

TITLE

[0001] Growth-Independent Detection of Cells.

CROSS REFERENCE TO RELATED APPLICATIONS

[0002] This application claims the benefit of U.S. Provisional Application No. 62/011,660, filed on June 13, 2014; which is hereby incorporated by reference, in its entirety, for all purposes.

SEQUENCE LISTING

[0003] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on June 10, 2015, is named 29449PCT_CRF_sequencelisting.txt and is 25 kilobytes in size.

BACKGROUND

[0004] In the research laboratory, many studies employing cells are conducted using cultured cells that often exhibit logarithmic growth. In nature, however, cells are rarely in a logarithmic growth-rate and often are in a stationary state. This phenomenon is an important consideration in the development of clinical diagnostics for the detection of cells since clinical samples can contain cells that are not in a metabolic state that supports optimal growth. In some cases, cells can be obtained that do not grow under the assay conditions intended to detect the cells. As such, when testing for the presence of cells directly from a clinical or environmental sample, it can oftentimes be important to employ an assay that operates independent of cell growth.

[0005] Even when cells are isolated and cultured in the laboratory there can still be situations in which individual strains of isolated cells can exhibit varying growth characteristics. When such cells exhibit sub-optimal growth, this can lead to a cell not being detected by an assay that generally requires growth. Example 9 of WO 2014/145899 exemplifies this situation (See page 80, line 2 - page 82, line 33, and Figure 26B). In that assay, *S. aureus* cells were monitored for growth in the presence of clindamycin. The assay was intended for distinguishing a clindamycin susceptible vs. resistant phenotype making the determination based on the growth rate of the bacteria in the presence of clindamycin. In the assay, one clindamycin-resistant isolate of bacteria was misinterpreted as clindamycin-sensitive because the isolate exhibited a sub-optimal growth rate.

[0006] As such, assays that generally require a minimum amount of growth or a minimum growth rate may not detect target cells that do not exhibit the required growth characteristics during the assay.

[0007] Related patent applications include: PCT/US2014/026536, filed on March 13, 2014, which is hereby incorporated by reference, in its entirety, for all purposes.

SUMMARY

[0008] Disclosed herein is a cellular detection method that operates independent of cell growth. While existing cellular detection methodologies benefit from cell growth, the methods disclosed herein demonstrate embodiments that are independent of cell growth.

[0009] The methods disclosed herein are generally independent of growth – which can be an important feature for detecting cells at a metabolic state that does not support adequate growth (e.g., cells encountered in clinical samples) and for strains of cells with lower than expected growth rates.

[0010] Disclosed herein are various methods, systems, and compositions for the growth independent detection of cells.

[0011] For example, a method disclosed herein can include a growth-independent method for detecting a microorganism of interest in a sample, comprising: contacting the sample with a plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces one or more microorganisms of interest in the sample, wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase; providing conditions for activation of the reporter nucleic acid sequence; and detecting a signal produced by the reporter nucleic acid sequence, wherein the presence of the signal indicates the presence of the one or more microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more microorganisms of interest.

[0012] As a further example, a composition disclosed herein can include a sample or a cell culture comprising a plurality of non-replicative transduction particles (NRTPs) and one or more microorganisms of interest, optionally wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase.

BRIEF DESCRIPTION OF THE SEVERAL VIEWS OF THE DRAWINGS

[0013] These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description, and accompanying drawings, where:

[0014] Figure 1 – Figure 1A summarizes the ratio of colony forming units (CFUs) counts of MRSA at the end of the assay and at the beginning of the assay where most samples exhibited minimal growth during the 4-hour assay (e.g., 1-2 divisions) with two samples showing a decrease in growth (48 and 60), one sample showