略(诸如例如NASBA、qRT-PCR、测序、纳米串,以及其他)。在一些实施方案中,来自从样品获得的细胞的RNA以数字格式进行定量。在一些实施方案中,通过单个细胞测量来定量每一个单独细胞中的基因靶表达水平。

[0122] 表1中提供了可用于评价细胞对药物(例如,环丙沙星)的响应的多重和/或单独用途的RNA靶的示例性列表。

[0123] 表1

[0124]

基因	对环丙沙星的响应
EcHS_A0999_dmsA_厌氧_二甲基_亚砜_还原酶，_A_亚基	相同或下调
EcHS_A1247_pepT_肽酶_T	相同或下调
EcHS_A1333_narK_亚硝酸盐_挤压_蛋白_1	相同或下调

[0125]

EcHS_A1334_narG_硝酸盐_还原酶，_α_亚基	相同或下调
EcHS_A1335_narH_硝酸盐_还原酶，_β_亚基	相同或下调
EcHS_A1557_fdnG_甲酸_脱氢酶，_硝酸盐_可诱导，_α_亚基，_含硒代半胱氨酸	相同或下调
EcHS_A1755_-_假定_蛋白	相同或下调
EcHS_A1987_flhC_转录_激活因子_FlhC	相同或下调
EcHS_A1988_flhD_转录_激活因子_FlhD	相同或下调
EcHS_A2346_napF_铁氧还蛋白-型_蛋白	相同或下调
EcHS_A2342_napH_醌醇_脱氢酶_膜_组分	显著下调
EcHS_A2343_napG_醌醇_脱氢酶_周质_组分	显著下调
EcHS_A2380_glpT_sn-甘油-3-磷酸_转运蛋白	显著下调
EcHS_A2381_-_假定_蛋白	显著下调
EcHS_A2382_glpA_sn-甘油-3-磷酸_脱氢酶_亚基_A	显著下调
EcHS_A2597_aegA1_氧化还原酶_Fe-S_结合_亚基	显著下调
EcHS_A3117_ansB_L-天冬酰胺酶_II	显著下调
EcHS_A3561_nirB_亚硝酸盐_还原酶_[NAD(P)H],_大_亚基	显著下调
EcHS_A3562_nirD_亚硝酸盐_还原酶_小_亚基	显著下调
EcHS_A3605_feoA_亚铁_离子_转运_蛋白_A	显著下调
EcHS_A3606_feoB_亚铁_离子_转运_转运_B	显著下调
EcHS_A3607_-_假定_蛋白	显著下调
EcHS_A3616_malT_转录_调控因子_MalT	显著下调
EcHS_A4395_frdD_富马酸_还原酶_亚基_D	显著下调
EcHS_A1940_-_DNA_损伤-可诱导_蛋白_YebG	显著上调
EcHS_A2835_recA_重组酶_A	显著上调
EcHS_A4283_lexA_LexA_阻抑蛋白	显著上调
EcHS_A4284_dinF_DNA-损伤-可诱导_SOS_响应_蛋白	显著上调
EcHS_A4300_uvrA_切除核酸酶_ABC_亚基_A	显著上调
EcHS_A2341_napB_柠檬酸_还原酶_细胞色素_c-型_亚基	要求评价
EcHS_A2344_napA_硝酸盐_还原酶_催化_亚基	要求评价
EcHS_A3610_gntX_葡萄糖酸_周质_结合_蛋白	要求评价
EcHS_A3612_-_假定_蛋白	要求评价
EcHS_A3821_secB_前蛋白_移位酶_亚基_SecB	要求评价
EcHS_A4404_rsgA_核糖体-相关_GTP 酶	要求评价
EcHS_A4424_-_23S_rRNA_(鸟苷-2'-O-)-甲基转移酶	要求评价
23S rRNA	要求评价
EcHS_A3544_-_假定_蛋白	要求评价
EcHS_A0467_phoB_转录_调控因子_PhoB	要求评价
EcHS_A0468_phoR_磷酸盐_调节子_传感_蛋白	要求评价
EcHS_A1067_sulA_SOS_细胞_分裂_抑制物	要求评价
EcHS_A3855_DNA-损伤-可诱导蛋白 D_DinD	要求评价
EcHS_A2357_双组分响应调控因子 RscB	要求评价
EcHS_A1184_dinI_DNA_损伤-可诱导_蛋白_I	要求评价
EcHS_A4417_FtsH_蛋白酶_调控因子_HflC	要求评价

[0126] 在装置的一些实施方案中，反应性物质(reactive species)可以用于检测每个单

个细胞中的氧化应激。因为无活力细胞不能维持细胞质中的还原环境，所以氧化敏感染料组成大部分存活力测定。

[0127] 在一些实施方案中，本文公开的装置和方法可以用于检测由OxyR和SoxR转录因子上调的基因表达的增加，作为抗生素诱导的氧化应激响应的证据。这些基因实例是诸如例如sodA(编码Mn-辅助的超氧化物歧化酶)和acrAB(编码多药外排泵)、soxS(次级转录因子)和KatG和Ahp基因、OxyS、RecA。

[0128] 在一些实施方案中，选择参与Fe离子转运和调控的RNA靶和/或其基因。在一些实施方案中，选择参与氧化应激响应的RNA靶和/或其基因。

[0129] FeoB是一种属于亚铁离子摄取(FeoB)转运蛋白家族的亚铁离子摄取系统(Kammler M,Schön C,Hantke K.Characterization of the ferrous iron uptake system of Escherichia coli.Journal of Bacteriology.1993;175(19):6212-6219)。FeoB是被报道在大肠杆菌分离株总RNA表达的RNAseq分析中响应于环丙沙星处理转录被下调的许多基因之一。(Shishkin,A.A.,G.Giannoukos,等(2015)."Simultaneous generation of many RNA-seq libraries in a single reaction."Nat Meth 12(4):323-325)。已知FeoB转录受到Fur的阻抑。在一些实施方案中，在由Fur活化或阻抑的那些基因中选出靶。

[0130] FeoB表达也被RstAB双组分系统活化。在一些实施方案中，将在由RastAB双组分系统活化或阻抑的那些基因中选出靶。

[0131] 在一些实施方案中，将细胞暴露于抗生素的培养基的pH和Mg2+浓度将结合本文描述的方法被增加或降低，以增强细胞对抗生素的响应。在一些实施方案中，以相对低Mg 2+、低pH(温和酸性条件)和不同的选择的受控水平的Fe处理细菌细胞，以增加FeoB表达响应于抗生素的变化幅度。

[0132] 在一些实施方案中，本文描述的方法包括以下的步骤：1)以低Mg和低Fe(2+)和低pH(温和酸性条件)预孵育细胞，以及2)连同高铁(2+)一起添加抗生素。在一些实施方案中，方法包括：1)以低Mg和高Fe(2+)和低pH(温和酸性条件)预孵育细胞，以及2)添加抗生素。

[0133] 在一些实施方案中，进行溶解氧、PH、Fe2+、银离子浓度、Mg2+浓度和其他盐浓度的调整，以通过测量FeoB表达水平来优化用于最快/最可靠的AST的条件。这些变化可以与本文档中提及的基因表达靶组合。这将通过定量这些靶来实现抗生素易感性的更高分辨率确定。在一些实施方案中，在抗生素的存在下，向被孵育的细菌提供空气/氧气。在一些实施方案中，经由添加过氧化物，增强细菌中的氧化应激。

[0134] 在一些实施方案中，靶分析物可以包含单独靶或一组靶，例如，来自编码具有响应于pH、Mg^{2+}和氧化应激的相似功能的蛋白的基因的组(诸如以上提及的并与铁代谢相关的基因的组)的RNA。在一些实施方案中，靶分析物将包含来自与氧化应激(oxidative stress)(氧化应激(oxidation stress))相关的一组基因的RNA。

[0135] 在一些实施方案中，定量recA基因和/或recA RNA以确定抗生素易感性。在一些实施方案中，SOS响应中的其他基因用作用于定量的靶以确定抗生素易感性。在一些实施方案中，定量FeoB RNA和/或recA RNA以确定抗生素易感性。在一些实施方案中，定量FeoB RNA和recA RNA，检测它们的比率，以确定抗生素易感性和耐受性。在一些实施方案中，响应于抗生素而被上调和下调的其他基因被一起定量并检测它们的比率，以确定微生物中的抗生

素易感性或耐受性。

[0136] 在一些实施方案中，装置使用前体rRNA和核糖体RNA标志物的组合用于以实时RT-PCR对单个细胞检测耐受和易感细胞或微生物。在一些实施方案中，装置可以集成标志物的组合以用于通用药物易感性和/或特异性药物耐受性检测。

[0137] 样品加工

[0138] 在一些实施方案中，将微生物暴露于药物以测定其对药物的响应并确定该微生物是否对药物耐受或易感。在一些实施方案中，分离株可以被预培养于或预暴露于多种基质(诸如例如细菌培养基或人类尿液以及其他)中，并且随后可以在多种药物(例如抗生素，诸如环丙沙星、呋喃妥因、甲氧苄啶、四环素和磺胺甲噁唑以及其他)的存在或不存在下孵育，其中在同一装置上实质上同时进行对多种药物和/或多种添加剂的暴露。在一些实施方案中，微生物在暴露于药物期间实质上不分裂。在一些实施方案中，被限制为单个细胞、或处于小组和/或细胞聚集体中的微生物比平均群体更快地响应药物。

[0139] 本文描述的装置和方法可以包括当微生物的一些被限制为单个细胞或作为小组和/或细胞聚集体诸如在同一隔室内少于3个细胞、少于10个细胞、少于30个细胞、少于100个细胞时的那些。隔室的体积可以在100fL至1nL、1nL至100nL、或100nL至500nL的范围内。

[0140] 在一些实施方案中，数字测定可以使用将来自样品的单个细胞限制到克隆分离区域。在一些实施方案中，将分离的细胞的每一个培养于克隆分离区域中以产生来自样品的多个克隆群体。在一些实施方案中，分离的细胞在扩增之前或之后用药物处理。在一些实施方案中，在培养分离的细胞并扩增之后，将克隆群体分为两个或更多个处理区域。如此，克隆群体的至少一部分可以用药物治疗，同时克隆群体的另一部分不用药物治疗。然后，可以进行本文描述的测定以确定克隆群体中的细胞是否对一种或更多种药物易感或耐受。

[0141] 在一些实施方案中，特定的培养条件用于加速微生物对药物的响应(例如群体感应分子(quorum sensing molecules)、气体分压、温度等等)。在一些实施方案中，微生物被暴露于气体或气体混合物，例如含H_2S、CO和NO的气体混合物。已知此类气体，例如，影响微生物对抗生素的易感性。此类气体可以用可以是厌氧、需氧或微氧的气体混合物稀释。此类气体混合物可以包含CO_2。

[0142] 在一些实施方案中，微生物与真核细胞和/或其他微生物的共培养用于加速微生物对药物的响应。

[0143] 在一些实施方案中，进行控制培养物中复制起始的平均数目和/或定位和/或时间的措施。例如，细胞可以在暴露于抗生素之前被置于高或低营养条件下。在一些实施方案中，细胞对生长条件变化(诸如，例如，将感染的血液与富含营养的培养基混合)的习服(acclimatization)将导致DNA复制速率的变化。在一些实施方案中，可以通过修改生长培养基来缓解该影响。在一些实施方案中，数据分析可以允许在该噪声内捕获复制速率的变化。在一些实施方案中，培养基对复制的影响可以用于增强药物处理的效果。

[0144] 在一些实施方案中，将细胞如下暴露于抗生素：将细胞在包含Bacto脑心浸液肉汤(BHI)、BHI和混合的人类尿液的混合物和/或完全人类尿液的多种基质中在37℃下预培养至多达10^9个细胞/mL的密度，然后被稀释并与抗生素孵育或不与抗生素孵育。用于处理的抗生素包括环丙沙星、呋喃妥因、甲氧苄啶、四环素和磺胺甲噁唑。孵育以范围从10^5-10^8个细胞/mL的起始浓度在37℃进行，并且然后根据处理用范围从0.75ug/mL-156ug/mL的抗生

素浓度进行处理。将细胞与抗生素以及不与抗生素孵育一定的时间段(包括10分钟、15分钟、20分钟、30分钟、45分钟、或60分钟)，然后将培养物的等分试样用于核酸提取。

[0145] 在一些实施方案中，将微生物暴露于药物持续以下的时间：少于4小时、少于3小时、少于2小时、少于1小时、少于30分钟、少于15分钟、少于10分钟、少于5分钟、少于3分钟、或少于1分钟。

[0146] 可以提取核酸，然后分析。用于提取核酸的准确方案取决于样品和待进行的准确测定。提取来自靶细菌的核酸通常包括细胞裂解步骤，随后是核酸纯化。细胞裂解步骤破坏细胞和核膜，释放遗传物质。这通常使用也使细胞中存在的大量蛋白变性的裂解去垢剂，诸如十二烷基硫酸钠来完成。

[0147] 然后可以用醇沉淀步骤(通常是冰冷的乙醇或异丙醇)或经由以下的固相纯化步骤纯化核酸：通常在高浓度的离液盐的存在下在柱、树脂中的二氧化硅基质上或顺磁性珠(paramagnetic beads)上，然后洗涤，并且然后在低离子强度缓冲液中洗脱。在核酸沉淀之前的任选的步骤是添加消化蛋白的蛋白酶以进一步纯化样品。

[0148] 在一些实施方案中，使用标准方法包括一步DNA提取缓冲液或一步RNA提取缓冲液(从Epicentre购得)提取核酸。在提取后，使用核酸扩增技术包括定量PCR和数字PCR定量核酸。

[0149] 在一些实施方案中，样品的微生物被裂解。在一些实施方案中，从样品中去除抑制物。在一些实施方案中，样品中的抑制物被失活。在一些实施方案中，将样品暴露于用于防止核酸降解的条件或试剂。在一些实施方案中，样品经历核糖体RNA耗尽。在一些实施方案中，从样品中去除不需要的RNA。在一些实施方案中，样品中的微生物用与游离核酸结合并阻止其扩增的试剂诸如PMA和EMA处理。在一些实施方案中，辐射样品以引发光化学反应以防止不需要的核酸的扩增。在一些实施方案中，在定量之前进一步纯化提取的核酸。

[0150] 在一些实施方案中，在定量反应之前样品制备花费少于2小时、少于1小时、少于30分钟、少于15分钟、少于10分钟、少于5分钟、少于3分钟、少于1分钟、或少于30秒。

[0151] 在一些实施方案中，在数字定量之前使用技术诸如复制的DNA片段的变性、限制性消化、片段化、消化。这实现单独基因到单独体积的增强的分离，以用于检测和扩增。在一些实施方案中，在基因之间的限制性消化用于促进携带靶基因的DNA分子分离到单独体积中。例如，在一些实施方案中，大肠杆菌中具有每基因组7个拷贝的rDNA用作靶，数字实验仅可以显示每大基因组片段的一个阳性体积。当基因组被变性、片段化和/或消化时，每一个基因可被分离为单独体积，得到24个至70个模板阳性体积用于在装置中从单个基因组的分析。

[0152] 在一些实施方案中，将使用抑制来自死细胞的核酸扩增的剂(例如，叠氮溴化丙锭(PMA)或叠氮溴化乙锭)。在一些实施方案中，细胞将在与抗生素孵育之前用此类抑制来自死细胞的核酸扩增的剂处理。在一些实施方案中，细胞将在与抗生素孵育之后用此类抑制来自死细胞的核酸扩增的剂处理。

[0153] 在一些实施方案中，在定量之前进行染色体DNA的限制性消化，诸如以分离含靶基因的多个复制拷贝的DNA片段，同时保存适于检测和定量的感兴趣的片段。

[0154] 在一些实施方案中，进行一个或更多个变性步骤，诸如以加倍阳性模板的数目(例如，如果在给定的样品中，大肠杆菌细胞在某些条件下可以具有从12个至35个拷贝的rDNA

基因(取决于生长速率、个体年龄和个体状态)-并且在变性之后产生,例如24-70个片段;这在一些情况下可以改善数字方法的可视化)。

[0155] 在一些实施方案中,变性或消化可以用于分离在复制叉的DNA片段-新复制的DNA的部分可以分区到不同的孔中-诸如以增加被抑制的细胞和活跃复制细胞之间的分辨。

[0156] 在一些实施方案中,数字测定可以使用将几个或单个靶核酸分子限制到单独反应体积。在复制完成之前,变性剂(诸如例如加热、尿素、盐酸胍、酸、碱、机械应力(mechanical strain)、酶、限制性酶以及其他)可用于增强靶DNA链到独立体积的分离。这例如在单个基因组内存在多个靶区域的情况中可以特别有用。

[0157] 在一些实施方案中,在进行数字定量之前进行整个染色体DNA的变性。

[0158] 扩增

[0159] 在一些实施方案中,进行核酸扩增反应以扩增靶分析物(例如,靶核酸)。扩增反应可以包括聚合酶链式反应(PCR)、基于核酸序列的扩增(NASBA)、自主序列复制(3SR)、环介导的等温扩增(LAMP)、链置换扩增(SDA)、全基因组扩增、多重置换扩增、链置换扩增、解旋酶依赖性扩增、切口酶扩增反应、重组聚合酶扩增、逆转录PCR或连接介导的PCR。用于核酸的扩增或检测方法可以包括但不限于PCR、RT-PCR或其他方法,包括等温扩增方法。等温核酸扩增方法可以包括但不限于链置换扩增(SDA)、转录介导的扩增、基于核酸序列的扩增(NASBA)、重组酶聚合酶扩增(RPA)、滚环扩增、网状分枝扩增(ramification amplification)、解旋酶依赖性等温DNA扩增、环介导的等温扩增(LAMP)、基于信号扩增和靶扩增两者的方法诸如基于分支DNA的检测方法、杂交链式反应或基于核酸的逻辑门(nucleic acid based logic gates)和DNA电路(参见,例如,Qian和Winfree,Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades,Science 2011;6034:1196-1201)。

[0160] 扩增反应测定可以是PCR。PCR在本领域中是熟知的,并且在E.van Pelt-Verkuil等,Principles and Technical Aspects of PCR Amplification,Springer,2008中提供了这种类型的反应的综合描述。

[0161] PCR是针对复杂DNA背景扩增靶DNA序列的强大的技术。如果RNA将被扩增(通过PCR),则必须使用称为逆转录酶的酶将其首先转录为cDNA(互补DNA)。然后,通过PCR扩增得到的cDNA。

[0162] 只要维持反应的条件是可接受的,进行的PCR就是指数过程。反应的组分是:

[0163] 1.引物对-具有与位于靶序列侧翼的区域互补的约10-30个核苷酸的短单链DNA

[0164] 2.DNA聚合酶-合成DNA的热稳定酶

[0165] 3.脱氧核糖核苷三磷酸(dNTP)-提供将被掺入到新合成的DNA链中的核苷酸

[0166] 4.缓冲液-为DNA合成提供最佳的化学环境。

[0167] 在使用PCR的实施方案中,反应的组分可以与样品接触。反应的组分可以被添加至容纳样品的容器中。反应的组分可以存在于容器中,并且可以添加样品。在一些实施方案中,试剂盒可以包含多个小容器,至少一个容器容纳PCR反应的组分。试剂盒可以包含滑动芯片和反应的组分。

[0168] PCR通常包括将这些反应物置于包含提取的核酸的小管(～10-50微升)中。将管置于热循环仪中;热循环仪是一种使反应经历一系列不同温度持续不同时间量的仪器。用于

每一个热循环的标准方案包括变性阶段、退火阶段和延伸阶段。延伸阶段有时被称为引物延伸阶段。除了此类三步方案之外，可以采用两步热方案，其中将退火和延伸阶段合并。变性阶段通常包括将反应温度升高至90℃-95℃以使DNA链变性；在退火阶段，将温度降低至～50℃-60℃，用于引物退火；然后在延伸阶段将温度升高至60℃-72℃的最佳DNA聚合酶活性温度，用于引物延伸。该过程周期性地重复约20-40次，最终结果是在引物之间创建靶序列的数百万个拷贝。

[0169] 扩增反应测定可以是PCR的变化形式。扩增反应测定可以选自标准PCR方案的变化形式的组，诸如多重PCR、接头引发PCR(linker-primed PCR)、直接PCR、串联式PCR、实时PCR和逆转录酶PCR以及其他，这已被开发用于分子诊断。

[0170] 扩增反应测定可以是多重PCR。多重PCR使用在单个PCR混合物内的多个引物组以产生对不同DNA序列特异性的不同大小的扩增子。通过同时靶向多个基因，可以从单个测试运行中获得否则将要求数个实验的另外的信息。

[0171] 在一些实施方案中，进行多重PCR反应，其中将多个引物组添加至反应混合物中，并且每一个引物组例如在同一体积内扩增它们指定的靶。在其他实施方案中，将样品分为多个较小的体积，将单个引物组引入到其中。

[0172] 扩增反应测定可以是接头引发PCR，也称为连接衔接子PCR(ligation adaptor PCR)。接头引发PCR是一种用于使复杂DNA混合物中基本上所有DNA序列的核酸扩增而不需要靶特异性引物的方法。该方法首先包括用合适的限制性内切核酸酶(酶)消化靶DNA群体。然后使用连接酶将具有合适悬垂末端的双链寡核苷酸接头(也称为衔接子)连接至靶DNA片段的末端。随后使用对接头序列特异性的寡核苷酸引物进行核酸扩增。以这种方式，可以扩增侧链有接头寡核苷酸的DNA源的所有片段。

[0173] 扩增反应测定可以是直接PCR。直接PCR描述了一种藉以直接对样品进行PCR而无任何或具有最少的核酸提取的系统。用适当的化学反应和样品浓度，以最小的DNA纯化进行PCR或直接PCR是可能的。用于直接PCR的对PCR化学反应的调整包括增加的缓冲液强度、使用具有高活性和持续性的聚合酶、以及与潜在的聚合酶抑制物螯合的添加剂。

[0174] 扩增反应测定可以是串联式PCR。串联式PCR利用两个不同轮次的核酸扩增以增加正确扩增子被扩增的概率。串联式PCR的一种形式是巢式PCR，其中使用两对PCR引物以单独轮次的核酸扩增来扩增单个基因座。扩增反应测定可以是巢式PCR。第一对引物与在靶核酸序列外部区域处的核酸序列杂交。在第二轮次扩增中使用的第二对引物(巢式引物)在第一PCR产物内结合并产生包含靶核酸的可以比第一PCR产物短的第二PCR产物。该策略背后的逻辑是，如果错误的基因座在第一轮次核酸扩增期间错误地被扩增，则其通过第二对引物第二次被扩增的概率非常低，并且因此增加特异性。

[0175] 扩增反应测定可以是实时PCR。扩增反应测定可以是定量PCR。实时PCR或定量PCR用于实时测量PCR产物的量。通过在反应中使用含荧光团的探针或荧光染料连同一组标准物一起，定量样品中核酸的起始量是可能的。这在分子诊断中特别有用，其中治疗选择方案可以根据样品中的病原体载量而不同。

[0176] 扩增反应测定可以是逆转录酶PCR(RT-PCR)。逆转录酶PCR(RT-PCR)用于从RNA扩增DNA。逆转录酶是将RNA逆转录为然后通过PCR扩增的互补DNA(cDNA)的酶。RT-PCR可以用于表达谱分析，以确定基因的表达或鉴定包括转录起始位点和终止位点的RNA转录物的序

列。它可用于扩增RNA病毒诸如人类免疫缺陷病毒或丙型肝炎病毒。

[0177] 扩增反应测定可以是等温的。等温扩增是核酸扩增的一种形式，其不依赖于扩增反应期间靶核酸的热变性，并且因此可不要求多次快速的温度变化。因此，等温核酸扩增方法可以在实验室环境内或外进行。已经开发了许多等温核酸扩增方法，包括但不限于链置换扩增(SDA)、转录介导的扩增(TMA)、基于核酸序列的扩增(NASBA)、重组酶聚合酶扩增(RPA)、滚环扩增(RCA)、网状分枝扩增(RAM)、解旋酶依赖性等温DNA扩增(HDA)、环状解旋酶依赖性扩增(Circular Helicase-Dependent Amplification)(cHDA)、环介导的等温扩增(LAMP)、单引物等温扩增(SPIA)、信号介导的RNA扩增技术(SMART)、自主序列复制(3SR)、基因组指数扩增反应(Genome Exponential Amplification Reaction)(GEAR)和等温多重置换扩增(IMDA)。此类扩增化学反应的其他实例描述于以下中：例如("Isothermal nucleic acid amplification technologies for point-of-care diagnostics:a critical review,Pascal Craw and Wamadeva Balachandrana Lab Chip,2012,12,2469-2486,DOI:10.1039/C2LC40100B,")，其通过引用以其整体并入本文。例如，如果限制性酶在典型的PCR操作温度下不够稳定，在低于PCR操作温度的温度下操作的等温扩增方法可以用于改善限制性酶与扩增过程的相容性。

[0178] 此外，可以在这种方法中使用基于信号扩增和靶扩增两者的检测方法，诸如基于分支DNA的检测方法。例如，对于基于分支DNA的检测方法，使用可以切割在用于结合捕获延长剂(extender)和标记延长剂的两个位置之间定位的位置中的靶的酶(例如，如在以下中描述的：Tsongalis,Branched DNA Technology in Molecular Diagnostics,Am J Clin Pathol 2006;126:448-453)，当限制性酶识别并切割靶时，可以减少测定中获得的信号。

[0179] 扩增反应测定可以是链置换扩增(SDA)。链置换扩增(SDA)可以依赖于某些限制性酶在半修饰DNA的未修饰链形成切口的能力以及5′-3′外切核酸酶缺陷型聚合酶(exonuclease-deficient polymerase)延伸和置换下游链的能力。然后可以通过偶联有义反应和反义反应来实现指数核酸扩增，其中来自有义反应的链置换用作用于反义反应的模板。例如，可以在该反应中使用不以传统方式切割DNA但在其中一条DNA链上产生切口的切口酶，诸如N.Alw1、N.BstNB1和Mly1的使用。通过使用热稳定限制性酶(Ava1)和热稳定Exo-聚合酶(Bst聚合酶)的组合已改善了SDA。已显示该组合将反应的扩增效率从10^8倍扩增增加至10^{10}倍扩增，使得使用该技术扩增独特的单拷贝分子是可能的。

[0180] 扩增反应测定可以是转录介导的扩增(TMA)。扩增反应测定可以是基于核酸序列的扩增(NASBA)。转录介导的扩增(TMA)和基于核酸序列的扩增(NASBA)可以使用RNA聚合酶复制RNA序列而非相应的基因组DNA。该技术可以使用两种引物和两种或三种酶，RNA聚合酶、逆转录酶和任选地RNA酶H(如果逆转录酶不具有RNA酶活性)。一种引物可以包含用于RNA聚合酶的启动子序列。在第一步核酸扩增中，该引物与靶核糖体RNA(rRNA)在限定位点杂交。逆转录酶可以通过从启动子引物的3′末端延伸而创建靶rRNA的DNA拷贝。所得RNA:DNA双链体中的RNA可以通过逆转录酶的RNA酶活性(如果存在)或另外的RNA酶H降解。然后，第二引物与DNA拷贝结合。通过逆转录酶从该引物的末端合成新的DNA链，创建双链DNA分子。RNA聚合酶识别DNA模板中的启动子序列并启动转录。新合成的RNA扩增子的每一个重新进入该过程并用作新一轮次复制的模板。

[0181] 扩增反应测定可以是重组酶聚合酶扩增(RPA)。在重组酶聚合酶扩增(RPA)中，特

异性DNA片段的等温扩增通过相对的寡核苷酸引物与模板DNA结合并且将其通过DNA聚合酶延伸来实现。不总要求加热来变性双链DNA(dsDNA)模板。而是,RPA可以采用重组酶-引物复合物扫描dsDNA并促进同源位点处的链交换。所得结构通过单链DNA结合蛋白与置换的模板链相互作用来稳定,因此防止引物通过分支迁移而喷射。重组酶分解使寡核苷酸的3′末端可访问链置换DNA聚合酶,诸如枯草芽孢杆菌的大片段Pol I(Bsu),并且接着发生引物延伸。指数核酸扩增通过该过程的周期性重复来完成。

[0182] 扩增反应测定可以是解旋酶依赖性扩增(HDA)。解旋酶依赖性扩增(HDA)模拟体内系统,因为它使用DNA解旋酶来产生用于引物杂交的单链模板,并且随后引物延伸通过DNA聚合酶进行。在第一步HDA反应中,解旋酶沿着靶DNA穿过,破坏连接两条链的氢键,该两条链然后被单链结合蛋白结合。通过解旋酶暴露单链靶区域允许引物退火。然后DNA聚合酶使用游离的脱氧核糖核苷三磷酸(dNTP)延伸每一个引物的3’末端以产生两个DNA复制。两个复制的dsDNA链独立地进入下一个HDA周期,导致靶序列的指数核酸扩增。

[0183] 扩增反应测定可以是滚环扩增(RCA)。其他基于DNA的等温技术包括滚环扩增(RCA),其中DNA聚合酶在环状DNA模板周围连续延伸引物,产生由许多环的周期拷贝组成的长DNA产物。到反应结束时,聚合酶产生数以千计的环状模板拷贝,其中拷贝链连接到原始靶DNA。这允许靶的空间分辨和信号的快速核酸扩增。1小时内可以产生多达10^{12}个模板拷贝。网状分枝扩增是RCA的一种变化形式,并且利用闭合的环状探针(C-探针)或锁式探针(padlock probe)和具有高持续性的DNA聚合酶在等温条件下指数地扩增C-探针。

[0184] 扩增反应测定可以是环介导的等温扩增(LAMP)。LAMP提供高选择性,并采用DNA聚合酶和识别靶DNA上总计六个不同序列的四个特别设计的引物的集合。包含靶DNA有义链和反义链序列的内引物启动LAMP。由外引物引发的随后的链置换DNA合成释放单链DNA。这用作由与靶的另一末端杂交的第二内引物和外引物引发的DNA合成的模板,产生茎环DNA结构。在随后的LAMP周期中,一个内引物与产物上的环杂交,并启动置换DNA合成,产生原始的茎环DNA和具有两倍长的茎的新的茎环DNA。周期反应继续,在少于一小时内积累了许多靶拷贝。最终产物是茎环DNA,所述茎环DNA具有靶的几个反向重复和具有通过在同一链中的靶的交替反向重复之间退火而形成的多个环的花椰菜样结构。

[0185] 在一些实施方案中,扩增是用于用进行的所有反应定量mRNA的一步数字逆转录环介导的等温扩增(dRT-LAMP)反应。LAMP通过在钙黄绿素中用镁替代锰产生明亮的荧光信号。在一些实施方案中,然后可以使用商业手机相机(cell phone camera)检测和计数该荧光。

[0186] 基于核酸的逻辑门和DNA电路可以用于核酸扩增。限制性酶与基于核酸的逻辑门和DNA电路的使用可以减少或停止DNA网络的内在泄漏问题。结合限制性酶和DNA网络两者的分子识别能力,限制性酶逻辑门可以是用于生物计算装置的设计和构建的高度活跃的组分(参见,例如Qian和Winfree,Scaling Up Digital Circuit Computation with DNA Strand Displacement Cascades,Science 2011;6034:1196-1201)。

[0187] 在一些实施方案中,采用的扩增可以在多种不同的培养基中进行,诸如例如水性溶液、聚合物基质、固体支撑物等等。

[0188] 检测

[0189] 测定结果可以包括从用于检测反应进程或结果的一系列读出(readouts)选择的

读出或检测机制，包括但不限于光学技术、电学技术或磁学技术。实例包括但不限于电化学读出、光学读出，包括例如荧光读出、比色读出、化学发光、电信号、猝灭、探针结合、探针杂交、金属标记、造影剂标记、吸光度、质谱、测序、侧向流动条(lateral flow strips)和异质物质(例如，沉淀、气泡)的产生。

[0190] 读出机制可以包括荧光。例如荧光染料可以用于标记核酸；与更多核酸产物的反应可以产生更多的荧光信号。荧光染料可以包括但不限于溴化乙锭、小檗碱、原黄素(proflavine)、道诺霉素、多柔比星(doxorubicin)、沙利度胺(thalidomide)、YOYO-1、SYBR Green I、SYBR Green II、噁唑黄(oxazole yellow)(YO)、噻唑橙(thiazole orange)(TO)、PicoGreen(PG)、TOTO、TO-PRO、SYTOX、SYTO、其他花菁染料和钙黄绿素。荧光强度可以在终点或实时测量，允许测量反应进程。例如，可以将给定的荧光水平设置为来自数字或准数字(quasi-digital compartment)隔室的阳性信号的阈值。

[0191] 在一些情况下，信号可以由具有报告物部分和亲和部分的分子产生，所述亲和部分应用于数字单元以结合捕获的靶分析物。报告物分子或报告物部分可以是荧光的。可以洗涤数字单元或捕获区域以去除未结合的报告物。在一些情况下，报告物分子可以是钙黄绿素或具有十六烷基三甲基溴化铵的钙黄绿素(钙黄绿素-CTAB)。在一些情况下，报告物可以是嵌入染料。靶分析物可以用酶进行标记，酶可以例如通过电活化可被氧化和还原的底物分子产生电信号。标记可以通过与例如如在夹心测定(sandwich assay)中的亲和剂结合而发生。标记可以通过嵌入染料发生。

[0192] 读出机制可以包括质谱。例如，可以通过质谱区分和/或计数不同大小的核酸(例如来自限制性消化或连接)。可选择地，读出机制可以在无质谱的情况下操作。

[0193] 读出机制可以包括电泳，包括凝胶电泳。例如，可以通过电泳鉴定或区分不同大小的核酸(例如来自限制性消化或连接)。可选择地，读出机制可以在无电泳的情况下操作。

[0194] 读出机制可以包括测序。测序或序列确定技术可以通过包括但不限于以下的方法进行：Sanger测序、Illumina(Solexa)测序、焦磷酸测序(pyrosequencing)、下一代测序、Maxam-Gilbert测序、链终止方法、鸟枪法测序或桥式PCR；下一代测序方法可以包括大规模并行签名测序(massively parallel signature sequencing)、群落测序、SOLiD测序、Ion Torrent半导体测序(Ion Torrent semiconductor sequencing)、DNA纳米球测序(DNA nanoball sequencing)、Heliscope单分子测序、单分子实时(SMRT)测序、纳米孔DNA测序、隧道电流DNA测序(tunneling currents DNA sequencing)、通过杂交的测序、用质谱的测序、微流体Sanger测序、基于显微术的技术、RNA聚合酶测序或体外病毒高通量测序。

[0195] 信号可以是电磁的。该信号可以包括物理目标(诸如珠)的存在或不存在。捕获的靶分析物可以用荧光剂或造影剂进行标记。靶分析物可以用可以产生荧光信号的酶进行标记。靶分析物可以用可以在底物中产生颜色变化、产生比色信号的酶标记。在一些情况下，信号可以从与亲和分子结合的报告物分子并应用于数字单元以结合捕获的靶分析物产生。

[0196] 测序读段(sequencing reads)可以用于鉴定反应产物，并且对于给定核酸产物产生的测序读段的数目可以用于评价反应。例如，可以将给定的测序读段数目设置为来自数字或准数字隔室的阳性信号的阈值。可选择地，读出机制可以在无测序的情况下操作。

[0197] 多路复用信号检测(Multiplexed signal detection)确保在多路复用信号检测中存在区分同一体积内扩增的许多信号的能力，以及区分来自不同体积的不同信号的能

力。

[0198] 二进制定量(Binary Quantification)/数字扩增

[0199] 在一些实施方案中,本文描述的方法和测定使用数字和二进制定量方法;数字方法能够在包含低浓度细胞的样品中定量靶(诸如例如,当样品(例如血液)具有在仍然是临床相关或甚至代表危及生命的疾病的范围的低浓度的病原体(例如每mL1个细菌细胞或10个细胞/mL)时)。在一些实施方案中,本文描述的数字方法可以用于确保易感细胞和耐受细胞之间的可靠定量分辨。

[0200] 二进制定量的过程开始于可以包含靶分析物的样品。靶分析物可以是待定量或探查的分子,例如特定核酸、特定核酸序列、基因或蛋白质。样品可以被分区为许多单独的反应体积。在一些实施方案中,反应体积是单独的分析区域。在一些实施方案中,单独的反应体积在例如单独的孔、室、载玻片表面上的区域、液滴、珠或等分试样中物理分离。在一些实施方案中,单独的反应体积可以在同一容器中,例如,靶分析物可以被固定至基底或被附着至珠。反应体积可以在珠上,在载玻片的表面上,或被附着至基底。将样品分配至许多单独的反应体积,使得一些反应体积但非全部反应体积产生阳性信号。

[0201] 将样品分配至许多单独的反应体积,使得每一个单独的反应体积包含低于或高于用于产生阳性信号的阈值(threshold value)的数目的靶分析物。来自反应体积的阳性信号的产生可以取决于通过该反应体积捕获、捕集或结合的靶分析物的数目或浓度。在一些情况下,通过反应体积捕获、捕集或结合的阈值数目的靶分析物允许阳性信号从该反应体积产生。允许阳性信号产生的阈值数目的靶分析物可以是至少约1个、2个、3个、4个、5个、6个、7个、8个、9个、10个、11个、12个、13个、14个、15个、16个、17个、18个、19个、20个、21个、22个、23个、24个、25个、26个、27个、28个、29个、30个、31个、32个、33个、34个、35个、36个、37个、38个、39个、40个、41个、42个、43个、44个、45个、46个、47个、48个、49个、50个或更多个靶分析物。允许阳性信号产生的阈值数目的靶分析物可以是至多约1个、2个、3个、4个、5个、6个、7个、8个、9个、10个、11个、12个、13个、14个、15个、16个、17个、18个、19个、20个、21个、22个、23个、24个、25个、26个、27个、28个、29个、30个、31个、32个、33个、34个、35个、36个、37个、38个、39个、40个、41个、42个、43个、44个、45个、46个、47个、48个、49个、或50个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从1个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从2个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从3个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从4个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从5个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从6个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从7个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从8个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从9个至19个靶分析物。在一些情况下,允许阳性信号产生的阈值数目的靶分析物可以是从10个至19个靶分析物。在一些情况下,通过反应体积捕获、捕集或结合的阈值浓度的分析物允许阳性信号从该反应体积产生。允许阳性信号产生的靶分析物的阈值浓度可以是至少约零仄摩尔/升(zeptomolar)(zM)、1zM、10zM、100zM、1阿摩尔/升(attomolar)(aM)、10aM、100aM、1飞摩尔/

升(femtomolar)(fM)、10fM、100fM、1pM、10pM、100pM、1nM、10nM、100nM、1μM、10μM、100μM、1mM、10mM、100mM、1M、或更多。允许阳性信号产生的靶分析物的阈值浓度可以是至多约1fM、10fM、100fM、1pM、10pM、100pM、1nM、10nM、100nM、1μM、10μM、100μM、1mM、10mM、100mM、1M、或更少。可以控制允许从反应体积产生阳性信号的靶分析物的阈值数目或浓度。抑制物可以与反应体积一起用于控制阈值数目或浓度。例如,靶分析物的数目或浓度可以被要求高于反应体积中抑制物的数目或浓度,以便信号从该反应体积产生。

[0202] 在一些情况下,从反应体积产生阳性信号的概率取决于被该反应体积捕获、捕集或结合的靶分析物的数目或浓度。可以控制从反应体积产生阳性信号的概率。例如,可以控制信号产生反应的效率,从而控制信号产生的概率;对于给定数目或浓度的靶分析物,较低效率的反应可以导致较低概率的信号产生。

[0203] 在一些实施方案中,将样品分配至许多单独的反应体积,使得每一个单独反应体积包含零个单独出现的靶分析物、或一个或更多个单独出现的靶分析物。一个或更多个分子可以意指非零数目的分子。一个或更多个分子可以意指一个分子。在一些实施方案中,一个或更多个分子可以意指一个分子、两个分子、三个分子、四个分子等等。在一些实施方案中,每一个单独的反应体积被包含在孔中。在一些实施方案中,样品被分配使得每一个反应体积包含平均少于一个单独分子的靶分析物。在一些实施方案中,样品被分配使得大部分反应体积包含零个分子或一个分子的靶分析物。然后,可以通过阅读离散的阳性反应体积和阴性反应体积的模式来进行定性的“是或否”测试以确定每一个反应体积是否包含一个或更多个靶分析物。阳性反应体积可以是被确定为包含一个或更多个靶分析物的反应体积。阳性反应体积可以是被确定为具有与一个或更多个靶分析物的存在相关的信号的反应体积。阳性反应体积可以是被确定为具有高于与一个或更多个靶分析物的存在相关的阈值的信号的反应体积。在一些实施方案中,阳性反应体积被定量为1,或1的简单倍数,诸如2、3等等,而阴性反应体积被定量为0或小于阈值。在一些实施方案中,阳性反应体积被定量为1,且阴性反应体积被定量为0。阴性反应体积可以是被确定为包含零个靶分析物的反应体积。阴性反应体积可以是不具有与一个或更多个靶分析物的存在相关的信号的反应体积。阴性反应体积可以是不具有高于与一个或更多个靶分析物的存在相关的阈值的信号的反应体积。将每一个反应体积确定和/或指定为阳性反应体积或阴性反应体积可以被称为二进制测定或数字测定。这种“是或否测试”或这样的测试可以被称为二进制测定。哪种反应体积是阴性反应体积和哪种反应体积是阳性反应体积的这种定性分析然后可以使用泊松分析转化为样品中靶分析物的定量浓度。通过使用许多反应体积可以实现高动态范围。可以通过使用具有不同大小的反应体积的装置来实现高动态范围。可以通过将样品分区到许多孔和/或到不同大小的孔实现高动态范围。

[0204] 该总体过程可以被称为核酸的二进制定量。该过程可以被称为靶分析物的计数。在一些实施方案中,二进制定量是将样品分区为多个反应体积的过程,使得每一个反应体积包含零个或非零个数目的靶分析物;确定和/或指定哪种反应体积是阳性反应体积,且哪种反应体积是相对于靶分析物的阴性反应体积;并将关于阳性反应体积和阴性反应体积的信息转化为关于样品中靶分析物的量或浓度的信息。在一些实施方案中,确定靶分析物的绝对数目。在一些实施方案中,关于哪种反应体积是阳性反应体积和哪种反应体积是阴性反应体积的信息向关于样品中靶分析物的量、绝对分子数或浓度的信息的转化被称为靶分

析物的数字定量。在一些实施方案中，靶分析物是核酸。在一些实施方案中，实现核酸的二进制定量。在一些实施方案中，确定核酸靶分析物的二进制定量，其中将样品分区为几个反应体积，其中反应体积是在滑动芯片上。

[0205] 在一些实施方案中，可以实现样品中靶分析物的二进制定量，而无需将样品空间分离为多个反应体积。在这些实施方案中，靶分析物可以通过提供信息的分离(informational separation)来计数。在一些实施方案中，样品中的靶分析物通过其中靶分析物用被扩增或复制的携带信息的分子的池加标签的过程经历二进制定量，并且被扩增或复制的不同的携带信息的分子的数目被计数以得到靶分析物的起始数目的定量(参见例如WO2012148477)。在一些实施方案中，携带信息的分子可以是化学条形码库。在一些实施方案中，携带信息的分子可以是核酸序列的集合。

[0206] 可以使用聚合酶链式反应(PCR)、重组聚合酶扩增(RPA)和环介导扩增(LAMP)作为定量RNA或DNA浓度的方法来实现数字分析。可以使用等温化学反应的扩增诸如RPA和LAMP可以良好地适用于家庭(home)和有限资源设施使用。特别地，LAMP化学反应是在家庭或有限资源设施平台使用的有吸引力的候选者，因为它可以具有相对宽的温度耐受(tolerance)范围，可以用简单和便宜的基于化学的加热器和相变材料工作，并且可以具有对阳性孔的荧光增益。

[0207] 稳健性

[0208] 稳健性可以是一系列重复定量测量在不同的实验条件下提供一组相似的测量的程度。例如，手机相机可以用于在见于现实世界中的多种条件下在滑动芯片上成功地进行相似的测量。相似的测量可以是相同的测量。相似的测量可以是相同的诊断。相似的测量可以是相同的答案。相似的测量可以意指在彼此的实验误差内的多于一个的测量。相似的测量可以产生具有统计学显著性的一致结果。相似的测量可以具有相似的数字大小，例如在彼此的5%、10%、15%、20%、25%、30%、40%、50%、60%、70%、80%、90%、100%、200%、1,000%内。稳健的测定可以产生通常比例如在给定组的条件下测量的实例的25%、30%、40%、50%、60%、70%、80%、85%、90%、95%、96%、97%、98%、99%、99.5%、99.9%、99.99%更多的相似的测量。

[0209] 不同类型的测定可以是稳健的测定。核酸扩增和定量测定可以是稳健的。检测蛋白或其他靶诸如细胞、外来体、脂质体、细菌、病毒等等的测定可以是稳健的。LAMP测定可以是稳健的。RT-LAMP测定可以是稳健的。dRT-LAMP测定可以是稳健的。二进制LAMP反应可以是稳健的。二进制的两步LAMP反应可以是稳健的。PCR反应可以是稳健的。qPCR测定可以是稳健的。定量核酸扩增反应可以是稳健的。定性核酸扩增反应可以是稳健的。基于核酸序列的扩增来诊断健康结果的方法可以是稳健的。滑动芯片内的一个过程可以是稳健的。在LAMP反应之后的滑动芯片的成像和分析可以是稳健的过程。

[0210] dRT-LAMP的绝对效率可以通过以下被提高10倍以上，例如从～2%至～28%：i)使用更有效的逆转录酶，ii)引入RNA酶H以破坏DNA-RNA杂交体，以及iii)在RT步骤期间仅添加BIP引物。dRT-LAMP可以与塑料滑动芯片装置相容，并使用该两步法定量HIV RNA。在一些情况下，dRT-LAMP定量结果对患者的HIV RNA的序列非常敏感。

[0211] 测定可以是相对于实验变量稳健的。测定可以是相对于给定的温度范围稳健的。测定可以是在一定温度范围内稳健的。在其内测定可以是稳健的一些非限制性范围包括例

如,1℃、2℃、3℃、4℃、5℃、6℃、7℃、8℃、9℃、10℃、11℃、12℃、16℃、20℃、24℃、28℃、32℃、40℃、50℃、60℃、80℃、100℃、150℃、200℃、250℃或300℃。测定是稳健的温度范围可以以绝对温标(temperature scale)内的温度为中心。可以是测定是稳健的温度范围的中心的一些非限制性温度包括例如-40℃、-30℃、-20℃、-10℃、0℃、10℃,20℃、室温、25℃、30℃、35℃、体温、37℃、40℃、45℃、50℃、55℃、60℃、65℃、70℃、80℃、90℃、100℃、110℃、150℃、或200℃。在一些实施方案中,二进制LAMP测定用于对样品中的核酸序列进行扩增并随后成像和定量。在这些实施方案中,测定可以是以约60℃为中心的9℃的温度范围内的核酸序列的稳健的定量。用于对样品中的核酸序列进行扩增和随后成像和定量的二进制LAMP测定可以在约55℃至约66℃的温度范围内是稳健的。在一些实施方案中,可以对滑动芯片进行成像,并且可以加工数据以在约5℃至约70℃的温度范围内给出稳健的结果。

[0212] 测定可以是相对于时间稳定的。测定可以在一定时间点范围内给出一致的结果。测定可以仅要求终点读出。二进制DNA扩增实验可以仅要求终点读出。终点读出可以在接近扩增完成时或在该时间点之后的时间获得。稳健的DNA扩增测定可以在接近反应结束的时间点和/或反应完成之后的时间点给出一致的结果。在其内测定可以是稳健的非限制反应时间范围包括例如,0.01min、0.1min、0.5min、1min、2min、3min、4min、5min、6min、7min、8min、9min、10min、12min、14min、16min、20min、24min、28min、32min、40min、45min、50min、1.0小时、2小时、3小时、4小时、5小时、6小时、8小时、10小时、12小时、16小时、18小时、1天、2天、3天、7天、1个月、或1年。在一些情况下,二进制DNA扩增实验不要求准确的时间知识。二进制DNA扩增的输出对超出最佳反应时间的反应时间的变化可以是稳健的。在一些实施方案中,例如,在LAMP反应开始之后的40分钟和60分钟之间的20分钟时间段内,滑动芯片上的d-LAMP测定是稳健的。

[0213] 测定可以是相对于大气湿度的变化稳健的。在一些实施方案中,无论大气湿度如何,测定可以是稳健的。在一些实施方案中,测定可以在一系列大气湿度内是稳健的。湿度范围可以是从约0%至100%的相对湿度。在其测定可以是稳健的大气湿度的范围可以是在约30℃下每立方米空气从约0至约40克水。在一些实施方案中,例如,测定可以从约0%湿度至约40%、50%、60%、70%、80%、90%或100%湿度是稳健的。在一些实施方案中,测定可以在约40%、50%、60%、70%、80%、90%或100%湿度的湿度范围内是稳健的。在一些实施方案中,在滑动芯片中运行的d-LAMP测定可以在从约0%至约100%大气湿度的湿度范围内作为稳健的测定进行成像和分析。

[0214] 在一些实施方案中,测定提供了定量分析测量。例如,本发明可以测量和展示样品内核酸序列的量和/或浓度作为定量的量。例如,该测量可以相对于在核酸序列的化学扩增期间、在光学数据测量期间和/或在数据加工期间存在的实验条件是稳健的。

[0215] 在一些实施方案中,可以通过定量两个或更多个基因对抗生素处理的响应,以更大的统计学显著性来解析靶的浓度的小差异。例如,在一个场景中,当将用药物处理的样品与未处理的样品进行比较时,其中两种基因经历浓度1.2倍变化。如果定量产生p值为约0.10的这种1.2倍的差异,则当独立分析时,该差异将不是统计学显著的。然而,使用费舍尔方法(Fisher's method)将来自具有相同总体零假设(即处理的和未处理的细菌核酸是相同的)的几个独立测试的结果相结合将导致比每一个单独测试更低的p值。用于组合来自单

独测试的p值的检验统计量是x^2,且公式是$\chi^2_{2k} = -2\sum_{i=1}^{k}\ln(p_i)$。

[0216] 例如,如果使用该方法组合了每一个p值为0.10的两个独立测试,则总体p值现在将是0.05,这在95%的置信水平是显著的。在一些实施方案中,数字NA(核酸)定量更适合于POC诊断。

[0217] 在一些实施方案中,来自多个基因的RNA的比率用于确定药物易感性。在一些实施方案中,该方法可以包括使用持家基因来测量基因表达的相对变化。在一些实施方案中,这包括使用响应于药物处理被上调的基因与被下调的基因之间的比率。在一些实施方案中,当在样品中存在少量细胞,在一些实施方案中少至1个细胞时,基因的比率用于实现较高灵敏度的响应定量。

[0218] 分析

[0219] 应当理解,本文描述的示例性方法和系统可以以多种形式的硬件、软件、固件、专用处理器或其组合来实现。这些指令和程序可以通过非瞬时计算机可读介质执行和/或存储于非瞬时计算机可读介质中。本文中的方法可以作为有形地体现在一个或更多个程序存储装置上的应用程序在软件中实现。应用程序可以通过包括合适架构的任何机器、装置或平台执行。应当进一步理解,由于本文描述的一些系统和方法是以软件实现的,系统组分(或处理步骤)之间的实际连接可以根据本发明被编程的方式而不同。

[0220] 可以基于从反应体积产生的信号计算样品中靶分析物的数目或浓度。阳性反应体积的数目、定位、类型或其组合可以用于计算样品中靶分析物的数目或浓度。阳性捕获区域的数目、定位、类型或其组合可以用于计算样品中靶分析物的数目或浓度。

[0221] 测定结果可以通过比较结果与理论模型来确定。例如,可以应用泊松统计分析来定量荧光区域和非荧光区域的数目。结合来自不同体积的孔的结果完全最小化标准误差,并跨越非常大的动态范围提供高质量的分析。

[0222] 本文描述的计算机组件、软件模块、功能、数据存储和数据结构可以彼此直接或间接连接,以便允许其操作所需的数据的流动。还应当注意,术语模块的含义包括但不限于进行软件操作的代码单元,并且可以例如作为子程序代码单元、或作为代码的软件功能单元、或作为对象(如在面向对象的范例中)、或作为小程序、或以计算机脚本语言、或作为另一类型的计算机代码被实现。软件组件和/或功能可以被定位于单个计算机上,或根据当前的情况分配在多个计算机上。在又另一方面,提供了计算机可读介质,所述计算机可读介质包括计算机可读指令,其中计算机可读指令指示处理器执行本文描述的方法。指令可以在软件运行时间环境(software runtime environment)中运行。在又另一方面,提供了可以使用网络传输的数据信号,其中数据信号包括在本文描述的方法的步骤中计算的数据。数据信号还可以包括通过有线网络或无线网络传输的封包化数据(packetized data)。在一方面,计算机可读介质包含计算机可读指令,其中当被执行时指令基于从样品获得的数据进行患者中医学状况概率的计算。计算机可读指令可以在处理器的软件运行时间环境中操作。在一些实施方案中,软件运行时间环境提供软件包所需的常用功能和设施。尽管存在运行时间环境的其他几个实例,软件运行时间环境的实例包括,但不限于,计算机操作系统、虚拟机或分布式操作系统。计算机可读指令可以作为软件产品、应用程序(app)或软件包的一部

分进行包装和销售。例如，说明书可以与测定试剂盒一起包装。

[0223] 计算机可读介质可以是存储单元。计算机可读介质也可以是可以通过服务器、处理器或计算机访问的任何可用介质。计算机可读介质可以作为基于计算机的系统的一部分并入，并且可以用于对医学状况的基于计算机的评价。

[0224] 在一些实施方案中，本文描述的计算可以在计算机系统上进行。计算机系统可以包括以下任何或全部：处理器、存储单元、软件、固件、网络通信装置、显示器、数据输入和数据输出。计算机系统可以是服务器。服务器可以是通过网络与多个输入装置和/或多个输出装置通信的中央服务器。服务器可以包括至少一个存储单元，诸如硬盘驱动器或用于存储将由处理器或外部装备访问的信息的任何其他装置，其中存储单元可以包括一个或更多个数据库。在一个实施方案中，数据库可以存储相应于数百至数百万个样品的数据的数百至数百万个数据点。存储单元还可以存储从外部数据库阅读或作为由使用者的输入的历史数据。在一个实施方案中，存储单元存储从正在与服务器通信或已与服务器通信的输入装置接收的数据。存储单元可以包括多个数据库。在一个实施方案中，多个数据库中的每一个相应于多个样品中的每一个。单独数据库还可以包括关于多个可能的样品容纳单元的信息。此外，计算机系统可以包括多个服务器。处理器可以从存储单元或从输入装置访问数据，以进行来自数据的输出的计算。处理器可以执行由使用者提供的或由计算机系统或服务器提供的软件或计算机可读指令。处理器可以具有用于直接从输入装置接收患者数据的工具(means)、将受试者数据存储于存储单元中的工具以及用于处理数据的工具。处理器还可以包括用于从使用者或使用者界面接收指令的工具。处理器可以具有存储器，诸如随机存储器。在一个实施方案中，提供了与处理器通信的输出。在进行计算之后，处理器可以提供输出，诸如从计算返回至例如输入装置或存储单元、至相同或不同的计算机系统的另一存储单元或至输出装置。来自处理器的输出可以通过数据显示来显示。数据显示可以是显示屏幕(例如，监视器或数字装置上的屏幕)、打印输出、数据信号(例如，数据包(packet))、报警器(例如，闪光灯或声音)、图形使用者界面(例如，网页)或上述任何一种的组合。在一个实施方案中，输出通过网络(例如，无线网络)传输至输出装置。输出装置可以被使用者使用以从数据处理计算机系统接收输出。在使用者接收到输出之后，使用者可以确定动作过程，或者可以进行一个动作过程，诸如当使用者是医学人员时的医学处理。在一些实施方案中，输出装置与输入装置是相同的装置。示例性输出装置包括，但不限于，电话、无线电话、移动电话、PDA、闪存驱动器、光源、声音发生器、计算机、计算机监视器、打印机和网页。使用者站可以处于与打印机或显示监视器通信，以输出由服务器加工的信息。

[0225] 在本发明的实施方案中可以使用客户端-服务器、关系数据库架构。客户端服务器架构是一种网络架构，其中网络上的每一个计算机或过程是客户端或服务器。服务器计算机通常是专用于管理磁盘驱动器(文件服务器)、打印机(打印服务器)或网络流量(网络服务器)的强大计算机。客户端计算机包括PC(个人计算机)、手机或使用者运行应用程序的工作站、以及本文公开的示例性输出装置。客户端计算机依赖于服务器计算机的资源，诸如文件、装置、以及甚至处理能力。在本发明的一些实施方案中，服务器计算机处理所有的数据库功能。客户端计算机可以具有处理所有前端数据管理的软件，并且还可以接收使用者输入的数据。

[0226] 受试者数据可以用用于被处理器或使用者识别的独特的标识符存储。在另一步骤

中，处理器或使用者可以通过为特定患者数据选择至少一个标准来进行存储数据的搜索。然后可以检索特定的患者数据。计算机系统中的处理器可以进行将输入数据与来自计算机系统可用数据库的历史数据进行比较的计算。计算机系统然后可以将来自计算的输出存储于数据库中和/或通过网络将输出与输出装置诸如网页、文本或电子邮件通信。在使用者从计算机系统接收到输出之后，使用者可以根据输出采取医学处理的过程。例如，如果使用者是医师，并且输出是高于阈值的癌症概率，则医师可以进行或下令(order)可疑组织的活检。一组使用者可以使用web浏览器将来自生物标志物测定的数据输入到网页的图形使用者界面中。网页是与前端服务器相关的图形使用者界面，其中前端服务器可以与使用者的输入装置(例如，计算机)和后端服务器通信。前端服务器可以包括存储装置或与存储装置通信，所述存储装置具有能够存储任何类型的数据(例如使用者帐户信息、使用者输入和待输出至使用者的报告)的前端数据库。然后可以将来自每一个使用者的数据发送至能够操作数据以产生结果的后端服务器。例如，后端服务器可以计算相似手机的修正或编译从相似样品收集单元产生的数据。后端服务器然后可以将操作或计算的结果发送返回至前端服务器，在那里它可以被存储于数据库中，或可以用于产生报告。结果可以从前端服务器传输至输出装置(例如，具有web浏览器的计算机或手机)，以被递送至使用者。不同的使用者可以输入数据并接收数据。在一个实施方案中，结果以报告来递送。在另一实施方案中，结果被直接递送至可以提醒使用者的输出装置。

[0227] 来自测定的信息可以是定量的并且被发送至本发明的计算机系统。信息也可以是定性的，诸如观察模式或荧光，其可以由使用者转换为定量量度或由阅读器或计算机系统自动地转换为定量量度。在一个实施方案中，受试者还可以向计算机系统提供除样品测定信息之外的信息，诸如种族、身高、体重、年龄、性别、眼睛颜色、头发颜色、家族医学史、身份、定位和可以对使用者有用的任何其他信息。

[0228] 在一些实施方案中，另外的信息由与装置相关的传感器提供。例如，可以通过包括图像传感器的装置测量全球定位数据、加速数据、空气压力或湿度水平。该另外的信息可以由本发明的计算机系统使用。

[0229] 信息可以由阅读或提供来自图像传感器的数据的装置自动地发送至计算机系统。在另一实施方案中，使用者(例如，受试者或医学专业人员)使用输入装置将信息输入到计算机系统中。输入装置可以是个人计算机、移动电话或其他无线装置，或者可以是网页的图形使用者界面。例如，以JAVA编程的网页可以包括使用者可以对其添加文本的不同输入框，其中由使用者输入的字符串然后被发送至计算机系统进行处理。受试者可以以多种方式输入数据，或使用多种装置输入数据。数据可以被自动地获得，并从另一计算机或数据输入系统输入计算机。将数据输入至数据库的另一方法是使用输入装置诸如键盘、触摸屏、跟踪球(trackbal)或鼠标，用于将数据直接输入到数据库。

[0230] 在一个实施方案中，计算机系统包括存储单元、处理器和网络通信单元。例如，计算机系统可以是个人计算机、膝上型计算机或多个计算机。计算机系统也可以是服务器或多个服务器。计算机可读指令，诸如软件或固件可以被存储于计算机系统的存储单元上。存储单元还可以包括用于存储和组织由计算机系统接收和产生的信息的至少一个数据库。在一个实施方案中，数据库包括历史数据，其中历史数据可以从另一数据库自动地填充(populated)或由使用者输入。

[0231] 在一个实施方案中，计算机系统的处理器访问至少一个数据库或直接从输入装置接收信息作为待处理的信息的来源。处理器可以对信息来源进行计算，例如进行动态筛选或概率计算方法。在计算之后，处理器可以将结果传输至数据库或直接传输至输出装置。用于接收结果的数据库可以与输入数据库或历史数据库相同。输出装置可以通过网络与本发明的计算机系统通信。输出装置可以是能够将处理结果递送至使用者的任何装置。

[0232] 本发明的装置或计算机系统之间的通信可以是任何数字通信包括，例如，通过因特网的方法。网络通信可以是无线的、基于以太网的、光纤的、或通过带电线(fire-wire)、USB或任何其他能够通信的连接。在一个实施方案中，可以对通过本发明的系统或方法传输的信息进行加密。

[0233] 还应当注意，系统和方法可以包括经由用于与一个或更多个数据处理或存储装置通信的网络(例如，局域网络、广域网络、因特网)、光纤介质、载波(carrier waves)、无线网络输送的数据信号。数据信号可以携带提供至装置或来自装置的本文公开的任何或全部数据。

[0234] 另外，本文描述的方法和系统可以通过包括通过装置处理子系统可执行的程序指令的程序代码在许多不同类型的处理装置上实现。软件程序指令可以包括源代码、目标代码、机器代码或可操作以使处理系统进行本文描述的方法的任何其他存储的数据。然而，还可以使用其他实现方式，诸如被配置为进行本文描述的方法和系统的固件或甚至适当设计的硬件。

[0235] 计算机系统可以与用于从受试者获得数值的仪器物理分离。在一个实施方案中，图形使用者界面也可以远离计算机系统，例如可以是与网络通信的无线装置的一部分。在另一实施方案中，计算机和仪器是相同的装置。

[0236] 计算机系统的输出装置或输入装置可以包括一个或更多个使用者装置，所述使用者装置包括图形使用者界面(graphical user interface)，所述图形使用者界面包括界面元件诸如如在本领域已知的图形使用者界面中常规发现的按钮、下拉菜单(pull down menus)、滚动条(scroll bars)、用于输入文本的字段等。在使用者界面上输入的请求被输送至系统中的应用程序(诸如Web应用程序)。在一个实施方案中，系统中的使用者装置的使用者能够使用由系统的Web浏览器和Web服务器提供的HTML界面直接访问数据。

[0237] 图形使用者界面可以通过作为die操作系统(die operating system)或服务器的一部分的图形使用者界面代码产生，并且可以用于输入数据和/或展示输入数据。处理数据的结果可以展示于界面或不同的界面中、印刷于与系统通信的打印机上、保存于存储装置中和/或通过网络传输。使用者界面可以指呈现给使用者的图形、文本或听觉信息，并且还可以指用于控制程序或装置的控制顺序，诸如按键、移动或选择。在另一实例中，用户界面可以是触摸屏、监视器、键盘、鼠标或允许使用者与本发明的系统交互的任何其他物品。

[0238] 抗生素易感性数据的使用

[0239] 在又另一方面，提供了一种由使用者采取医学行动的过程的方法，所述方法包括基于样品分析启动医学行动的过程。医学行动的过程可以是将医学处理递送至所述受试者。医学处理可以选自由以下组成的组：药物、手术、器官切除术和放射疗法。药物可以包括，例如，用于癌症疗法的化学治疗化合物。医学行动的过程可以包括，例如，医学测试的管理、所述受试者的医学成像、设置用于递送医学处理的具体时间、活检和与医学专业人员的

咨询。医学行动的过程可以包括，例如，重复以上描述的方法。方法还可以包括由所述使用者以所述样品诊断受试者的医学状况。系统或方法可以包括递送医学处理或启动医学行动的过程。如果疾病已通过本发明的方法或系统估计或诊断，医学专业人员可以评价该估计或诊断并根据他的评价来递送医学处理。医学处理可以是意指处理疾病或疾病症状的任何方法或产品。在一个实施方案中，系统或方法启动医学行动的过程。医学行动的过程通常由评价来自本发明的计算机系统的处理器的结果的医学专业人员确定。例如，医学专业人员可以接收通知他受试者具有患有特定医学状况的97％概率的输出信息。基于该概率，医学专业人员可以选择最适当的医学行动，诸如活检、手术、医学处理或无动作。在一个实施方案中，本发明的计算机系统可以在数据库中存储医学行动的过程的多个实例，其中处理的结果可以触发待输出至使用者的一个或多个示例性的动作过程的递送。在一个实施方案中，计算机系统输出医学行动的信息和示例性过程。在另一个实施方案中，计算机系统可以启动适当的医学行动的过程。例如，基于处理的结果，计算机系统可以与可以将药物递送至受试者的装置通信。在另一实例中，计算机系统可以基于处理结果与紧急人员或医学专业人员联系。患者可以采取的医学行动的过程包括自主管理药物、应用软膏(ointment)、改变工作时间表、改变睡眠时间表、休息、改变饮食(diet)、去除装饰(dressing)、或安排约会和/或访问医学专业人员。医学专业人员可以是例如医师、紧急医学人员、药剂师、精神病学家、心理学家、按摩师(chiropractor)、针灸师(acupuncturist)、皮肤科医师(dermatologist)、泌尿科医师(urologist)、直肠科医师(proctologist)、足科医师(podiatrist)、肿瘤学家(oncologist)、妇科医师(gynecologist)、神经病学家(neurologist)、病理学家、儿科医师(pediatrician)、放射科医师(radiologist)、牙科医师、内分泌学家、胃肠病学家、血液学家、肾病学家(nephrologist)、眼科医师、物理治疗师、营养学家、物理治疗师或外科医师。

[0240] 在已确定了阳性孔或反应室的数目后，则使用泊松统计和关于所讨论的芯片的先前知识来处理该数目以确定芯片中样品的原始浓度。然后，该信息将经由电子邮件自动地发送至任何有效的电子邮件帐户，并且然后由拍摄图像的原始人员接收，无论其相对于进行图像分析的计算机在世界的何处。尽管实际时间受到手机网络上的上传速度和计算机网络上的下载速度影响，在拍摄图像和收到电子邮件确认之间逝去的时间已正好(in well)在1分钟内进行。这很重要，因为如果在分析过程中检测到误差，诸如无法发现所有4个点，用户需要快速被提醒，必须拍摄另一图像。该软件已被编程为这样做，并且使用者通常在1分钟内知道拍摄另一图像。通过电子邮件通知的能力可以给出经由文本通知的能力。手机提供商可以具有将电子邮件的正文作为文本发送至特定使用者的服务。其他服务器可以被借助(leveraged)作为SMS信使。分析过程可以使用计算机自动化以通知使用者是否可以使用图像。该通知可以是例如SMS消息、电子邮件消息、电话呼叫、web发布或电子消息。在一些实施方案中，从上传图像直到通知使用者的时间量可以被称为分析过程。分析过程可以花费，例如，少于5min、4min、3min、2min、1min、50秒、45秒、40秒、30秒、20秒、10秒、9秒、8秒、7秒、6秒、5秒、4秒、3秒、2秒、1秒、0.5秒、0.4秒、0.3秒、0.2秒、或0.1秒。在一些实施方案中，分析过程花费少于1min。

[0241] 在一些实施方案中，可以提供用于提供校准发射的至少一个校准来源和用于感测校准发射的至少一个校准光电二极管，其中控制电路具有用于从检测光电二极管输出的每

一个中减去校准光电二极管输出的差分电路(differential circuit)。

[0242] 通信界面可以是通用串行总线(USB)连接,使得外壳(outer casing)被配置为USB驱动器。

[0243] 在一些情况下,信息被传输返回至用于成像的移动装置。例如,可以获得图像,将其发送至单独的计算机用于分析,并且然后可以将图像或与图像相关的日期传输返回至移动装置。在一些实施方案中,将图像和/或经处理的图像和/或所得数据通过使用者传输至单独的装置,例如医师的移动装置可以接收该信息。在一些情况下,两个或信息的集合被传输至两个或更多个装置。两组或更多组信息可以是相同的信息,或者在一些实施方案中,将单独的数据发送至每一个使用者。例如,患者可以接收与图像相关的一些信息,同时患者的医师接收更适于医师分析的信息。

[0244] 尽管将图像分析卸载至“云”提供了许多益处,包括原始数据的可追溯性和存档、全局访问以及与几乎所有智能手机操作系统的相容性,但其要求具有足够高带宽的无线数据连接;因此,在一些场景下,直接的电话上分析(direct on-phone analysis)可以是优选的。

[0245] 平台/装置

[0246] 本文提供了装置(例如,微流体装置)和方法,所述装置(例如,微流体装置)和方法可以快速地鉴定细胞(包括癌细胞)或微生物(包括病原体),定量它们的载量,并诊断它们对药物诸如抗生素的易感性或耐受性。在一些实施方案中,装置可以使用可以起源于多种样品类型(包括临床或环境样品)的单独微生物或细胞来实现药物易感性或药物耐受性的表型检测和代谢分析。这些样品类型可以包括,但不限于,血液、脑脊髓液(CSF)、唾液和尿液,并且还可以包括诸如来自水或医院表面的环境样品。在一些实施方案中,装置能够使细胞与药物诸如抗生素孵育,并然后在无污染的平台快速地提取和定量核酸或其他分子。装置可以在微流体装置中使用数字单个分子测量,所述单分子测量提供改善检测限值同时提供定量数据的超敏感的测量,对区分病原体与污染物并且能够较早区分药物耐受与易感生物体或细胞非常重要。在一些实施方案中,这些装置可以区分来自临床样品的单独微生物或细胞的状态,并且了解它们对药物(诸如例如抗生素)的单独响应的时机,提供超快的药物-易感性测量。

[0247] 在一些实施方案中,装置和方法可以用于评价每一个给定细胞的基因重复(gene duplication)测量,如当响应于抗生素应激时一些微生物(microbes)可以将抗生素耐受基因(如内酰胺酶)复制到100个拷贝,允许它们进行进化。

[0248] 在一些实施方案中,装置和方法允许从细菌池(诸如临床样品)鉴定药物耐受细菌,所述细菌池可以包括药物易感细菌和/或药物耐受细菌和/或具有病原性和/或非病原性细菌的污染-或这些类型的一些组合。在一些实施方案中,装置可以用于将细胞与药物孵育,并且然后在无污染的平台中快速提取和定量核酸,诸如例如RNA,以确定药物易感性。在一些实施方案中,本文提供的方法和装置使得能够在CLIA临床实验室外进行微生物和细胞鉴定和药物易感性测试。

[0249] 装置可以包括通道和流路(flowpaths),诸如微流体通道。装置可以包括入口、出口或其任何组合。装置可以包括孔、储库(reservoir)或其任何组合。装置可以包括反应体积。装置可以包含预加载的试剂。在一些情况下,微流体装置包括如例如在以下中描述的滑

动芯片装置:在美国专利申请号13/257,811中、在PCT申请号PCT/US2010/028361中、在美国专利公布号20120329038 A1中和在国际专利公布号WO 2013072069 A1中,其的每一个通过引用以其整体并入本文。在一些实施方案中,样品与用于进行反应的试剂接触。如本文使用的,"接触"指将物质引入或维持物质在包含待与该物质接触的实体的区域或溶液中。例如,将用于扩增靶核酸的区域与用于进行反应的试剂接触可以包括将试剂单独地或已经连同靶核酸一起流动到室中,或者使试剂已经预加载到该区域,以用于扩增。当两种物质在结合、碰触(touch)、促进反应条件下相互作用或以其他方式在期望条件下维持邻近时,接触可以发生。

[0250] 平台可以包括流体处理机制(fluid handling mechanism),其使得能够对样品体积、试剂体积和其他流体进行加载、卸载、混合及其他处理。例如,可以使用微流体装置,所述微流体装置包括用于将流体加载到孔或液滴中、用于混合孔或液滴的内容物、或用于卸载孔或液滴的内容物的通道。

[0251] 如本文描述的,一些平台可用于以数字或准数字格式进行测定。例如,孔、孔板、微孔、微流体液滴、乳液、珠和微阵列可以提供用于进行数字或准数字测定的有用的平台。在此类测定中,隔室可以包括单独的孔、液滴、珠或微阵列斑点(microarray spots)。

[0252] 在一些实施方案中,本文描述的装置并入滑动芯片数字扩增技术和滑动芯片样品制备技术(Shen,等,2010,Analytical Chemistry,4606-4612,Shen,等,2010,Lab on a Chip,2666-2672,Shen,等,2011,Analytical Chemistry,3533-3540,Shen,等,2011,J Am Chem Soc,17705-17712),并且可以进行快速单个分子鉴定和定量来自细胞和微生物(诸如例如肺炎克雷伯菌(Klebsiella pneumonia)、铜绿假单胞菌和肠外病原性大肠杆菌)的核酸。样品可以从多种人类体液,诸如例如血液、血浆、唾液、CSF或尿液,或者多种环境样品,诸如例如水或医院表面获得。在一些实施方案中,这可以实现在一些实施方案中提供感染诸如例如UTI的鉴别诊断的工作流程的临床验证。在一些实施方案中,该装置可以提供总细胞载量的定量和物种和/或菌株鉴定。在一些实施方案中,测定的结果可以在以下内可用:少于45分钟、少于30分钟、少于20分钟、少于10分钟、少于5分钟、少于3分钟、或少于1分钟。

[0253] 在一些实施方案中,本文描述的技术使得能够鉴定和定量核酸,诸如例如从掺有(spiked with)参考菌株的细胞培养物的尿液样品提取的细菌DNA和RNA。参考菌株是临床实验室常规用作其质量控制方案的一部分的那些菌株,诸如例如肺炎克雷伯菌(K.pneumoniae)(ATCC 700603)、铜绿假单胞菌(P.aeruginosa)(ATCC 27853)和大肠杆菌(ATCC 25922)(Institute.,2012,M07-A09)。在一些实施方案中,本公开内容中描述的技术和装置可以与标准实验台方法(bench method)和试剂盒(Qiagen Qiamp DNA试剂盒)集成在一起以提取核酸,并且先前公布的通用引物可以用于靶向基因,诸如例如16S基因的保守区域,以定量核酸浓度(Clifford,等,2012,PLoS ONE,e48558,Nadkarni,等,2002,Microbiology,257-266)。例如,该技术可以用于靶向16S基因的超变区以定量和鉴定微生物(Baker,等,2003,Journal of Microbiological Methods,541-555,Hansen,等,2013,PLoS ONE,e61439,Spilker,等,2004,Journal of Clinical Microbiology,2074-2079)。

[0254] 在一些实施方案中,本文描述的装置允许从细菌池(诸如临床样品)鉴定药物耐受细菌,所述细菌池可以包括药物易感细菌和/或药物耐受细菌和/或具有病原性和/或非病原性细菌的污染、或其任何组合。

[0255] 在一些实施方案中，该技术可以以例如三倍分辨率和95％置信区间在例如1,000个至1x10^{7}个拷贝/mL的动态范围内使用数字滑动芯片用于细菌DNA和RNA扩增测定。该动态范围被商业制造的数字滑动芯片充分覆盖，所述数字滑动芯片包含10,240个0.84nL孔，具有450个至9.7x10^{7}个拷贝/mL的动态范围和120个拷贝/mL的检测限值。

[0256] 图5中示出了根据本发明的一个实施方案确定尿液样品中的细胞对药物的耐受性或易感性的工作流程。在一些实施方案中，该装置实现进行样品制备(例如，将样品中的生物体暴露于药物并且提取来自样品的靶核酸)和数字扩增(例如，分配和扩增靶核酸以定量存在于样品中的靶核酸以确定生物体对药物的易感性或耐受性)两者的测定。

[0257] 集成装置

[0258] 在一些实施方案中，本文提供的装置是包含一个或更多个模块的集成装置。这些模块包括，但不限于，孵育模块、样品制备模块、扩增模块和读出模块。本文描述了这些模块的每一个。在一些实施方案中，集成装置组合2个或更多个模块以提供用于检测细胞中响应于药物的生物体性能的简化处理流程。在图6A、6B和6C中描绘了包括孵育室的集成装置和用于调控样品流入孵育室的装置的实例。

[0259] 孵育模块

[0260] 在一些实施方案中，集成装置包括孵育模块。孵育模块可以包含用于例如将微生物暴露于药物的孵育室(参见图6A、6B和6C)。在一些实施方案中，孵育模块包含允许插入流体，诸如样品、培养基、试剂或其他溶液的入口。这些溶液通过一些工具被推入滑动芯片样装置中，所述工具的一个实例是来自泵盖或柱塞的正压力，如图6C中示出的。在一些实施方案中，与滑动芯片层相邻的层包含样品可以流动通过其的通道。在一些实施方案中，最上层具有用于排出和控制压力的一个或更多个开口。在一些实施方案中，滑动芯片层的开口用溶液填充，将样品分为不同的体积。然后将该层滑动，移动滑动芯片层的开口以与单独层形成连接，例如至孵育室的开口(opening to incubation chambers)。样品可以从中央滑动芯片层转运并与包含在孵育室内的溶液诸如培养基或抗生素混合。在一些实施方案中，孵育室可以处于加热的浴中或以其他方式与加热元件接触以用于孵育。在一些实施方案中，膜诸如疏水薄膜用于排出。在一些实施方案中，一个或更多个开口存在于用于控制压力的装置的一个或更多个层中。在一些实施方案中，包含孵育室的层也可以滑动。在一些实施方案中，来自孵育室的流体被转运至单独的模块，诸如扩增模块。在一些实施方案中，一个或更多个滑动芯片层用于例如在具有至样品制备模块或其他模块的开口的孵育室下方。在一些实施方案中，集成装置具有另外的孵育模块。

[0261] 在一些实施方案中，孵育模块中的孵育室用于抗生素易感性测试。在一些实施方案中，通过过滤器对细胞进行预分选。在一些实施方案中，过滤器用于捕获细菌细胞。在一些实施方案中，哺乳动物细胞被选择性裂解而不裂解感兴趣的细菌细胞。在一些实施方案中，将选择性裂解和过滤步骤组合。在一些实施方案中，孵育室包括用于限制细胞的子室阵列。

[0262] 在一些实施方案中，孵育室是被配置为进行以下一个或更多个的模块：(i)将样品分区、(ii)将药物/抗生素分区、(iii)将样品与药物/抗生素合并、(iv)混合、(v)孵育样品、或(vi)将流体转运至单独的室。

[0263] 在一些实施方案中，数字测定可以使用将来自样品的细胞限制到孵育室或克隆分

离区域。在一些实施方案中，将分离的细胞培养于单独的孵育室中以产生来自样品的多个克隆群体。在一些实施方案中，分离的细胞在扩增之前或之后用药物处理。在一些实施方案中，在培养分离的细胞并扩增之后，将克隆群体分成两个或更多个处理区域。如此，克隆群体的至少一部分可以用药物治疗，同时克隆群体的另一部分不用药物治疗。然后，可以进行本文描述的测定以确定克隆群体中的细胞是否对一种或更多种药物易感或耐受。

[0264] 在一些实施方案中，分开通过使用死端(dead-end)填充的滑动芯片设计进行。在一些实施方案中，为了用水性溶液实现死端通道填充，在通道末端可以使用疏水膜，其阻塞水性溶液流动但允许非水性液体和气体流动通过。

[0265] 在一些实施方案中，滑动(即，层的运动)可以使用旋转轴自动地致动。在一些实施方案中，该轴可以由马达控制，所述马达任选地被编码或编程至特定速度、方向和/或旋转角度。

[0266] 在一些实施方案中，样品与药物/抗生素的合并和混合通过将一种或更多种溶液转运到另一溶液的室的增压来进行。在一些实施方案中，室的增压通过泵盖产生，所述泵盖创建密封并改变该隔室中的体积；这可以手动地或通过旋转轴以及其他致动而被致动。在一些实施方案中，流体进出生长室模块的转运可以通过，诸如例如通过泵盖(手动地或自动地)、阀的打开、或通过其他方法，诸如例如移液(pipetting)对腔室增压来进行。

[0267] 在一些实施方案中，孵育室模块包含加热元件。加热元件控制的一些实例包括具有控制温度和计时或相变材料的工具的电路板。

[0268] 在一些实施方案中，孵育室被设计为在1mL和10mL之间进行处理。在一些实施方案中，孵育室被设计为在100μL和3mL之间进行处理。在一些实施方案中，孵育室被设计为在10μL和100mL之间进行处理。在一些实施方案中，孵育室被设计为处理可能包含单个细胞的样品。

[0269] 样品制备模块

[0270] 在一些实施方案中，装置包括样品制备模块。在一些实施方案中，样品可以被分到可以包含或可以不包含感兴趣的药物(诸如抗生素)的不同的隔室。在一些实施方案中，在将样品与/不与抗生素药物孵育之后，将感兴趣的细胞(诸如例如细菌病原体的细胞)裂解(诸如通过裂解缓冲液)以提取RNA和/或DNA。在一些实施方案中，样品制备模块可以用于自动地提取来自尿液的核酸。在一些实施方案中，对于样品制备，过滤柱(filter column)可以用于捕获来自裂解的样品的RNA/DNA，并洗去裂解的样品中存在的细胞碎片或其他组分。

[0271] 在一些实施方案中，提供了自动化或半自动化样品制备模块。在一些实施方案中，自动化或半自动化样品制备模块在密封环境中对裂解的样品产生压力，迫使裂解的样品通过过滤柱(例如，核酸结合柱，诸如玻璃纤维柱)。

[0272] 在一些实施方案中，样品制备模块被设计为在1mL和10mL之间进行处理。在一些实施方案中，样品制备模块被设计为在100μL和100mL之间进行处理。在一些实施方案中，样品制备模块被设计为在10μL和1L之间进行处理。在一些实施方案中，样品制备模块被设计为在1μL和1mL之间进行处理。在一些实施方案中，样品制备模块可以由塑料制成，并且在一些实施方案中不要求外部电源(external power)或主动使用者干预。

[0273] 数字定量模块

[0274] 在一些实施方案中，装置包含数字(或二进制)定量模块，诸如包含多个检测元件

的模块，其中每个检测元件捕获0个、1个或更多个靶(例如RNA/DNA)分子并且适于集成。

[0275] 在一些实施方案中，从装置中回收富集的靶(例如DNA/RNA)以用于在随后或并行实验中使用。在一些实施方案中，集成装置具有多个定量模块。

[0276] 在一些实施方案中，定量模块被设计为在30μL和60μL之间进行处理。在一些实施方案中，定量模块被设计为在1μL和100μL之间进行处理。在一些实施方案中，定量模块被设计为在100nL和1mL之间进行处理。在一些实施方案中，定量模块被设计为在10nL和10mL之间进行处理。

[0277] 试剂存储

[0278] 在一些实施方案中，集成装置包括存储的试剂。在一些实施方案中，装置(包括以上描述那些)中的液体可以被存储，诸如在泡罩包装、试剂包装或其中密封它们的其他类型的容器中(图7)。

[0279] 在一些实施方案中，装置包含适于制备细胞或微生物的核酸以用于核酸定量反应的试剂包装。在一些实施方案中，装置包含适于核酸定量反应的试剂包装。在一些实施方案中，试剂包装包含用于进行核酸定量反应的酶。在一些实施方案中，试剂包装包含用于进行核酸定量(例如，用于PCR或等温扩增)的引物。

[0280] 试剂包装中的示例性试剂可以包括，但不限于，裂解溶液、洗涤溶液、洗脱溶液、再水合溶液(rehydration solutions)、酶溶液(例如，核酸扩增酶、聚合酶、限制性酶)、缓冲液、液体、粉末、团粒(pellet)、凝胶、微珠、探针、引物、核酸、DNA、RNA、多肽、核苷三磷酸(NTP)、抗体、牺牲试剂(sacrificial reagent)或其任何组合。牺牲试剂可以包括水性溶液、润滑剂、油、水不混溶的液体(aqueous-immiscible liquid)、凝胶、气体、氟碳油(fluorocarbon oil)、表面活性剂、气体、空气或其任何组合。例如，空气可以用于产生气泡以用于混合。作为另一实例，空气和不混溶液体(immiscible liquid)可用于去除基质中剩余的溶液(leftover solution)(死体积)。试剂可以被混合以改变其组成。例如，一种类型的缓冲液可以与另一种缓冲剂或干燥试剂混合以将其组成改变为另一缓冲液。

[0281] 在一些实施方案中，装置包括用于测定酶存在和/或活性的试剂包装，诸如，例如单溴二胺(monobromobimane)、7-二乙基氨基-3-(4'-马来酰亚胺苯基)-4-甲基香豆素、N-(7-二甲基氨基-4-甲基香豆素-3-基))马来酰亚胺)、NiWa蓝(1-苄基4-甲基5-(叔丁氧基羰基氨基)-2-(2,5-二氧代-2,5-二氢吡咯-1-基)对苯二甲酸酯)、NiWa蓝II(二甲基5-乙酰氨基-2-(2,5-二氧代-2,5-二氢吡咯-1-基)对苯二甲酸酯)、NiWa橙(二甲基2-氨基-5-((2,5-二氧代-2,5-二氢-1H--吡咯-`-基)乙基)氨基)对苯二甲酸酯)、Ellman试剂/DTNB(5,5'-二硫代双-(2-硝基苯甲酸))、伞形酮(Umbelliferone)衍生的头孢菌素、荧光素衍生的头孢菌素、试卤灵(Resorufin)衍生的头孢菌素、罗丹明衍生的头孢菌素、亚胺培南和对硝基苯酚释放物质。

[0282] 在一些实施方案中，装置包括包含一种或更多种浓度的一种或更多种药物的试剂包装。在一些实施方案中，装置包括包含一种或更多种浓度的一种或更多种抗生素的试剂包装。在一些实施方案中，装置包括包含适于加速细胞或微生物对药物的响应的组分(例如群体感应分子等等)的试剂包装。在一些实施方案中，装置包括包含培养基以增强细胞生长的试剂包装。

[0283] 在一些实施方案中，装置包括包含气体或气体混合物(包含H_2S、CO和NO)的试剂包

装。已知此类气体，例如，影响微生物对抗生素的易感性。此类气体可以用可以是厌氧、需氧或微氧的气体混合物稀释。此类气体混合物可以包含CO_2。在一些实施方案中，装置包括包含裂解试剂以暴露细胞间组分的试剂包装。

[0284] 在一些实施方案中，这些容器避免了溶液的蒸发。在一些实施方案中，试剂可以通过在适当的时间刺穿容器的某些区域或者选择性地打开容器的一部分而从一个或更多个容器中释放。在一些实施方案中，装置中产生的压力可以释放泡罩/容器的内容物；在一些实施方案中，这些流体被转运至装置的其他部分。在一些实施方案中，试剂(诸如例如扩增试剂)作为干燥的试剂、在装置上、在泡罩包装中或在容器中(或其某种组合)存储。试剂可以冻干、用糖(例如蔗糖、海藻糖以及其他)存储、和/或用珠存储。

[0285] 操作模块

[0286] 在一些实施方案中，装置包含操作模块，诸如例如操作装置(包括集成装置)的模块。在一些实施方案中，操作模块可以进行以下功能中的一种或更多种：马达控制、轴旋转、加热、图像捕获和图像加工。在一些实施方案中，操作模块可以使用一个或更多个马达和轴。在一些实施方案中，操作模块可以使用一个或更多个旋转阀。

[0287] 在一些实施方案中，操作模块由一个或更多个组件组成，其中一个或更多个组件可以由弹簧或马达驱动/旋转(图8)。在一些实施方案中，弹簧或马达可以用于旋转螺纹部分，所述螺纹部分可以与前一部分的反向螺纹一起连接至单独的盖/柱塞(如在泵盖专利申请中描述的)。在一些实施方案中，当弹簧或马达旋转第一螺纹部分时，柱塞/盖可以移动并改变存在于一个或更多个腔中的体积和压力。在一些实施方案中，盖/柱塞在一个或更多个腔内形成气密密封。在一些实施方案中，产生的压力可以用于使存在于腔中的液体样品通过过滤柱流动。

[0288] 流体管理

[0289] 在一些实施方案中，装置被配置为将一定体积的裂解样品转运出装置以用于单独或并行的实验。在一些实施方案中，用于产生压力以控制溶液流动通过装置(诸如其中不同的溶液流动通过柱或暂时被阻止流动通过柱)的阀被并入装置中。在一些实施方案中，在装置操作期间利用负压。在一些实施方案中，装置内的室可以在装置启动之前增压或减压。

[0290] 在一些实施方案中，可以诸如通过将过滤柱放置于装置的旋转层中来控制一步或多步过程。在一些实施方案中，当过滤柱在装置中旋转时，样品可以被移动至装置中可以包含不同试剂的不同室；这些流体可以顺序地或不连续地流动通过过滤柱。在一些实施方案中，存在于一个或更多个室中的液体将被移动(诸如经由泵盖的产生的压力)并且溶液将流动通过过滤柱。在一些实施方案中，当装置中的过滤柱处于第一位置时，溶液可以被泵出第一腔；在一些实施方案中，当装置被移动到第二位置时，第二溶液被泵送。装置可以包含过滤柱和一个或更多个包含液体的室的一个或更多个重叠。在一些实施方案中，可以通过装置设计来编程不同重叠位置之间的距离和/或角度。

[0291] 在一些实施方案中，裂解的样品可以流动通过过滤柱以在过滤柱上捕获靶(例如DNA或RNA)。在一些实施方案中，随后的步骤包括洗涤-例如，对耐受或易感细胞特异性的捕获的DNA或RNA可以诸如用洗涤缓冲液洗涤，并且用洗脱缓冲液从柱洗脱出以从柱释放。

[0292] 在一些实施方案中，在靶(例如DNA/RNA)从柱上富集和洗脱出之后，洗脱的DNA/RNA可以从柱转运到还被称为定量模块或装置的下一个室或模块，在一些实施方案中其适

于定量靶(例如DNA/RNA)分子。

[0293] 其他模块和元件

[0294] 在一些实施方案中,装置包含操作模块,诸如例如操作装置(包括集成装置)的模块。在一些实施方案中,操作模块被配置为进行以下功能中的一种或更多种:马达控制、轴旋转、加热、图像捕获和图像加工。在一些实施方案中,操作模块包括一个或更多个马达和轴。在一些实施方案中,操作模块包括一个或更多个旋转阀。在一些实施方案中,一个或更多个层被连接至轴并且能够旋转。在一些实施方案中,一个或更多个层被配置为与轴断开。在一些实施方案中,一个或更多个层包括制动球(detent ball),并且在低转矩下保持连接至轴,但在高转矩(即,当物理地停止层时)断开。

[0295] 在一些实施方案中,一个或更多个层被预编程以旋转指定的程度。在一些实施方案中,操作模块包括物理地停止一个或更多个层的停止组件。

[0296] 在一些实施方案中,装置包含富集或预浓缩模块。在一些实施方案中,富集模块浓缩生物体的数目。在一些实施方案中,这些装置包含阈值模块。在一些实施方案中,截断模块将生物体的数目限制为阈值。在一些实施方案中,将富集和阈值模块组合。在一些实施方案中,富集模块被设计为在1mL和10mL之间进行处理。在一些实施方案中,富集模块被设计为在100μL和100mL之间进行处理。在一些实施方案中,富集模块被设计为在100μL和1L之间进行处理。

[0297] 在一些实施方案中,装置包含孵育室和邻近的至少一个检测室,诸如彼此在10cm以内。在一些实施方案中,装置包含孵育室和邻近的至少一个检测室,诸如彼此在20cm以内。在一些实施方案中,装置包含孵育室和邻近的至少一个检测室,诸如彼此在1m以内。

[0298] 在一些实施方案中,集成装置集成了以下的一个或更多个:富集模块、截断模块(cutoff module)、孵育模块、样品制备模块、定量模块和操作模块。在一些实施方案中,集成装置将孵育模块与自主装置(autonomous device)和数字滑动芯片集成。在一些实施方案中,模块具有互锁特征。在一些实施方案中,模块是可互换的。在一些实施方案中,可用于样品制备和处理的模块被包括在装置中。

[0299] 在一些实施方案中,集成装置具有小于10x10x10cm的尺寸,或者集成装置具有小于20x20x20cm的尺寸。

[0300] 在一些实施方案中,装置包括柔性材料,诸如,但不限于PDMS、Tango+(3D-可打印软材料)或橡胶。柔性材料使得装置能够在模块间(例如,在样品制备模块至扩增模块之间)进行样品的防泄漏液体移动。在一些实施方案中,装置包括用于界面模块的通道/微通道。在一些实施方案中,装置包括注射泵/特氟隆管。在一些实施方案中,装置包括3D打印部分中的嵌入通道。在一些实施方案中,装置包括3D打印的阀。这些组件还可以使得装置能够在模块间进行样品的防泄漏液体移动。

[0301] 在一些实施方案中,定量装置具有一系列具有不同体积的隔室。在一些实施方案中,定量装置在加载一系列单个体积隔室之前并入一系列芯片上稀释物(dilutions)。在一些实施方案中,这些装置能够实现大的动态范围而无需异常大的隔室尺寸。

[0302] 作为实例,可以连续四次以1:10稀释样品。每一种稀释物被加载到一系列单个体积数字隔室。400个隔室可以以1-对数动态范围定量和分辨1.5倍的浓度差异。因此,示例性装置由四组各400个隔室组成。可以将四种10x稀释物加载到这四组单个体积隔室中,并且

将能够以4-对数动态范围实现1.5倍分辨率和抗生素易感性测试。

[0303] 在一些实施方案中，装置包括产生读出的读出模块或机制。平台可以与一个或更多个读出或检测机制相容。例如，平台可以部分地或全部地是透明的或半透明的，允许荧光测量、沉淀物或气泡的检测或者其他视觉观察。平台可以包括视觉检测器，诸如CCD、CMOS传感器、照相机、光子检测器和其他传感器。在另一实例中，平台可以包括电传感器，诸如定位于微孔内的电极。平台可以与卸载样品相容以用于分析。例如，平台可以允许卸载液滴或孔的内容物用于质谱、测序或电泳。

[0304] 在一些实施方案中，阅读定量模块的方法包括，但不限于，荧光显微术、明视野显微术、照相机、数字照相机或手机相机以及其他。在一些实施方案中，具有相机的基站用于捕获图像。图像处理可以被集成于基站中，或者图像文件可以被转运至计算机。在一些实施方案中，拍摄手机图片。手机软件可以用于处理图像，或者可以将图像上传至服务器以用于图像处理。

[0305] 在一些实施方案中，将加热元件嵌入装置中，诸如可以插入孵育模块的那些，诸如例如包含高导热性材料的浴。在一些实施方案中，靶(例如RNA/DNA)的扩增和检测可以包括装置上的加热元件。加热元件的实例可以包括，但不限于，通过使用电力、化学反应或相变材料进行加热。在一些实施方案中，可以使用透明导热材料，使得可以通过加热单元捕获扩增区域的图像。

[0306] 在一些实施方案中，这些装置并入混合方法。在一些实施方案中，喷射宏观混合(jet macro-mixing)可以用于混合溶液(Nealon,A.J.,O'Kennedy,R.D.,Titchener-Hooker,N.J.,&Lye,G.J.(2006).Quantification and prediction of jet macro-mixing times in static microwell plates.Chemical engineering science,61(15),4860-4870)。在此类方法中，一种或更多种溶液被增压，并且它们之间的开口允许一种溶液流入另一种溶液，或者两种溶液相互碰撞。在一些实施方案中，一种或更多种溶液是气态混合物，并产生气泡以形成气泡混合物(mixer)。在一些实施方案中，混合用于混合液体样品和干燥试剂。可选择地，正压和负压可以用于改善混合。在一些实施方案中，可以，例如通过叶轮、磁珠搅拌、重力、被动结构等等、或此类方法的组合机械地实现混合。

[0307] 在一些实施方案中，这些装置包含用于合并或混合试剂的室或室的阵列。

[0308] 在一些实施方案中，这些装置包含特殊材料。在一些实施方案中，装置中的一种或更多种材料与生物体相容(例如，负面地影响或不负面地影响存活力的材料)。在一些实施方案中，出于一个或更多个以下目的，材料或表面涂层可以被添加至材料：增强导热性能、控制表面化学反应/表面张力(疏水性)、增强生物体存活力、改变扩散性能、防止吸附、密封、防止浸出(leeching)(即塑料)、改变刚度以及其他。

[0309] 在一些实施方案中，这些装置利用一个或更多个手动步骤—诸如但不限于添加样品、插入试剂、调整设置、滑动、旋转、附着泵盖、致动泵送、插入材料、连接模块、启动装置、启动加热、转运流体、取出捕获图像的模块等等。

[0310] 在一些实施方案中，可以通过许多方法制造装置，所述许多方法包括3D打印、注射成型、压花，湿蚀刻和本领域已知的其他方法。

[0311] 装置使用

[0312] 装置的一些实施方案中的数字平台允许检测比目前基于核酸的技术更低丰度的

细菌，这使得能够对血液和CSF样品进行更稳健的分析。增加的数字技术精确度改善了患者对疗法响应的监测。

[0313] 样品制备模块可以在5min内提供快速的核酸提取和纯化，减少不稳定RNA的降解。

[0314] 现场护理(point-of-care)模块可以允许在跨越广泛的感染范围的临床群体中以及在临床试验和监视两者中进行广泛的测试(broad-based testing)。

[0315] 数字扩增模块可以允许在比培养方法更近的时间帧更清楚地了解低水平感染，并且可以告知对抗微生物剂的响应的异质性。

[0316] 在一个实施方案中，装置可以与样品制备技术一起使用以从靶微生物或细胞诸如例如肺炎克雷伯菌、铜绿假单胞菌和肠外病原性大肠杆菌快速且以高产率地分离细菌DNA和RNA，并且装置可以包括临床样品，并且可以包括多种体液，诸如例如尿液。先前已验证了样品制备滑动芯片用于从包含沙眼衣原体(Chlamydia trachomatis)的尿液样品的细菌DNA和RNA提取和纯化。还已验证了从包含HCV或HIV病毒颗粒的血浆样品的病毒RNA提取和纯化。在一个实施方案中，装置可以用于以小于5min进行样品制备，并且其中样品处理一定范围的体积，诸如例如多达0.5mL。在一些实施方案中，装置可以用于快速检测包含低细菌载量的样品。

[0317] 在一些实施方案中，装置可以用于，在一些情况下在极短的时间段内(诸如例如小于5分钟)，且在一些情况下以与标准实验台方法相比高于80％的产率，且以适于数字定量和实时定量两者的质量，从掺有细菌靶(诸如例如肺炎克雷伯菌、铜绿假单胞菌或大肠杆菌)的样品(例如尿液样品)提取和纯化核酸，诸如细菌DNA和RNA。

[0318] 已知细菌RNA不稳定，并且表达水平可以快速变化。在其中纯化的RNA的数量和质量可能受损的情况下，本公开内容中描述的装置可以用于进一步缩短样品制备方案，诸如例如少于3min。该时间帧先前已被证明用于分离来自血浆的病毒RNA。在一些实施方案中，裂解步骤可以通过添加另外的去垢剂或抑制物以最小化RNA酶的活性来修改。

[0319] 在一些实施方案中，装置可以组合滑动芯片样品制备和数字定量技术，以用于以3倍分辨率快速、可重复和定量测量掺有，例如，从100CFU/mL至1x10^{6}CFU/mL的肺炎克雷伯菌、铜绿假单胞菌和大肠杆菌的样品(例如，临床尿液样品)中的核酸，诸如例如细菌RNA水平。在一些实施方案中，本文描述的装置具有大于99％特异性的性能。

[0320] 在一些实施方案中，装置或平台组合多于一个模块以定量和鉴定例如临床尿液样品中的靶微生物。

[0321] 在一些实施方案中，装置将使得能够进行样品制备和数字扩增两者的测定。在一些实施方案中，总测定时间可以少于60min、少于30分钟、少于15分钟。通过优化变性时间，变性温度，或通过合并退火和延伸步骤(Bio-Rad)可以缩短目前的PCR方案。使用优化的扩增酶或快速热循环仪，可以实现10分钟的扩增(Wheeler，等，2011，Analyst，3707-3712，Neuzil，等，2006，Nucleic Acids Research，e77)。在一些实施方案中，这些装置可以进行在少于5min内的样品制备、少于5min的逆转录步骤和少于35min的数字滑动芯片工作流程，以实现例如少于45min的工作流程。在另一实施方案中，该RT数字PCR仪器可以用于辅助优化芯片热循环方案。

[0322] 在一些实施方案中，装置可以使得能够使用直接起源于临床样品的小数目细胞实现快速诊断药物耐受性。在一些实施方案中，本文描述的装置可以用于回答以下问题，所述

问题对于不依赖于许多轮次的细胞分裂的快速AST测定的设计是必需的，并且对于当有限数目的细胞是可用的时(如通常在血液和CSF情况)进行AST测定是必需的：(i)在药物暴露之后不久，药物易感和药物耐受的微生物群体或细胞的单个细胞生长、表型和基因表达谱是什么?(ii)是否存在可以非常快速地(在小于15分钟或甚至7分钟内)预测群体或细胞的易感性或耐受性的“岗哨”细胞?(iii)为了可靠地预测临床相关细菌群体的药物易感性或耐受性，需要多少细胞?(iv)当对混合的细胞进行测量时，可靠预测抗生素易感性和耐受性的基因表达签名和最短药物暴露是什么，以及该测量能够多么接近单个细胞测定的表现?

[0323] 在一些实施方案中，本文描述的装置可以用于监测来自响应于药物暴露的靶生物体或细胞的单个细胞。在一些实施方案中，这些装置具有在单个细胞水平监测生长、代谢活性、表型变异和基因表达水平的能力。

[0324] 在一些实施方案中，装置使得能够测量在药物诸如例如环丙沙星(氟喹诺酮)或氨苄青霉素(诸如β-内酰胺)的标准稀释物的存下易感菌株或细胞的生长速率分布和倍增时间。临床医师认为环丙沙星具有立即阻止生长的能力(Barcina，等，1995，Journal of Microbiological Methods，139-150)，而氨苄青霉素的作用方式被认为允许在裂解发生之前多达5代细胞分裂(Rolinson，1980，Journal of General Microbiology，317-323)。熟知的是，抗生素可以在塑料装置，诸如用于Fluidigm芯片的PDMS中快速损失。因此，在一些实施方案中，我们的装置可以由玻璃构成，并且在一些实施方案中，它们可用于回收流体(Ma，等，2014，PNAS，Ma，等，2014，Integrative Biology)以测量测定后抗生素浓度。

[0325] 在一些实施方案中，装置可以通过定量存在于单个细胞中的RNA来测量基因表达。滑动芯片装置可以分析来自单独孔的活细菌的DNA(Ma，等，2014，PNAS，Ma，等，2014，Integrative Biology)，并且可以定量每一个孔中的RNA拷贝数。在一些实施方案中，装置可以组合这些应用以创建单个细胞基因表达测定。

[0326] 在一些实施方案中，装置可以使用报道的响应于抗生素被差别调控的基因，诸如recA和LexA(Barczak，等，2012，Proceedings of the National Academy of Sciences，6217-6222)，由OxyR和SoxS响应于抗生素诱导的氧化应激诱导的基因(Dwyer，等，2014，Proceedings of the National Academy of Sciences，E2100-E2109)，并且测量在复制起点(oriC)附近的用靶向DNA复制抗生素处理之后变得扩增的基因(Burgess，2014，Nat Rev Genet，362-362，Slager，等，2014，Cell，395-406)。

[0327] 在一个实施方案中，装置可以用于特异性定量基因表达标志物(诸如例如16S RNA和rRNA前体)，以鉴定药物易感性(Mach，等，2011，The Journal of Urology，148-153，Rolain，等，2004，Journal of Antimicrobial Chemotherapy，538-541)。在另一个实施方案中，装置可以用于在多种孵育条件和时间(诸如例如少于10min、或约10min、20min或30min)下用至少20种临床分离株建立基因表达中可检测的最小变化。

[0328] 在一些实施方案中，本文描述的技术可以优化和/或加速这些用于临床样品的耐受性定量的方法。在一些实施方案中，在本文描述的装置上进行的测定的结果将在几分钟而不是几小时内可用。在一些实施方案中，本发明使得能够实现在不同的体积量表(诸如例如样品制备至单个细胞操作、单个分子定量和手机读出)上的不同的操作集合。

[0329] 在一些实施方案中，本发明使用数字单个分子测量以解决抗微生物剂易感性并提供可以从根本上改善检测限值且可以提供定量数据的超灵敏测量，这对区分病原体与污染

以及实现药物耐受和易感生物体的更早区分是重要的。在一些实施方案中，本发明可以用于响应于抗生素的表型、代谢和基因表达谱分析测量，并且可以使用单独细菌细胞并且可以使用起源于临床环境的样品。

[0330] 在一些实施方案中，本发明在诊所中使用高通量单个细菌细胞测量以确定抗生素易感性。在一些实施方案中，本发明允许区分单独微生物或细胞(诸如例如从临床人类样品或从环境样品获得的样品)的状态，并且可以允许短暂定量其对抗生素的单独响应及因此超快速的药物易感性测量。

[0331] 在一些实施方案中，装置可以用于快速鉴定细胞、定量其载量并提供其易感性谱。在一些实施方案中，装置可以用于将细胞与药物孵育，并且然后在无污染的平台中快速提取和定量核酸，诸如例如RNA，以确定药物易感性。在一些实施方案中，本发明使得能够在CLIA临床实验室外进行微生物和细胞鉴定和药物易感性测试。

[0332] 在一些实施方案中，装置使得能够在多个步骤内处理大的样品体积，实现快速、有效的核酸提取。先前已验证了样品制备滑动芯片用于提取和纯化来自掺有沙眼衣原体(C.trachomatis)的尿液的细菌RNA和来自掺有HCV病毒颗粒的血浆的病毒RNA。这一装置可以处理0.5mL临床样品，并且从原始样品至纯化的核酸的整个工作流程可以在5分钟内完成，无需使用者干预。已验证了该装置用于分析来自掺有沙眼衣原体的尿液样品的细菌DNA、来自掺有HIV病毒颗粒的血浆的病毒RNA和来自流感病毒培养物的病毒RNA。

[0333] 在一些实施方案中，本发明包括并入核酸定量的用于确定细胞或微生物的药物耐受性的装置。在一些实施方案中，本发明包括其中将来源于与药物预孵育的生物体的样品引入以上描述的装置的装置。

[0334] 在一些实施方案中，装置测量在亚致死剂量的药物暴露之后基因诸如例如接近OriC的基因(诸如例如感受态基因)的表达或基因拷贝数的变化。在一些实施方案中，可以将基因表达诸如OriC邻近基因的表达与持家基因(household gene)或选择的基因(诸如例如定位于终止子(ter)位点处的基因)的表达进行比较。

[0335] 在一些实施方案中，装置可以评价响应于亚致死剂量的药物的细胞感受态本身作为用于药物易感性的测定。

[0336] 在一些实施方案中，装置可以通过经由随机限制(例如经由小尺寸，诸如皮升或纳升的液滴或孔)将细胞或微生物划分到小体积来测定细菌易感性，诸如例如对β-内酰胺抗生素的易感性。在一些实施方案中，这些样品取自血液或尿液或CSF。在一些实施方案中，细胞处于抗生素和荧光报告物的存在下。在一些实施方案中，装置可以检测耐药株和遗传型上耐受、但表型沉默的细胞，诸如例如通过诱导耐受表型进行检测。

[0337] 在一些实施方案中，本文的装置和方法可以用于分析样品中微生物的基因表达的时间过程。例如，该分析可以监测基因表达响应于药物的变化。例如，可以使用在用药物处理之前立即和在将药物添加至微生物之后的一个或更多个时间间隔(此类时间间隔可以包括以下的一个或更多个：约3分钟、约5分钟、约7分钟、约10分钟、约15分钟、约20分钟)的基因表达的时间点。

[0338] 在一些实施方案中，本文的装置和方法可以包括在受控温度诸如例如35℃、37℃、38℃、39℃或40℃孵育微生物。

[0339] 使用单个细胞分析将使得能够观察随机响应时间，使异常细胞(outlier cells)

能够快速响应,以提供比平均早的结果。

[0340] 在一些实施方案中,装置提供了进行单个分子和单个细胞测量以检测相对核酸浓度的小变化和对病原性细菌中抗生素处理的异质细胞响应的机制(图9)。首先,使一组细胞经历抗生素处理,然后通过两种方法定量选择的基因的表达的变化。对于单个分子数字扩增,将细胞在一起裂解,然后以高分辨率定量RNA,以检测小的总体变化。对于单个细胞测量,将细胞限制在微流体装置中的单独孔中,并且使用对每一个细胞的qPCR以利用“岗哨”细胞中的大的、早期响应。

[0341] 在一些实施方案中,装置可以用于在我们的装置中测定来自分离株和临床样品的细菌,并且评价单个细胞分析是否揭示比在混合细胞分析中可能的更早预测易感性或耐受性的“岗哨细胞”(图9)。在一些实施方案中,装置可以用于优化装置内的培养基和生长条件,以确定确保所有活力细胞已显示生长迹象所需的最小时间量。在一些实施方案中,装置可以用于设计和优化快速测定以确定药物易感性和药物耐受性,例如肺炎克雷伯菌、铜绿假单胞菌或肠外病原性大肠杆菌的抗生素易感性和耐受性。在一些实施方案中,装置可以检测异质耐受性(hetero-resistance)(其中受试样品包含易感菌株或细胞和耐受性菌株或细胞两者)。尽管异质耐受性的检测不被认为是革兰氏阴性细菌的常见临床问题,对于革兰氏阳性细菌(Musta,等,2009,Journal of Clinical Microbiology,1640-1644,Kim,等,2002,Journal of Clinical Microbiology,1376-1380,Wong,等,1999,Clinical Infectious Diseases,760-767,Ariza,等,1999,The Lancet,1587-1588,Editors,2001,Journal of Medical Microbiology,1018-1020)和一些革兰氏阴性细菌(Pournaras,等,2007,Journal of Medical Microbiology,66-70)的该现象被充分地记载,并且该现象与患者治疗和结果相关。在一些实施方案中,当观察到异质耐受性时,本文公开的装置可以用于鉴定样品类型的异质耐受性,使得它们可以被靶向以用于诸如例如通过单个细胞平台分析。

[0342] 在一些实施方案中,装置可以用于孵育微生物或细胞,诸如例如将肺炎克雷伯菌的分离株与例如头孢唑啉、头孢曲松、环丙沙星、哌拉西林-他唑巴坦、甲氧苄啶-磺胺甲噁唑和美罗培南的抗生素样品孵育以及将铜绿假单胞菌与环丙沙星、哌拉西林-他唑巴坦和美罗培南孵育。

[0343] 在一些实施方案中,可以集成装置,使得例如样品制备滑动芯片和数字滑动芯片被集成以改善工作流程/效率并避免污染。两个芯片的集成可以防止RNA降解并消除污染且简化工作流程。在一些实施方案中,可以集成装置,使得例如装置将鉴定装置和AST装置组合以鉴定病原体、其载量及其抗生素易感性(图10)。

[0344] 在一些实施方案中,本发明鉴定和定量细菌并且评价抗微生物剂易感性。在一些实施方案中,平台借助了见于大多数实验室的现有热循环仪,使得甚至是最小的临床实验室也能以“开放”格式实现低成本的数字PCR能力,所述“开放”格式将允许扩展测试项目单(menu)。在装置的一些实施方案中,样品制备可以在不要求外部功率的宏观流体的一次性芯片上进行。

[0345] 在一些实施方案中,本发明将定量和评价抗微生物剂易感性的能力并入CLIA豁免结构(CLIA-waived structure),以便在初级护理和全球健康设施中提供对该技术的访问。在一些实施方案中,本发明使用等温酶,其已经在滑动芯片平台上被充分地研究,并且可以

允许在一系列环境条件下进行无设备测试。另外，在一些实施方案中，结果是手机可读的，并且可以被受训练最少的使用者获得。

[0346] 在一些实施方案中，可以修改该装置以与血液一起使用，并且还可以在测试套件中包括鲍曼不动杆菌(Acinetobacter baumannii)。在一些实施方案中，装置可以与低丰度样本(specimens)一起使用并且具有RT PCR能力，这允许甚至在低丰度样本中也能更准确地测试抗微生物剂易感性。这些能力在医院设施中提供了显著的临床价值。

[0347] 在一些实施方案中，装置可以通过物种鉴定和定量快速诊断感染，诸如例如尿路感染(UTI)，并且在一些实施方案中，装置还可以表征导致感染的物种的药物耐受性。因此，装置可以显著地增强临床医师提供抗生素管理(stewardship)的能力，并且当UTI不存在时或当细菌计数不批准疗法时也避免不适当的治疗的能力。

[0348] 在一些实施方案中，装置可以定量在样品收集时存在的细菌的数目，以确定细菌载量是否批准疗法，因为在较低的细菌浓度医师通常不进行治疗。在一些实施方案中，还可以对装置进行验证。来自装置的细菌浓度可以与使用用于细菌检测和表征的目前“金标准”培养方法获得的菌落计数相关联。在一些实施方案中，装置可以用于使用数字芯片格式上的核酸检测来鉴定存在哪些细菌。

[0349] 在一些实施方案中，装置可以用于评价鉴定的生物体的抗微生物剂耐受性，以帮助指导疗法的选择。在一些实施方案中，样品诸如尿液样品可以与一系列药物诸如抗生素一起孵育，并且基因表达测试可以确定哪种药物方案最适合于治疗感染(例如通过鉴定细菌对哪种药物易感)。

[0350] 在一些实施方案中，装置可以以大于99％的特异性鉴定细菌物种并且以优于3倍的分辨率定量尿液中在100CFU/mL至1,000,000CFU/mL的动态范围内的细菌载量。

[0351] 在装置和方法的一些实施方案中，工作流程允许在少于95分钟、少于30分钟、少于15分钟、少于5分钟内针对一种或更多种药物的组，对一种或更多种物种或菌株或细胞类型进行快速AST(抗微生物剂易感性测试)。

[0352] 在一些实施方案中，装置是能够进行样品制备和数字定量的集成装置，使得其可以用于定量细菌载量和定量AST的基因表达两者，具有至少80％的核酸回收率。

[0353] 在一些实施方案中，装置能够实现组合鉴定装置和AST装置的工作流程以鉴定生物体、定量其载量并确定其抗生素易感性。

[0354] 与现有Qiagen样品制备试剂盒相比，用于从尿液的样品制备的装置具有与标准核酸相比的至少80％的核酸回收率。在一些实施方案中，样品制备可以在少于5min的时间内自动地进行而无需离心，而Qiagen方法花费30min。

[0355] 在一些实施方案中，与培养相比，细菌物种鉴定可以以对生物体大于99％的特异性进行。

[0356] 在一些实施方案中，与常规培养方法相比，可以以优于3倍的分辨率进行诸如尿液中在100CFU/mL至1,000,000CFU/mL的动态范围内的活细菌载量的定量。

[0357] 在一些实施方案中，本发明能够实现允许在少于95min内针对一种药物或者两种或更多种抗生素的组，对一种或多种物种进行快速AST(抗微生物剂易感性测试)的工作流程。

[0358] 在一些实施方案中，本发明能够实现组合制备模块或装置、鉴定和定量模块或装

置和AST模块或装置的工作流程以在少于2.5小时、少于2小时、少于1小时、少于30分钟内鉴定生物体、定量其载量和/或确定其抗生素易感性。

[0359] 在一些实施方案中，装置以与通常用于参考实验室的设备等同的质量提供基于CLIA和有限资源设施(LRS)中核酸或蛋白的多重的和定量的诊断测量。

[0360] 在一些实施方案中，平台是“开放的”，使得可以添加用于其他/另外的细菌的试剂并扩大测量范围。

[0361] 在一些实施方案中，装置能够实现具有10^5的动态范围的HCV和HIV病毒载量的多重和数字的基于核酸的定量测量。在一些实施方案中，装置能够实现病原体(包括细菌和真菌)的多重检测。

[0362] 在一些实施方案中，装置是微流体装置诸如滑动芯片，其能够实现具有一个或更多个以下特征的样品制备：满足或超过用于低成本制造的商业标准的装置、最终将不要求使用者干预的装置、最终将不要求电力来操作的装置、在少于5min内能够实现HCV、衣原体和淋病和流感的样品制备的样品制备模块或装置、使用便宜的塑料并且适合于大量产生(mass-production)技术的装置、可以由未经训练的孩子进行操作和成像的装置、以及可以使用自动的基于云的分析和数据传输来进行操作和成像的装置。

[0363] 在一些实施方案中，装置可以以大于99％的特异性鉴定细菌物种并且以优于3倍的分辨率定量尿液中在100CFU/mL至1,000,000CFU/mL的动态范围内的细菌载量。

[0364] 在一些实施方案中，装置可以鉴定和定量地测量来自生物体诸如例如来自临床尿液样品的肺炎克雷伯菌、铜绿假单胞菌和肠外病原性大肠杆菌的细菌DNA和RNA，并且可以适于也提供细菌载量的定量测量。

[0365] 在一些实施方案中，装置是用于鉴定和定量从掺有肺炎克雷伯菌、铜绿假单胞菌和大肠杆菌的尿液样品提取的细菌DNA和RNA的数字微流体装置，诸如数字滑动芯片。在一些实施方案中，装置可以对生物体或细胞的DNA/RNA扩增具有高于99％的特异性，并且以3倍分辨率提供在1,000个至$1x10^7$拷贝/mL的动态范围内的提取的DNA/RNA的定量。

[0366] 在一些实施方案中，装置是可以用于通过定量16S rRNA来定量尿液样品中的活细菌载量的数字微流体装置，诸如数字滑动芯片。在一些实施方案中，装置可以并入快速孵育步骤(少于10min)，并且可以评价在多种存储条件下对不同样品类型的基因表达的重新归一化。

[0367] 在一些实施方案中，装置是能够定量使用标准实验台方法纯化的RNA标志物以定量活细菌载量的数字微流体装置，诸如数字滑动芯片。在一些实施方案中，装置是可以具有对参考菌株和肺炎克雷伯菌、铜绿假单胞菌和大肠杆菌的临床分离株高于80％的DNA和RNA两者的回收率的样品制备装置，诸如样品制备滑动芯片。在一些实施方案中，装置可以回收适于数字装置应用和实时qPCR两者的纯化的DNA和RNA的质量。

[0368] 在一些实施方案中，与标准实验台方法相比，装置可以在少于5分钟内，以高于80％的回收率提取和纯化来自掺有肺炎克雷伯菌、铜绿假单胞菌和大肠杆菌的参考菌株的尿液样品中的细菌DNA和RNA。

[0369] 在一些实施方案中，方法采用具有多个功能的集成装置，并且在其他实施方案中，方法采用非集成装置，诸如用于鉴定和定量的单独的样品制备装置和数字装置。在一些实施方案中，用于鉴定的集成的样品制备和数字装置的组合以大于99％的特异性进行，并且

能够以3倍分辨率定量在100CFU/mL至1,000,000CFU/mL的动态范围内的细菌载量。

[0370] 在一些实施方案中,装置可以通过将样品与药物孵育短的时间段,然后定量RNA表达标志物,对生物体诸如肺炎克雷伯菌、铜绿假单胞菌和大肠杆菌进行快速抗微生物剂易感性测试(AST)。在一些实施方案中,装置可以提供样品,以符合少于2.5小时或少于2小时或少于1小时的周转。在一些实施方案中,装置可以与临床分离株或临床样品一起使用。

[0371] 在一些实施方案中,装置可以定量在100个拷贝/mL至10,000,000个拷贝/mL的动态范围内的基因表达标志物。在一些实施方案中,装置可以用于鉴定在相同动态范围内的可检测的最小差异,并且这将为用于特定细胞或微生物的孵育时间和抗微生物剂易感性测试提供指导。

[0372] 在一些实施方案中,装置可以用于在氨苄青霉素的存在或不存在下将样品与大肠杆菌的至少20种临床分离株孵育。在装置的一些实施方案中,可以制备细菌RNA并且可以定量一个或更多个RNA标志物。在装置的一些实施方案中,结果将具有与标准临床方法至少95%的一致性。

[0373] 在一些实施方案中,装置可以用于将大肠杆菌与药物诸如抗生素,包括头孢唑啉、头孢曲松、环丙沙星、哌拉西林-他唑巴坦、甲氧苄啶-磺胺甲噁唑和美罗培南孵育。在装置的一些实施方案中,可以制备细菌RNA,并且使用数字方法且在少于95min、少于60分钟、少于30分钟、少于15分钟的时间帧内定量RNA标志物的表达水平。在一些实施方案中,装置可以用于在头孢唑啉、头孢曲松、环丙沙星、哌拉西林-他唑巴坦、甲氧苄啶-磺胺甲噁唑和美罗培南的存在和不存下孵育掺有大肠杆菌的至少20个临床分离株的阴性尿液。在一些实施方案中,装置可以以小于5%的大错误(major error)和无严重错误(very major error)的工作流程性能来定量RNA标志物的表达水平。

[0374] 在一些实施方案中,装置可以在临床相关药物组的药物的存在和不存在下孵育至少一种临床分离株的样品。在一些实施方案中,装置可以定量细菌DNA中的RNA标志物的表达水平以进行AST并且具有小于5%的大错误且无严重错误。

[0375] 在一些实施方案中,装置集成了样品制备和数字能力。装置的集成可以防止降解并消除污染,并且能够以高于99%的特异性且在少于55分钟、少于30分钟、少于15分钟内实现核酸提取、纯化和数字定量。

[0376] 在一些实施方案中,装置可以以高于99%的特异性且在少于55分钟内提供诸如来自掺有肺炎克雷伯菌(ATCC 700603)、铜绿假单胞菌(ATCC27853)和大肠杆菌(ATCC 25922)的尿液样品的细菌DNA和RNA的定量测量。在一些实施方案中,装置或集成装置可以以大于3倍的分辨率提供在100CFU/mL至1,000,000CFU/mL的动态范围的细菌载量的定量。

[0377] 在一些实施方案中,装置可以用于定量在选择的药物的存在和不存在下孵育的至少20种、至少50种或至少100种临床分离株的基因表达水平。在一些实施方案中,包括细菌载量和AST的装置性能与CLSI参考方法是可比较的。在一些实施方案中,性能错误是小于5%的小错误(minor error)、小于2.5%的小错误、小于1%的小错误。

[0378] 在一些实施方案中,性能具有小于1%的大错误、小于0.5%的大错误、小于0.1%的大错误。在一些实施方案中,装置不具有严重错误。

[0379] 试剂盒

[0380] 试剂盒可以包括滑动芯片装置和选择用于参与核酸扩增的一批试剂。在一些实施

方案中，试剂可以被设置在适于与第一组分的导管、第二组分的导管或两者接合的容器中。此类容器可以是移液管、注射器等。在一些实施方案中，试剂盒包括加热器。

[0381] 装置的一些实施方案可以用于使用核酸标记物检测不同的生物靶，诸如，例如蛋白、细菌、病毒、感染因子(infectious agent)等等。在一些实施方案中，靶用可以用于检测的寡核苷酸进行加标签。寡核苷酸标签可以使用许多不同的核酸扩增策略(诸如例如，PCR、LAMP、RPA、NASBA、RCA等等)的任一种进一步扩增。寡核苷酸标签也可以使用例如如Chen示出的荧光探针来可视化(Huang、Suxian、和Yong Chen.“Polymeric Sequence Probe for Single DNA Detection.”Analytical chemistry 83.19(2011):7250-7254.)。

实施例

[0382] 仅为了说明性目的提供这些实施例，而不是限制本文提供的权利要求的范围。

[0383] 实施例1:滑动芯片的形成

[0384] 使用钠钙玻璃(soda lime glass)制造期望的玻璃滑动芯片的程序是基于先前的研究。两步暴露蚀刻方案适于创建两种不同深度的孔(热膨胀孔为5μm，所有其他孔为55μm)。在蚀刻之后，玻璃板用食人鱼酸(piranha acid)和DI水彻底清洗，并用氮气干燥。然后将玻璃板在等离子体清洁器中氧化10分钟，并立即转运至干燥器中持续1小时硅烷化。将它们用氯仿、丙酮和乙醇彻底冲洗，并且用氮气干燥，然后使用。

[0385] 塑料聚碳酸酯滑动芯片装置在从microfluidic ChipShop GmbH接收到之后，在等离子体清洁器中直接氧化15分钟，并且然后转运至干燥器中持续90分钟硅烷化。将它们在65℃下在十四烷(tetradecane)中浸泡15分钟，并且然后用乙醇彻底冲洗，然后用氮气干燥，然后使用。塑料滑动芯片装置不被重复使用。

[0386] 将滑动芯片在脱气油(矿物油:十四烷1:4v/v;Fisher Scientific)下组装。将顶部板和底部板两者浸入油相中并面对面放置。将两个板在立体镜(Leica,Germany)下对准并使用粘合剂夹(binder clip)固定。在顶部板上钻了两个通孔(through-hole)以用作为流体入口。试剂溶液通过移液经由入口来加载。

[0387] 实施例2:滑动芯片中的数字扩增。

[0388] 根据Shen等2010)描述的方案在滑动芯片上进行数字PCR。Feng Shen,Wenbin Du,Jason E.Kreutz,Alice Fok和Rustem F.Ismagilov,"Digital PCR on a SlipChip,"Lab Chip 2010 10:2666-2672。

[0389] 简言之，在这种公布的方法中，经由光刻和HF蚀刻将顶部和底部滑动芯片板蚀刻出微流体通道和孔。将顶部板和底部板在20％矿物油/80％十四烷的混合物中对准，并且然后夹紧以保持在适当位置。将来自以上描述的提取物的DNA添加至PCR主混合物中。将该溶液用移液管(pipette)加载到滑动芯片装置中。在加载之后，滑动将通道分成1280个独立的3nL隔室。然后，将滑动芯片夹紧、密封并放置于热循环仪上用于以下温度:92℃持续3分钟，40个以下循环:92℃持续20秒、62℃持续20秒，以及72℃持续25秒。对滑动芯片进行成像，计数阳性和阴性孔，并使用泊松统计计算靶DNA的浓度。

[0390] 实施例3:检测处理/未处理的易感大肠杆菌之间rDNA随时间的差异

[0391] 在一个实施例中，将来自患有尿路感染的患者的环丙沙星敏感的大肠杆菌分离株在Bacto脑心浸液(BHI)培养基中预培养至高细胞密度，然后稀释并用和不用2.5ug/mL环丙

沙星处理10分钟、20分钟和30分钟。来自处理的和未处理的样品的DNA用Epicentre QuickExtract DNA提取缓冲液提取。定量PCR用对23S靶rDNA基因特异性的引物进行。使用以下对23S rDNA特异性的引物：

[0392] 5′-TGCCGTAACTTCGGGAGAAGGCA-3′(SEQ ID NO:1)

[0393] 5′-TCAAGGACCAGTGTTCAGTGTC-3′(SEQ ID NO:2)

[0394] 在10分钟、20分钟和30分钟时，测量到处理的(在每一个时间点的右侧柱)与未处理的(在每一个时间点的左侧柱)样品之间rDNA拷贝数的1.37、1.58和1.70倍变化(图11)。

[0395] 实施例4：通过qPCR和数字PCR检测处理的/未处理的易感和耐受大肠杆菌之间的rDNA随时间的差异

[0396] 在一个实验中，将来自患有尿路感染的患者的环丙沙星易感的大肠杆菌分离株在Bacto脑心浸液培养基中预培养至高细胞密度，然后稀释并用和不用2.5ug/mL环丙沙星处理30分钟。在0分钟、15分钟和30分钟时，来自处理的和未处理的样品的DNA用Epicentre QuickExtract DNA提取缓冲液提取。用如实施例3中提供的对靶23S rDNA基因特异性的引物对提取的DNA进行定量PCR。在15分钟时，测量到来自易感分离株的处理的(在每一个时间点的右侧柱)和未处理的(在每一个时间点的左侧柱)样品之间rDNA拷贝数的1.27倍变化(图12A)。

[0397] 在滑动芯片中用如实施例3中提供的对靶23S rDNA基因特异性的引物对提取的DNA进行数字。一个芯片包含来自未处理的细菌的DNA，同时另一个芯片包含来自用抗生素处理的细菌的DNA。比较浓度并计算差异倍数。数字PCR检测到rDNA拷贝数的1.5倍变化。对耐受分离株未观察到rDNA拷贝数的显著变化(图12B)。

[0398] 对来自患有尿路感染的患者的也对环丙沙星易感的不同的大肠杆菌分离株重复该实验。在15分钟时，qPCR测量到rDNA拷贝数的1.8倍变化，且数字PCR检测到rDNA拷贝数的1.5倍变化。

[0399] 实施例5：通过数字PCR检测处理的/未处理的易感大肠杆菌之间的rDNA随时间的差异

[0400] 在一个实验中，将来自患有尿路感染的患者的环丙沙星易感的大肠杆菌分离株在混合的人类尿液中预培养，然后稀释并用有和没有0.75ug/mL环丙沙星的1:1BHI:尿液处理15分钟。来自处理的和未处理的样品的DNA用Epicentre QuickExtract DNA提取缓冲液提取。用如实施例3中提供的对靶23S rDNA基因特异性的引物对提取的DNA使用定量PCR测量到处理的和未处理的样品之间rDNA拷贝数的2.61倍变化。还使用如实施例3中提供的对靶23S rDNA基因特异性的引物对提取的DNA进行数字PCR。数字PCR检测到rDNA拷贝数的2.4倍变化。数字PCR数据的结果示于图13中。

[0401] 实施例6：检测用四环素和甲氧苄啶/磺胺甲噁唑处理的/未处理的易感和耐受大肠杆菌的差异

[0402] 在一个实验中，将来自患有尿道感染的患者并且对多种抗生素易感的大肠杆菌分离株在BHI或尿液中预培养，然后稀释，并且用有和没有抗生素的1:1BHI:尿液处理持续多种处理时间。处理的和未处理的样品用Epicentre QuickExtract DNA提取缓冲液提取。用如实施例3中提供的对靶23S rDNA基因特异性的引物使用定量PCR测量处理的和未处理的样品之间rDNA拷贝数的可检测倍数变化。在四环素处理下，在30分钟时观察到rDNA拷贝数

的1.55倍变化(图14A),在呋喃妥因处理下,在15分钟时观察到rDNA拷贝数的1.62倍变化,且在甲氧苄啶/磺胺甲噁唑处理下,在45分钟时观察到rDNA拷贝数的1.35倍变化(图14B)。

[0403] 实施例7:比较qPCR和数字PCR检测耐受和易感细胞之间的差异

[0404] 在该实施例中,示出了数字扩增相比于qPCR增强的分辨率。具体地,与未处理的细菌相比,用抗生素处理的细菌中发现的核酸的差异可以用数字核酸定量(包括PCR或LAMP)来分辨,而qPCR方法(或qLAMP)可能无法可靠地分辨差异和/或可能在不使用更多重复的情况下无法可靠地分辨差异。

[0405] 将来自患有尿路感染的患者的环丙沙星易感的大肠杆菌分离株在混合的人类尿液中预培养,然后稀释并用有和没有0.75ug/mL环丙沙星的1:1BHI:尿液处理30分钟。来自处理的和未处理的样品的DNA用Epicentre QuickExtract DNA提取缓冲液提取。

[0406] 用如实施例3中提供的引物、用对靶32S rDNA基因特异性的引物对每一个样品进行定量PCR(每样品3次重复),并且获得了以下Cq:处理的--22.84+/-0.53;未处理的--22.13+/-0.07。使用Weaver等描述的方法"Taking qPCR to a higher level:Analysis of CNV reveals the power of high throughput qPCR to enhance quantitative resolution,"Methods 201050:271-276,rDNA拷贝数的1.6倍差异不是统计学上显著的,除非对每一个样品进行10次qPCR重复而不是3次。

[0407] 将相同的样品以1:10稀释并加载到滑动芯片中用于数字扩增。一个芯片包含来自未处理的细菌的DNA,同时另一个芯片包含来自用抗生素处理的细菌的DNA。使用实施例3中提供的引物对提取的DNA进行数字PCR,以扩增23S rDNA。

[0408] 测量结果,并且使用泊松统计计算以下浓度:处理的--148,031+/-7,103个拷贝/mL;未处理的---84,964+/-5,171个拷贝/mL。使用Kreutz等在"Theoretical Design and Analysis of Multivolume Digital Assays with Wide Dynamic Range Validated Experimentally with Microfluidic Digital PCR,"Analytical Chemistry 2011 83:8158-8168中描述的统计方法,计算出具有2e-13的p值的统计学上显著差异。

[0409] 因此,数字PCR成功地分辨了处理的与未处理的细菌DNA,而qPCR无法以典型的重复数(3)分辨浓度差异。

[0410] 与该实验一起描述的统计方法更普遍适用。从qPCR实验,Cq中0.16或更大的标准偏差是典型的。为了以该标准偏差在两个样品之间实现1.25倍的分辨率,qPCR可以要求18次重复(Weaver等(2010))。对于相同的分辨率(1.25),只要数字PCR实验中的隔室数目大于1200(同前),就可以进行一次数字PCR实验。

[0411] 实施例8:基因相对于复制起点的靶映射

[0412] 在与UTI患者相关的16种可用的基因组测序的大肠杆菌菌株的子集中将feoB、recA和DnaA抑制物蛋白基因的位置相对于复制起点进行映射。结果显示出FeoB基因邻近复制起点定位的趋势,而recA和DnaA抑制物蛋白基因被定位于距离复制起点更远(图15)。作为复制起始事件的结果,预期feoB基因连同在与feoB基因距离复制起点相似的距离处定位的接近复制起点定位的所有其他基因(例如,malB基因(GDB J01648))一起在早期进行复制。

[0413] 实施例9:DNA标志物在不同时间点的染色体复制数据

[0414] 在该实验中,一种易感UTI大肠杆菌分离株和一种耐受UTI大肠杆菌分离株用

2.5ug/m环丙沙星处理15分钟。它们的核酸用QuickExtra DNA提取试剂盒(Epicenter)提取,并且用rDNA(23S)、FeoB基因、DnaA抑制蛋白基因和RecA基因特异性引物经由qPCR定量DNA。

[0415] 图16示出了在BHI培养基中暴露(“处理的”)于2.5ug/ml环丙沙星持续15分钟之后的易感和耐受的细菌中,用于定量UTI临床分离株大肠杆菌基因组中的DNA片段拷贝数的循环阈值(Ct)。表2提供了易感和耐受的处理的/未处理的样品之间的每一个靶的循环阈值和变化倍数。

[0416] 表2

[0417]

	23S CT 未处理的/处理的	23S 变化倍数	FeoB 未处理的/处理的	FeoB 变化倍数	dnaAinh CT 未处理的/处理的	dnaAinh 变化倍数	recA CT 未处理的/处理的	recA 变化倍数
易感的	20.19/20.84	1.57	23.3/23.71	1.33	23.93/23.97	1.03	24.81/24.65	0.89(多达1.13)
耐受的	20.22/20.13	0.94(多达1.06)	23.19/23.1	0.94(多达1.06)	23.68/23.89	1.16	27.19/27.05	0.91(多达1.10)

[0418] 在易感菌株中,与未用抗生素处理的细胞相比,用环丙沙星处理的细胞显示出23S DNA的1.57倍减少、FeoB DNA的1.33倍减少、dnaAinh DNA无变化和recA的1.13倍增加。

[0419] 在耐受菌株中,与未用抗生素处理的细胞相比,用环丙沙星处理的细胞显示出23S DNA和FeoB DNA无变化、dnaAinh DNA的1.16倍减少和recA的1.10倍增加。

[0420] 在该实施例中,与耐受分离株相比,在易感分离株的基因组中观察到较少的rDNA(23S DNA)拷贝和较少的FeoB基因拷贝;同时对于被定位于距离复制起点更远的其他基因片段-dnaA抑制物基因和recA基因,药物处理的和未处理的细菌之间不存在统计学上的严格差异。

[0421] 实施例10-用于确定药物耐受性或易感性的靶mRNA

[0422] 我们的药物易感性测定使用几个感兴趣的靶进行验证。这些包括对环丙沙星耐受的分离株中的靶FeoB和RecA RNA。

[0423] Feo B表达在环丙沙星存在下被下调。尽管以低水平被下调,但它在是易感(未分裂或死亡)的细菌中被下调,因此与未处理的或耐受细胞相比,这将确保良好的分辨率。

[0424] 在该实施例中,在BHI培养基中用2.5ug/mL环丙沙星处理20min之后,FeoB和RecA RNA水平用于确定对环丙沙星临床分离株易感或耐受的细胞。在该实施例中,用FeoB和RecA基因特异性引物通过qRTPCR进行RNA定量。在该实施例中,在抗生素处理之后,FeoB表达在易感分离株中被下调。同一实验中的RecA表达在易感分离株中未显著被下调。

[0425] 在抗生素处理20分钟之后,feoB和recA的RNA比率允许区分易感和耐药UTI大肠杆菌分离株。

[0426] 在一个实施例中,将来自过夜培养物的一种易感UTI大肠杆菌分离株和一种耐受UTI大肠杆菌分离株以1:100在温热的BHI培养基中稀释(在1mL中10μL),并在37℃生长持续

～3-4小时。取每一个孔混合培养物的两个样品用于抗生素暴露测定。将它们添加至预热的1.7mL VWR微量离心管中，该1.7mL VWR微量离心管中包含A)温热(37℃)BHI培养基(对照)或B)具有以2.5μg/mL浓度添加的环丙沙星的温热(37℃)BHI培养基；用于所有管的架也被预热至37℃以使温度变化最小化。将对照和包含抗生素的样品两者在37℃孵育20分钟。之后，将样品A和B立即放置于冰上，并以1/5在RLT缓冲液(Qiagen)中稀释。它们的总核酸使用Qiagen RNAeasy微型试剂盒柱和缓冲液来提取，并且在0.3U/μL RNA酶抑制物Superase In(Ambion)和1μg/μL BSA(Roche)的存在下使用Maxima H-RT和SSO快速EvaGreen PCR混合物经由一步RT qPCR来分析以定量FeoB和Rec A RNA。在以下图中，从耐受分离株获取的样品被标记为UTI R，且从易感样品获取的样品被标记为UTI S。在环丙沙星的存在下孵育的样品在其名称中添加"cipro"。

[0427] 图17A和图17B示出了对比于在无抗生素的BHI培养基中20分钟，在BHI培养基中用2.5ug/mL环丙沙星处理20分钟之后，对环丙沙星易感(S)和耐受(R)的UTI临床分离株中FeoB和RecA基因表达的RNA定量。相对于暴露于环丙沙星的易感细胞，未处理的易感细胞显示出FeoB靶的表达的4.86倍增加，观察为2.28的循环差异。耐受细胞中未观察到基因表达的显著变化。耐受细胞和易感细胞两者均未显示出recA表达的显著变化。

[0428] 实施例11-环丙沙星对FeoB和RecA的RNA表达的影响

[0429] 获得来自UTI患者的临床分离株并且在TSA血液琼脂培养基上进行恢复。将来自血液琼脂平板的菌落接种到3mL BHI培养基中，生长过夜。将来自过夜培养物的一种易感UTI大肠杆菌分离株和一种耐受UTI大肠杆菌分离株以1:100在温热的BHI培养基中稀释(在1mL中10μL)，在37℃生长持续约3小时。取每一个孔混合培养物的两个样品用于抗生素暴露测定。将它们添加至预热的1.7mL VWR微量离心管中，该1.7mL VWR微量离心管中包含A)温热(37℃)BHI培养基(对照)或B)具有以10μg/mL浓度添加的环丙沙星的温热(37℃)BHI培养基；用于所有管的架也被预热至37℃以使温度变化最小化。将对照和包含抗生素的样品两者在37℃孵育20分钟。之后，将样品A和B立即放置于冰上，并将30μL的每一个样品添加至130μL冷的RLT缓冲液(Qiagen)中。它们的总核酸使用Qiagen RNAeasy微型试剂盒柱和缓冲液来提取，并且在RNA酶抑制物和BSA的存在下使用Maxima H-RT(和SSO快速EvaGreen PCR混合物经由一步RT qPCR来分析以定量FeoB和RecA RNA。

[0430] 图18示出了对比于在无抗生素的BHI培养基中20分钟，在BHI培养基中用10ug/mL环丙沙星处理20分钟之后，对环丙沙星易感和耐受的UTI临床分离株中的FeoB和RecA基因表达的RNA定量。将结果提供于表3中。

[0431] 表3

[0432]

	FeoB		RecA		
	CT 未处理的/处理的	下调的变化倍数	CT 未处理的/处理的	上调的变化倍数	变化倍数比率
易感的 1	20.88/24.45	11.88	20.87/18.11	6.77	80.45
易感的 2	23.99/26.21	4.66	23.00/20.21	6.91	32.22
易感的 3	22.28/24.73	5.46	22.08/19.42	6.32	34.54
耐受的	20.45/20.53	1.06	22.54/22.57	0.98	1.04

[0433] 相对于未用环丙沙星处理的易感细胞，处理的易感细胞显示出FeoB基因的显著下调。三种不同的易感分离株显示出该RNA在细胞中的存在的11.88倍、4.66倍、和5.46倍减少。耐受分离株未显示出显著变化。相对于未用环丙沙星处理的易感细胞，处理的易感细胞显示出RecA基因的显著上调。三种不同的易感分离株显示出该RNA在细胞中的存在的6.77倍、6.92倍、和6.32倍增加。耐受分离株未显示出显著变化。这些变化中的任一个都可以单独地用于区分易感细胞与耐受细胞，然而，采取FeoB与RecA的变化比率提供在耐受和易感细胞之间甚至更大的分辨率。受试的易感菌株显示出了80.45倍、32.22倍、和34.54倍的变化比率，而在耐受细胞中，该比率为1.04。

[0434] 虽然本文已经显示和描述了本发明的优选实施方案，但对于本领域技术人员将明显的是，这类实施方案仅通过示例的方式提供。本领域技术人员现在将想到许多变型、变化和替换，而不偏离本发明。应当理解，在实践本发明时可以采用本文描述的本发明的实施方案的各种替代选择。意图是，以下权利要求界定本发明的范围，并且从而涵盖在这些权利要求范围内的方法和结构及其等同物。

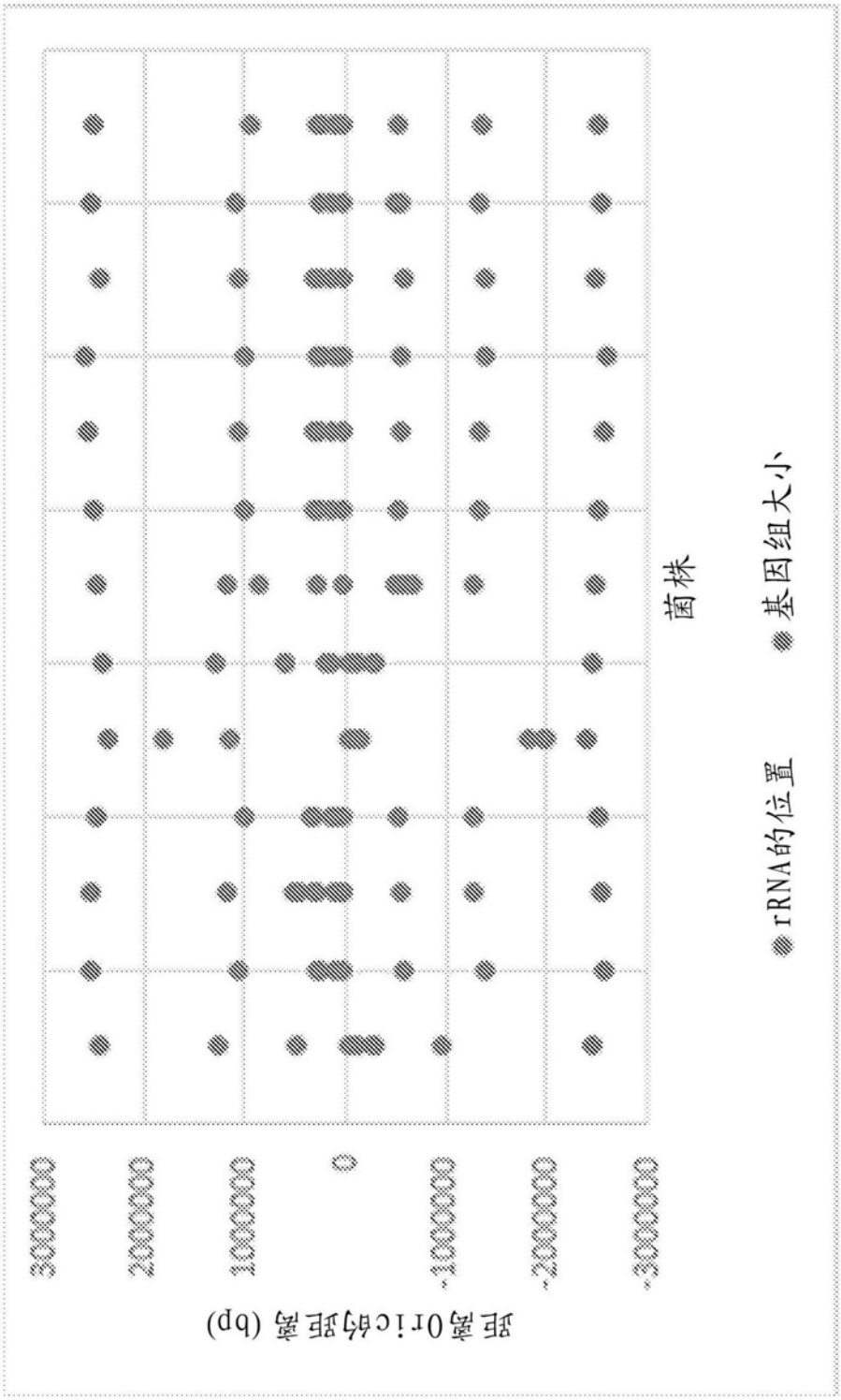

距离Ori的距离 (bp)
3000000
2000000
1000000
0
-1000000
-2000000
-3000000
菌株
rRNA的位置
基因组大小

图1

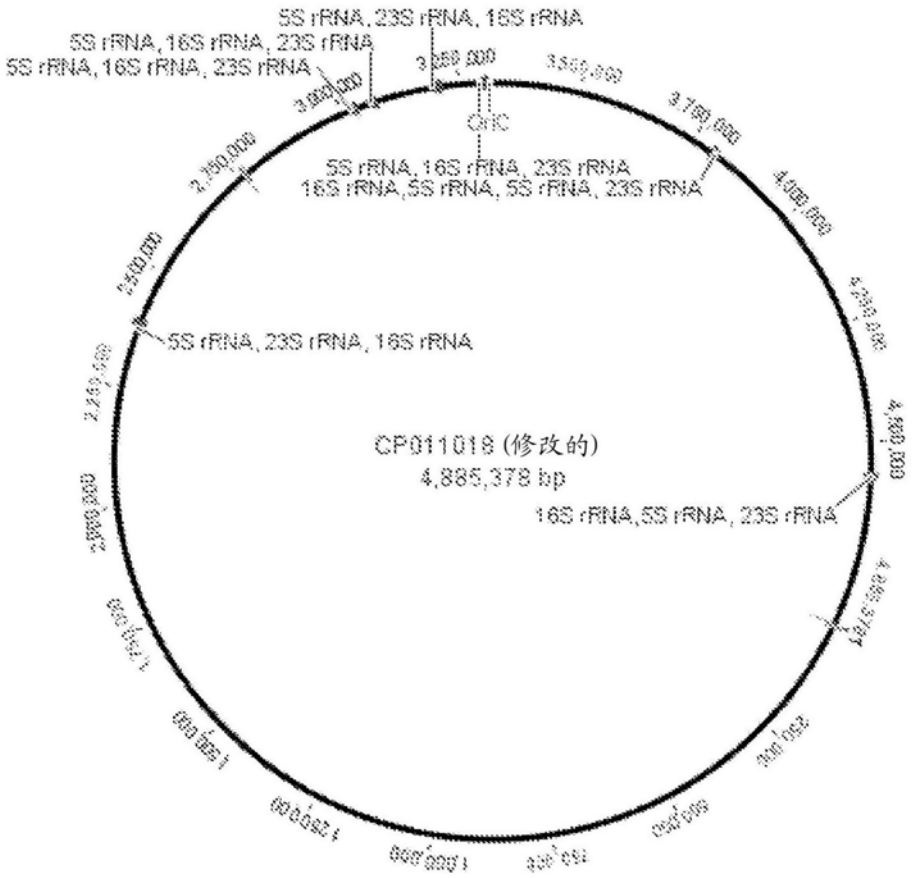

5S rRNA, 23S rRNA, 16S rRNA
5S rRNA, 16S rRNA, 23S rRNA
5S rRNA, 16S rRNA, 23S rRNA
OriC
5S rRNA, 16S rRNA, 23S rRNA
16S rRNA, 5S rRNA, 5S rRNA, 23S rRNA
5S rRNA, 23S rRNA, 16S rRNA
CP011018 (修改的)
4,885,378 bp
16S rRNA, 5S rRNA, 23S rRNA

图2

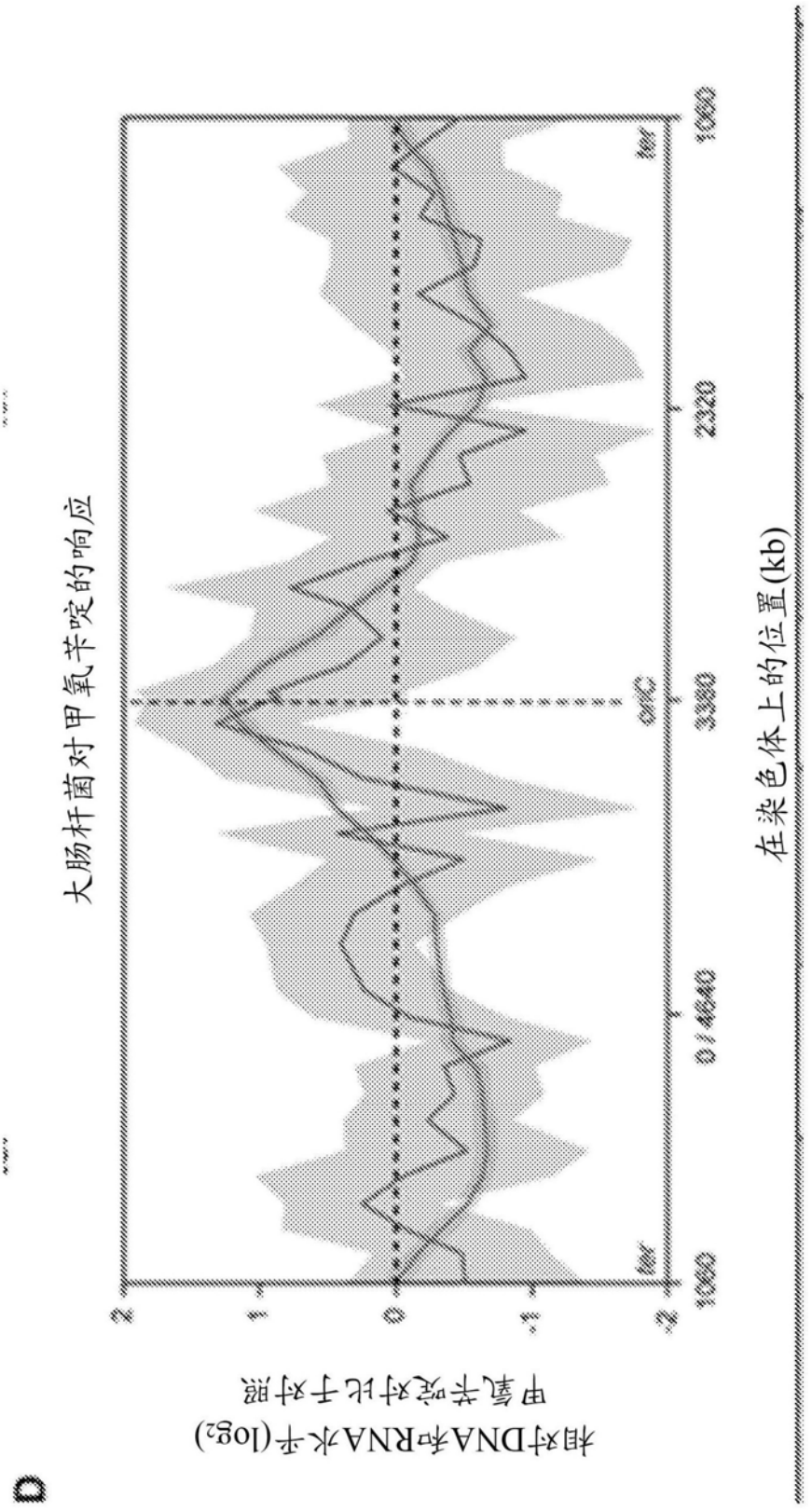

D
大肠杆菌对甲氧苄啶的响应
相对DNA和RNA水平(log2)
甲氧苄啶对比于对照
在染色体上的位置(kb)
ter
oriC
ter
1060
0 / 4640
3380
2320
1060
2
1
0
-1
-2

图3

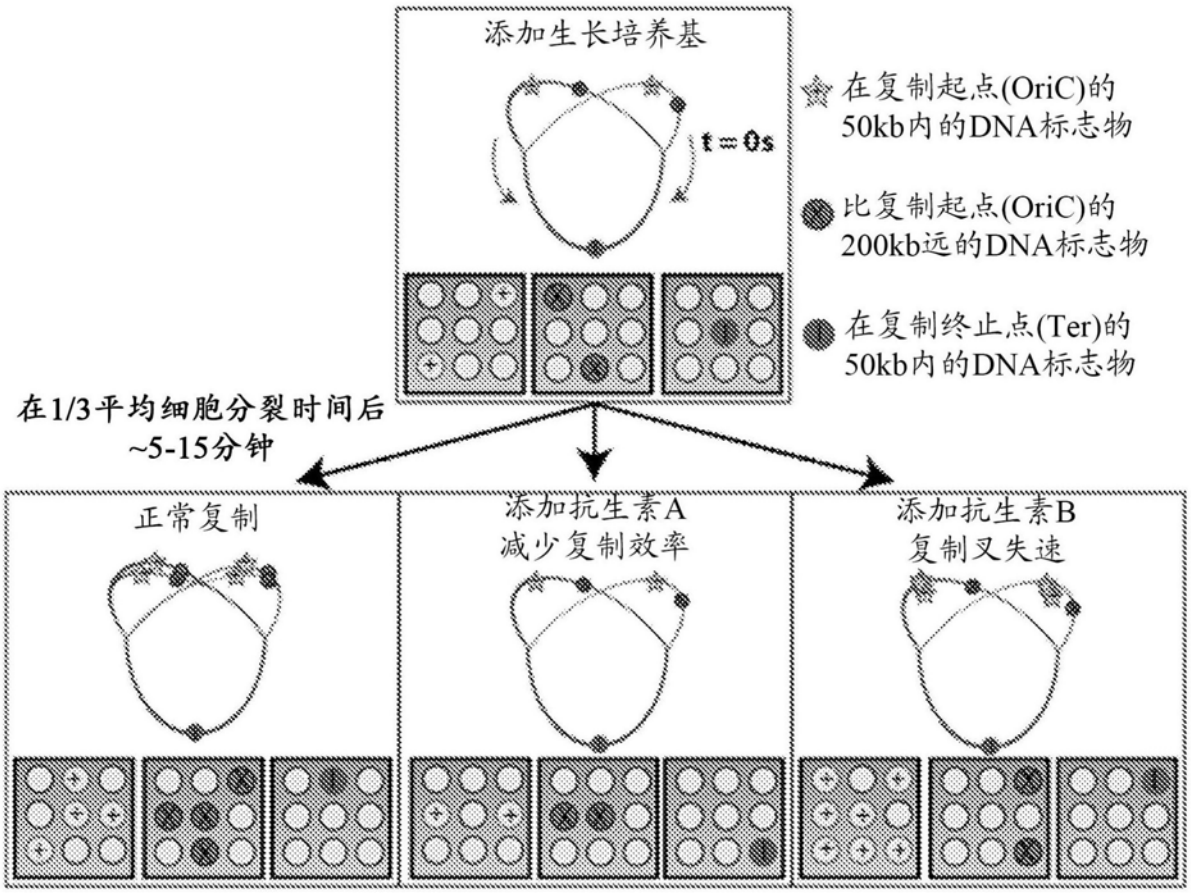

添加生长培养基
t = 0s
在复制起点(OriC)的
50kb内的DNA标志物
比复制起点(OriC)的
200kb远的DNA标志物
在复制终止点(Ter)的
50kb内的DNA标志物
在1/3平均细胞分裂时间后
~5-15分钟
正常复制
添加抗生素A
减少复制效率
添加抗生素B
复制叉失速

图4

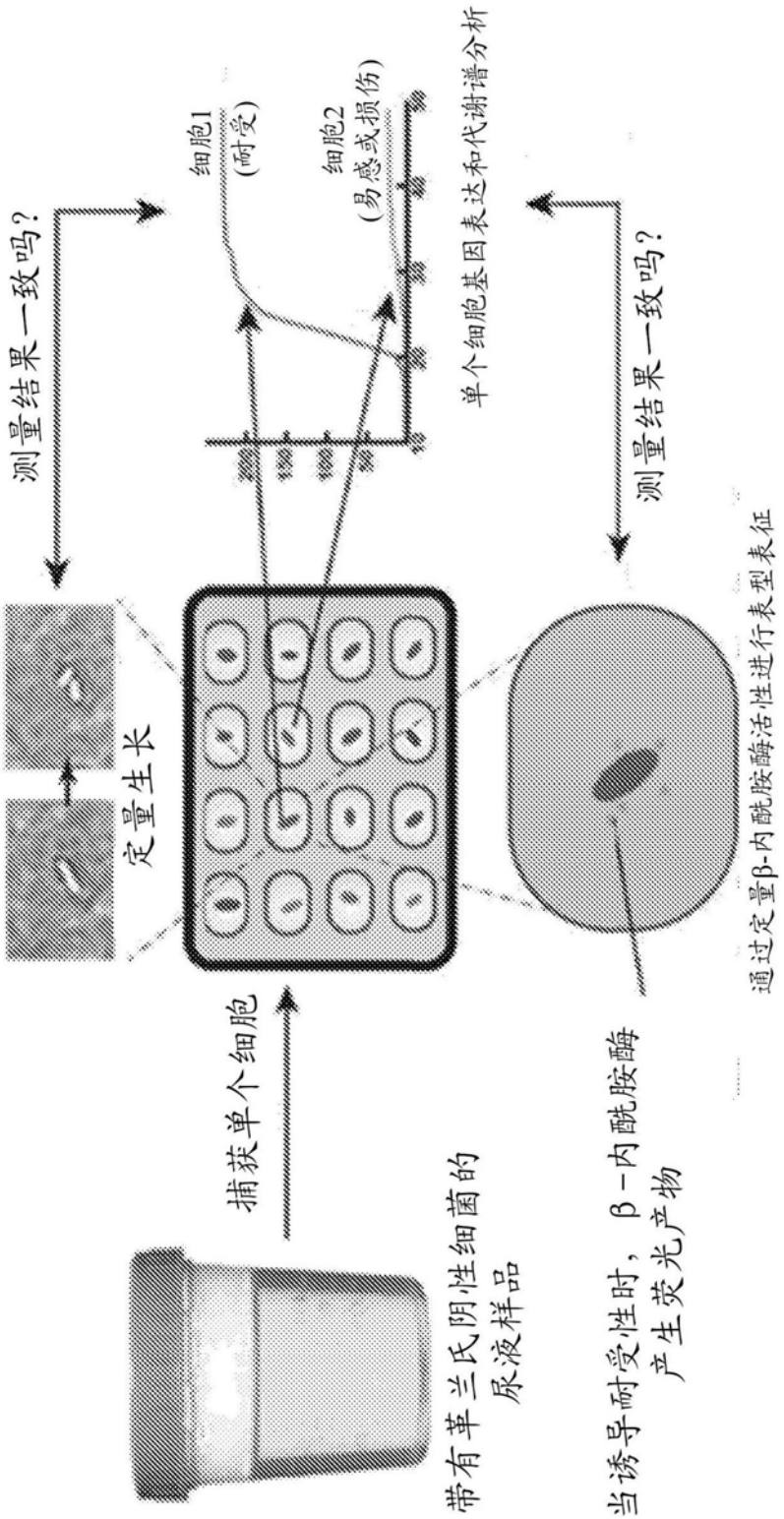

测量结果一致吗?
细胞1
(耐受)
细胞2
(易感或损伤)
单个细胞基因表达和代谢谱分析
定量生长
捕获单个细胞
测量结果一致吗?
带有革兰氏阴性细菌的
尿液样品
当诱导耐受性时，β-内酰胺酶
产生荧光产物
通过定量β-内酰胺酶活性进行表型表征

图5

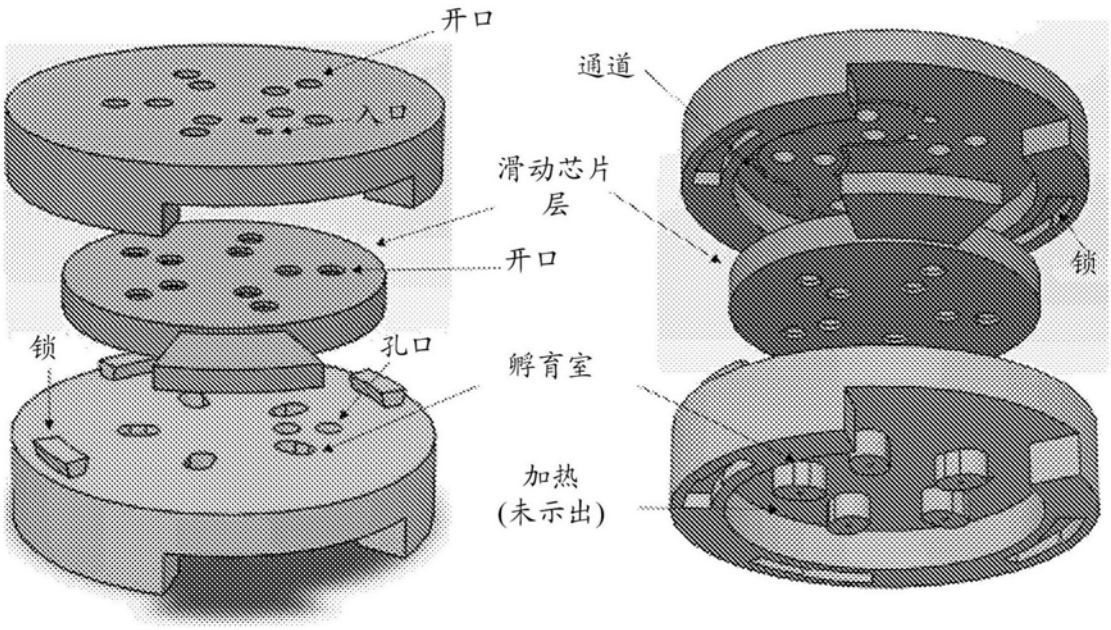

开口
入口
通道
滑动芯片层
开口
锁
孔口
锁
孵育室
加热
(未示出)

图6A

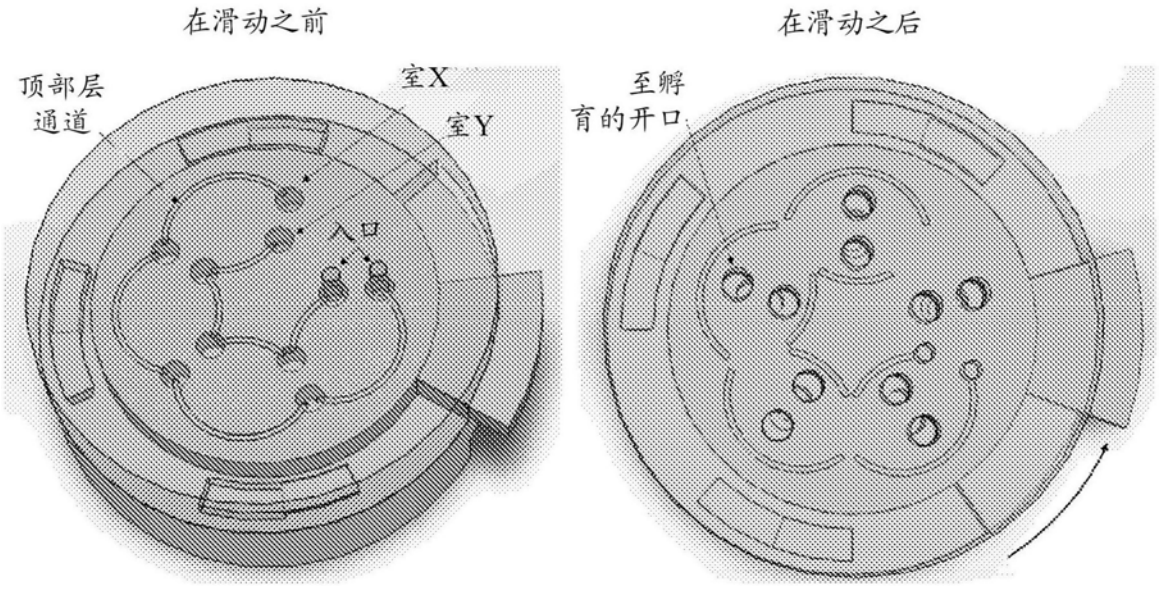

在滑动之前
在滑动之后
顶部层
通道
室X
室Y
入口
至孵
育的开口

图6B

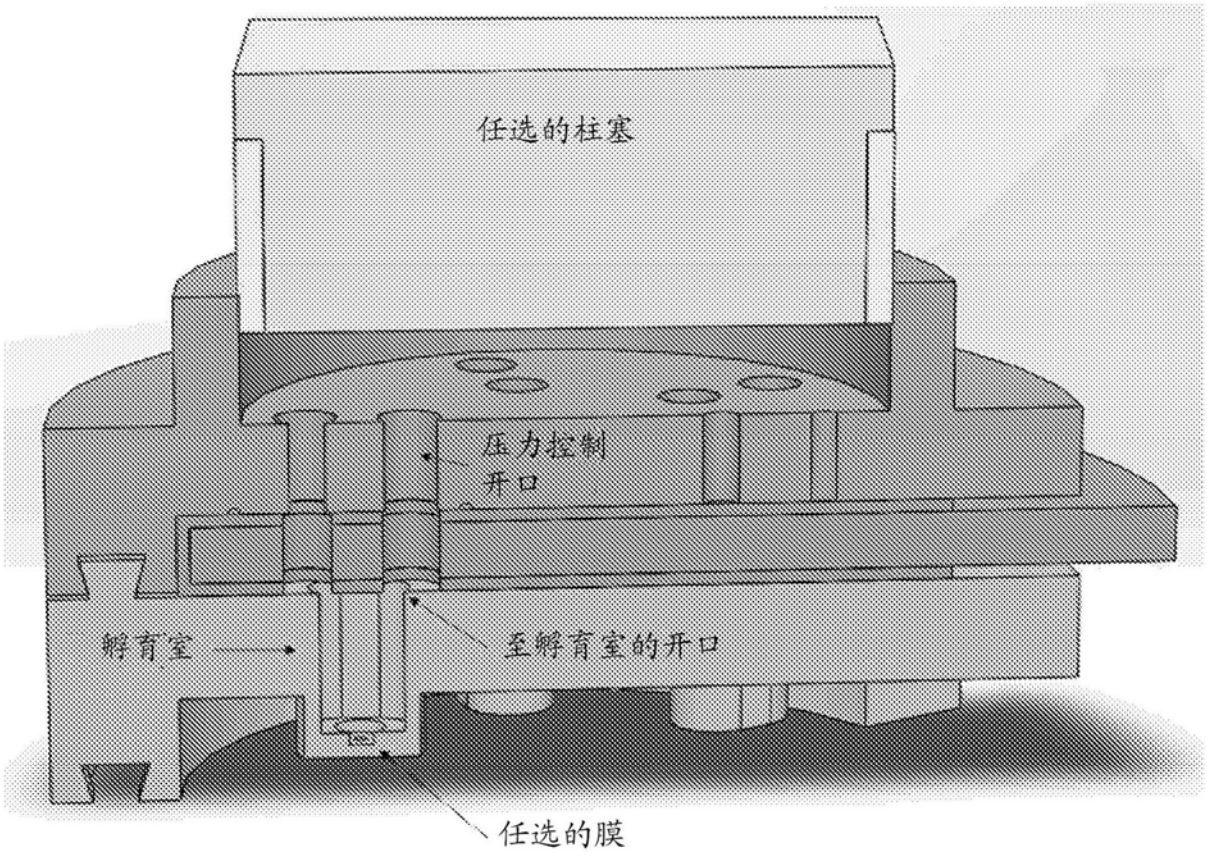


图6C

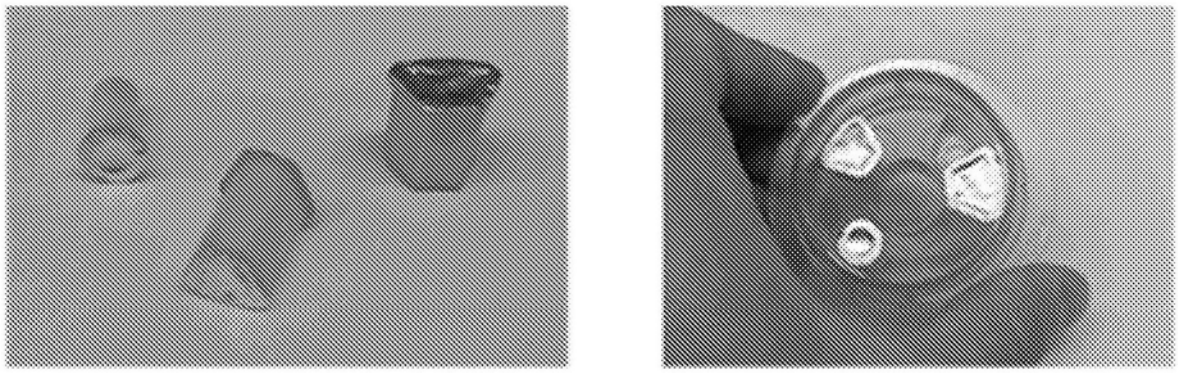


图7

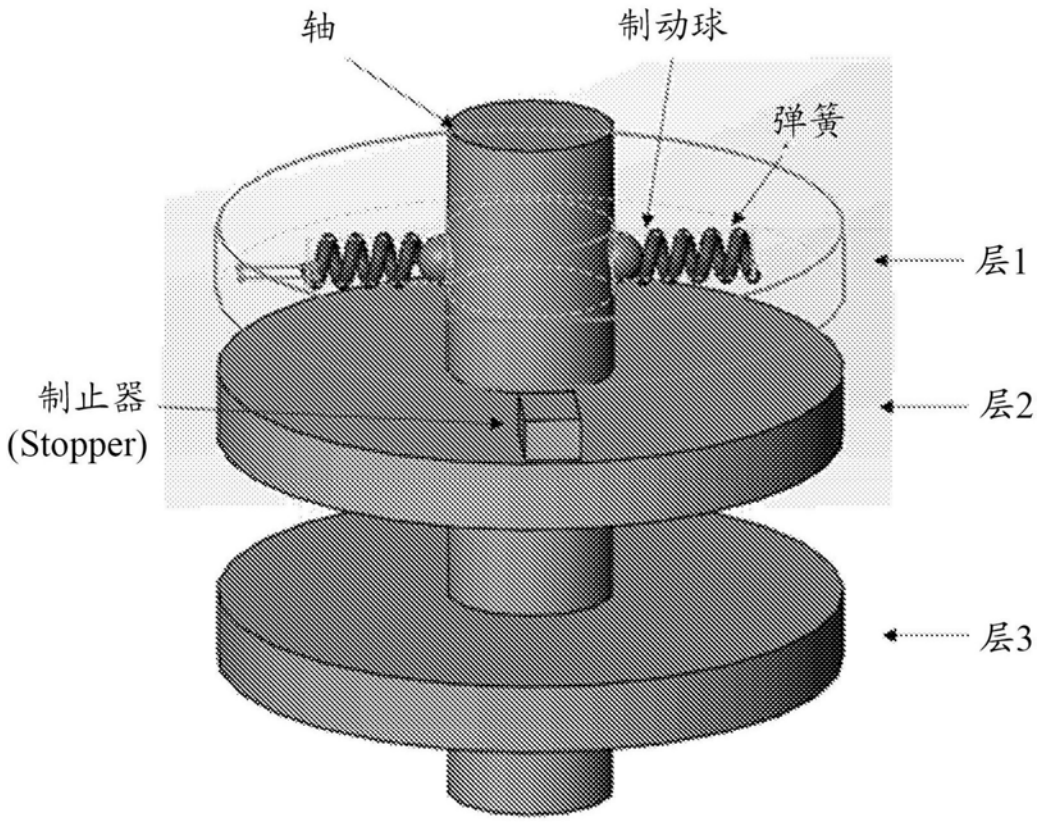


图8

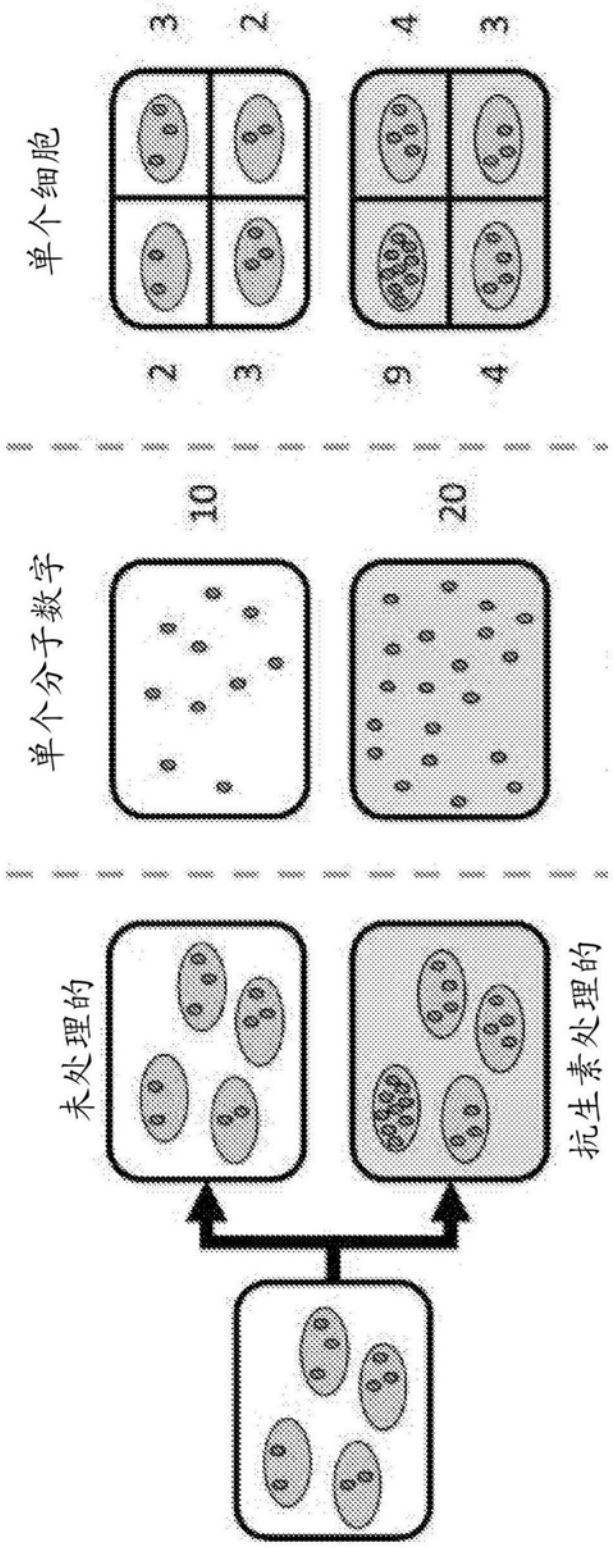


图9

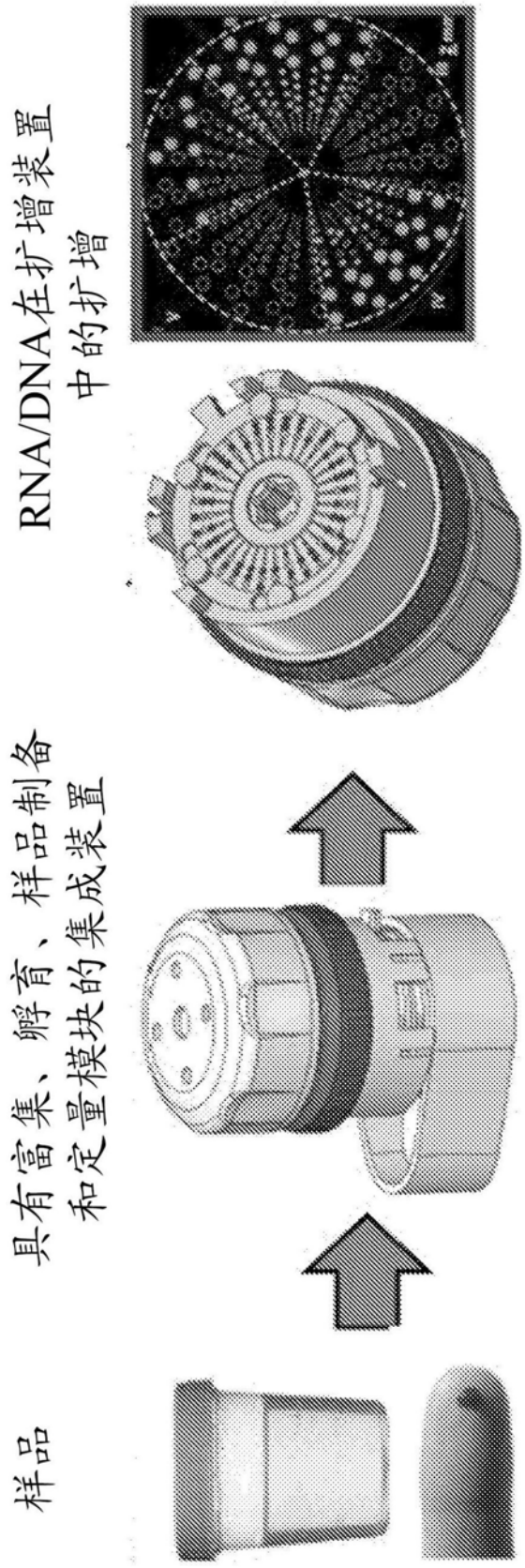


图10

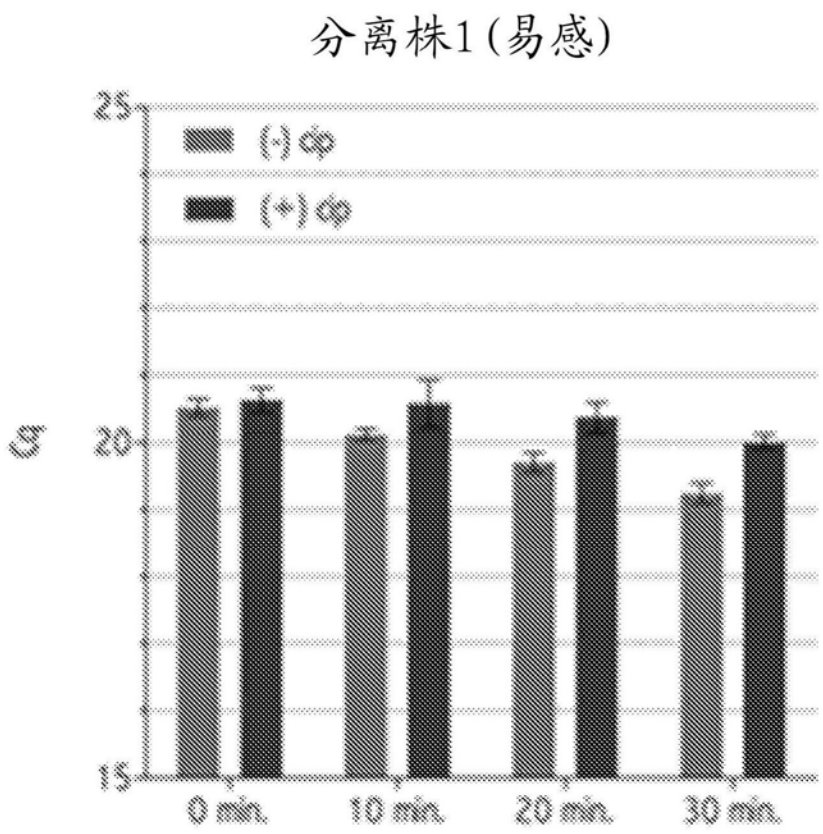

分离株1(易感)
25
20
15
S
(-)dp
(+)dp
0 min.
10 min.
20 min.
30 min.

图11

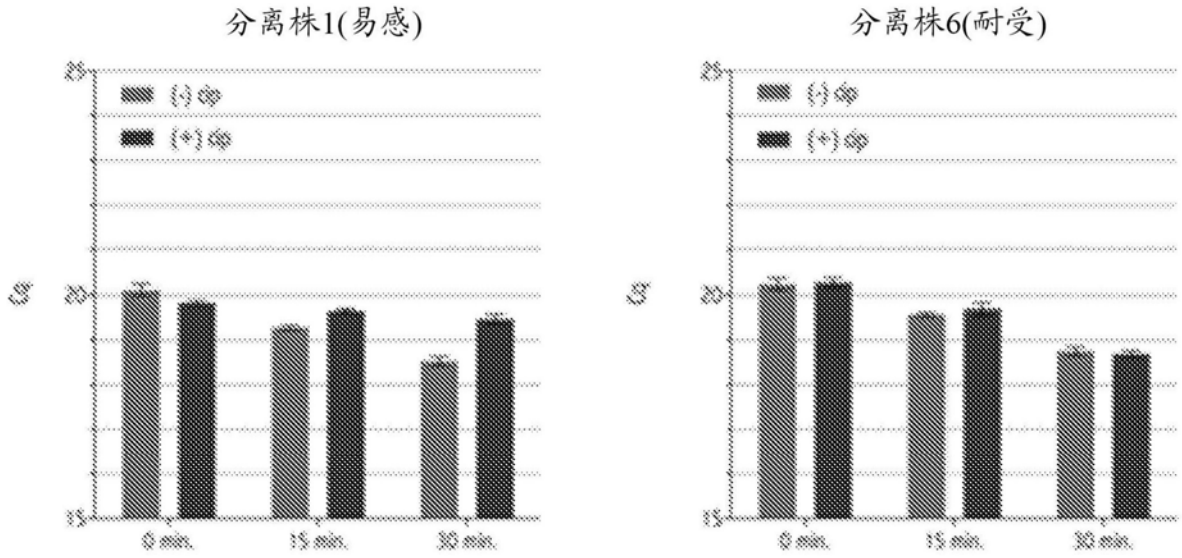

分离株1(易感)
分离株6(耐受)
S
(-)dp
(+)dp
0 min.
15 min.
30 min.

图12A

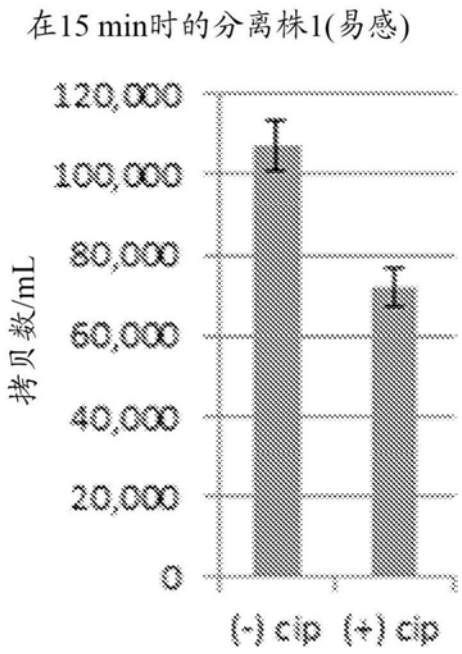

在15 min时的分离株1(易感)
120,000
100,000
80,000
60,000
40,000
20,000
0
拷贝数/mL
(-) cip
(+) cip

图12B

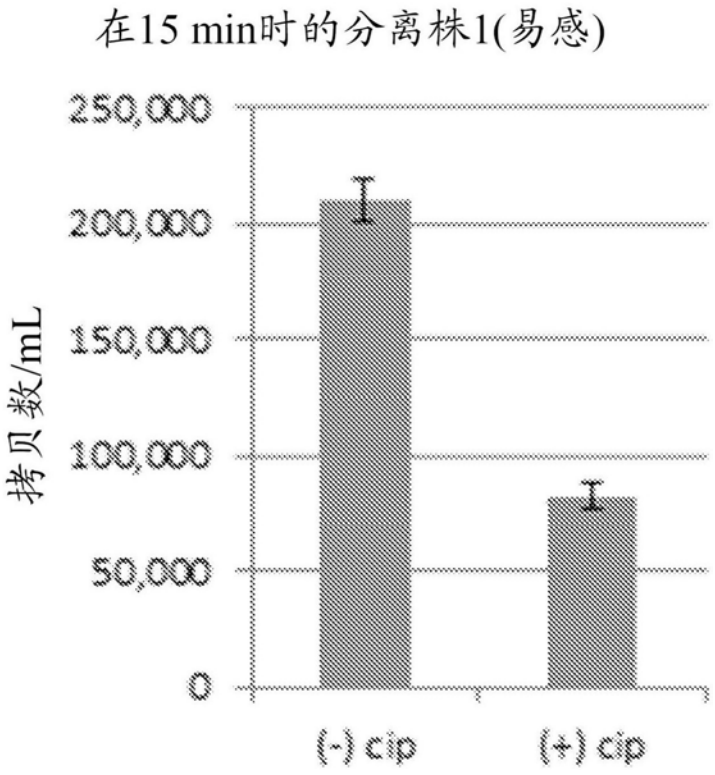

在15 min时的分离株1(易感)
250,000
200,000
150,000
100,000
50,000
0
拷贝数/mL
(-) cip
(+) cip

图13

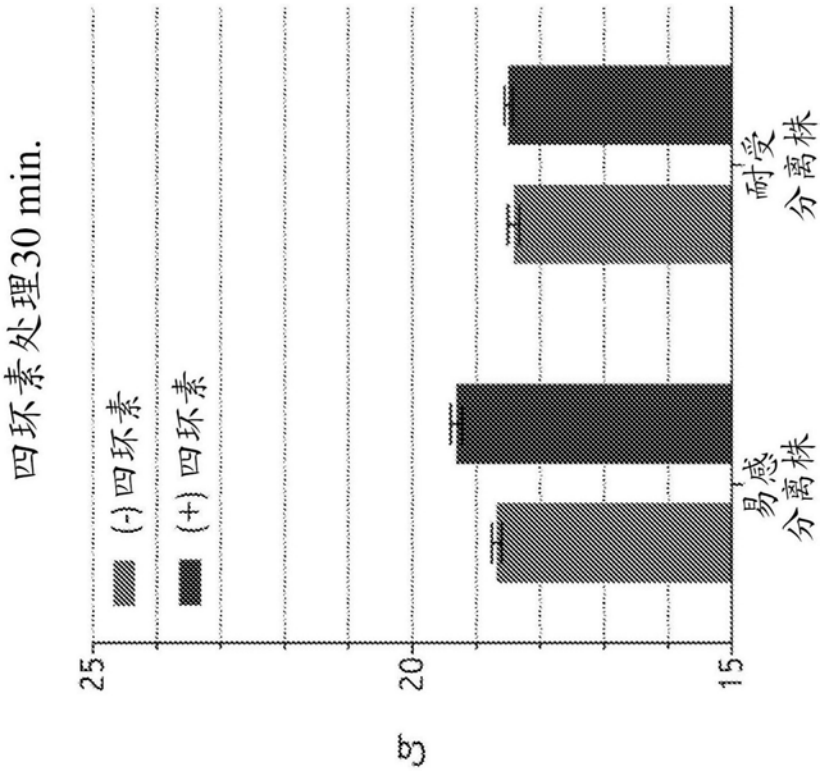

四环素处理30 min.
(-) 四环素
(+) 四环素
25
20
15
g
易感分离株
耐受分离株

图14A

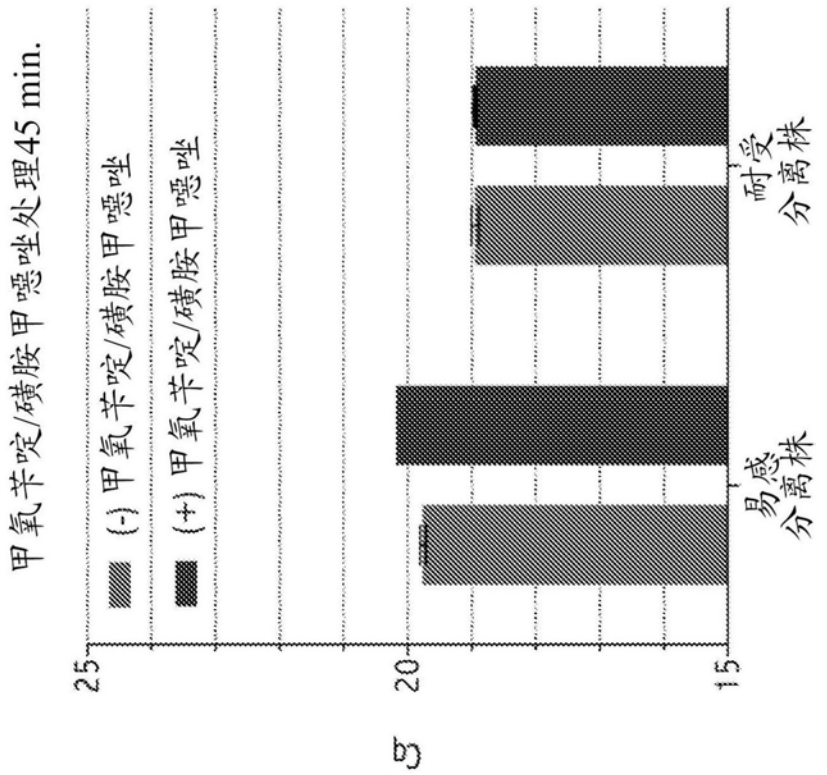

甲氧苄啶/磺胺甲噁唑处理45 min.
(-) 甲氧苄啶/磺胺甲噁唑
(+) 甲氧苄啶/磺胺甲噁唑
25
20
15
g
易感分离株
耐受分离株

图14B

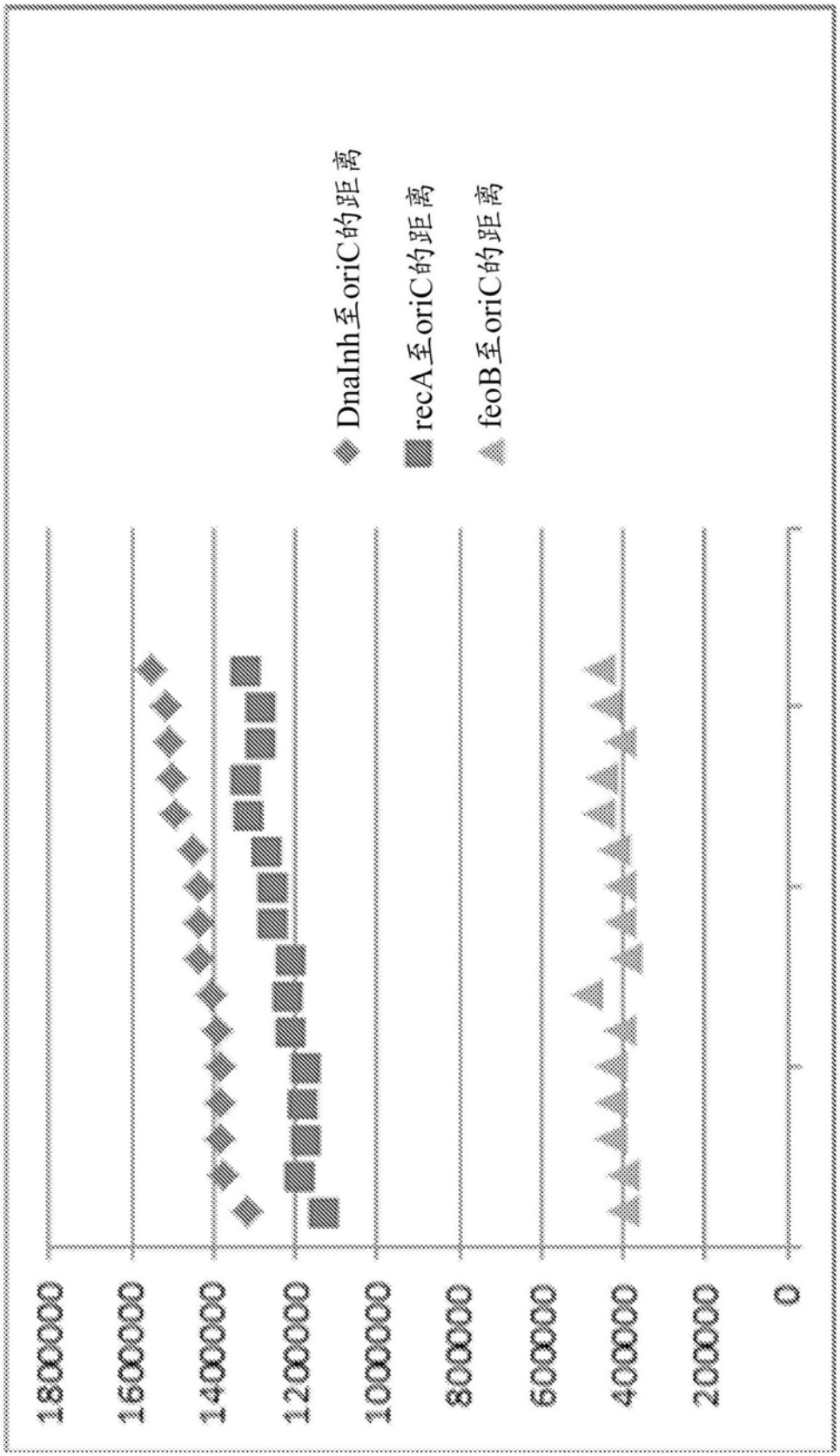

DnaInh至oriC的距离
recA至oriC的距离
feoB至oriC的距离
1800000
1600000
1400000
1200000
1000000
800000
600000
400000
200000
0

图15

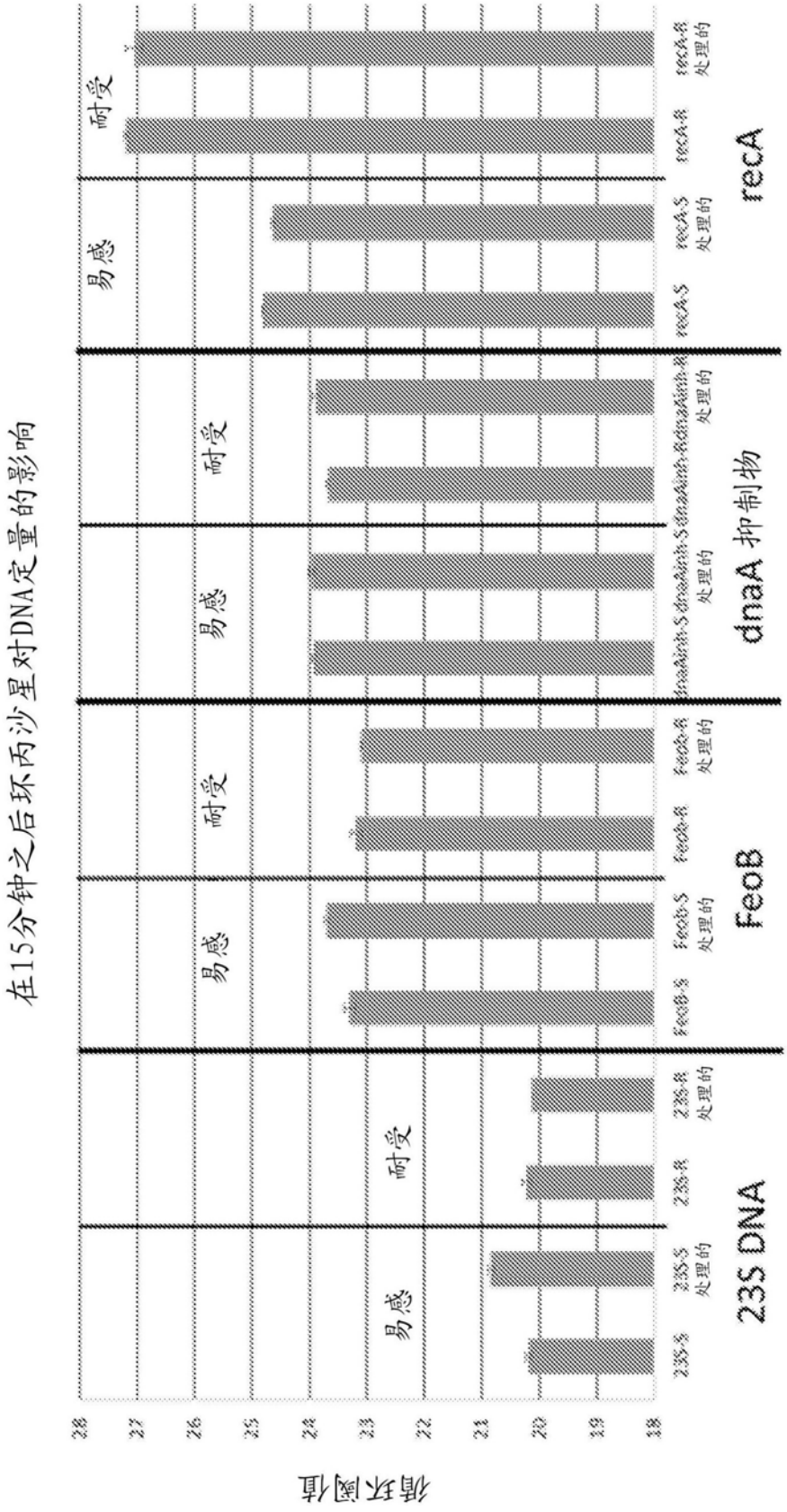

在15分钟之后环丙沙星对DNA定量的影响
循环阈值
28
27
26
25
24
23
22
21
20
19
18
23S DNA
FeoB
dnaA 抑制物
recA
易感
耐受
23S-S
23S-R
23S-S 处理的
23S-R 处理的
FeoB-S
FeoB-R
FeoB-S 处理的
FeoB-R 处理的
dnaA-S
dnaA-R
dnaA-S 处理的
dnaA-R 处理的
recA-S
recA-R
recA-S 处理的
recA-R 处理的

图16

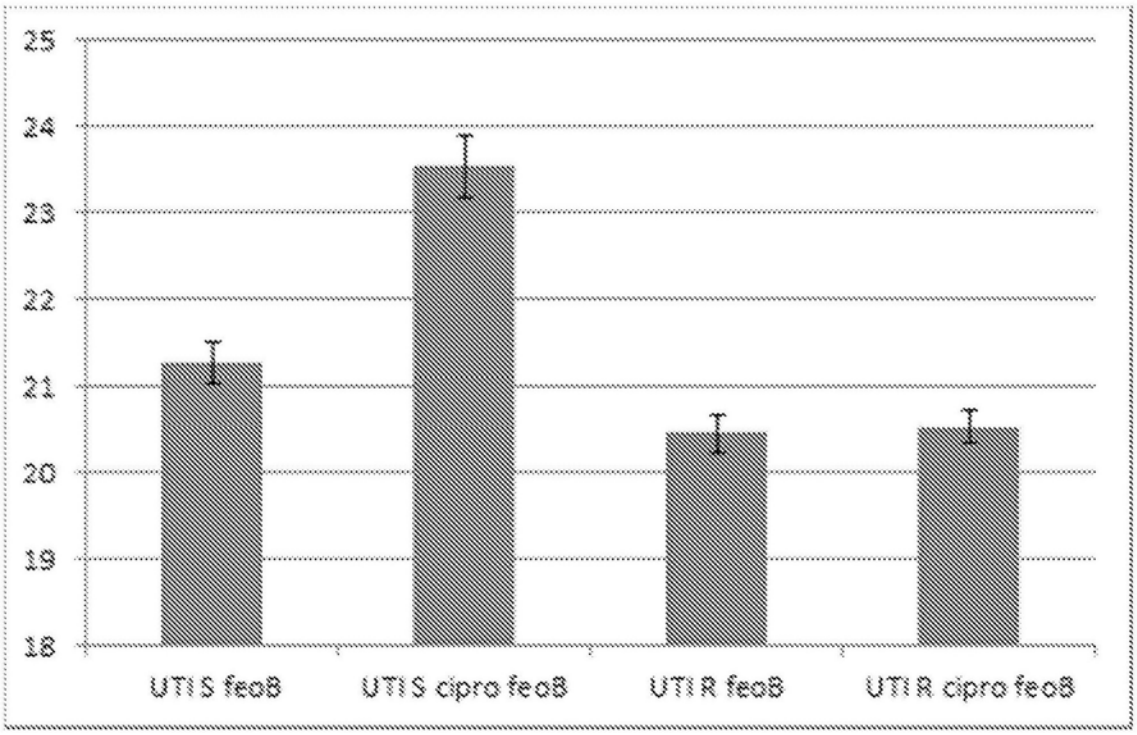

25
24
23
22
21
20
19
18
UTI S feoB
UTI S cipro feoB
UTI R feoB
UTI R cipro feoB

图17A

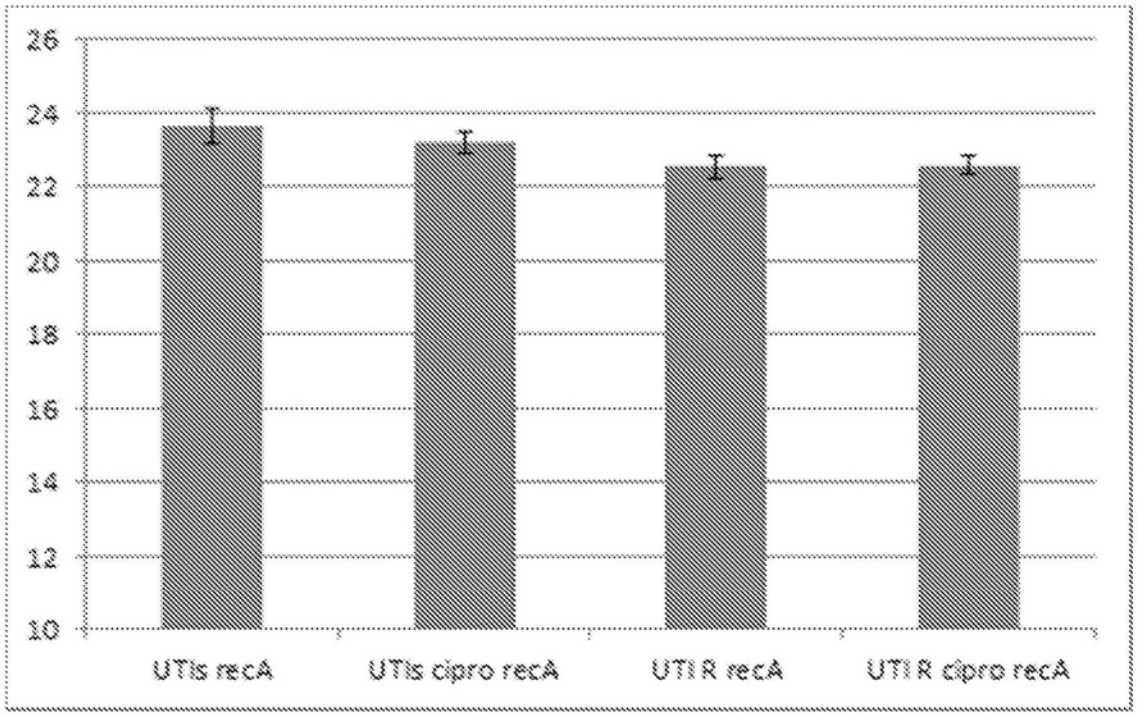

26
24
22
20
18
16
14
12
10
UTIs recA
UTIs cipro recA
UTI R recA
UTI R cipro recA

图17B

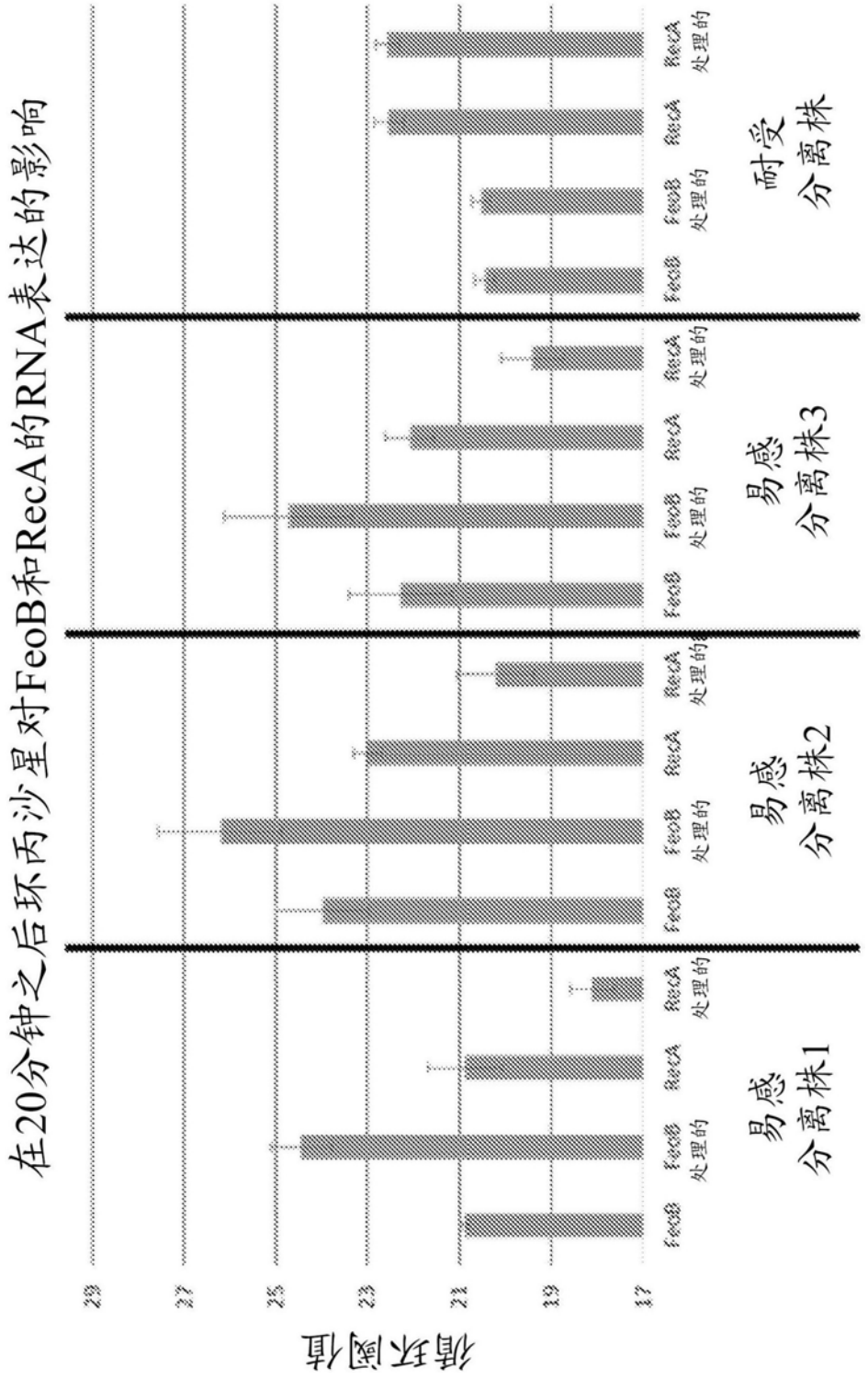

在20分钟之后环丙沙星对FeoB和RecA的RNA表达的影响
循环阈值
29
27
25
23
21
19
17
FeoB 处理的
RecA 处理的
易感
分离株1
易感
分离株2
易感
分离株3
耐受
分离株

图18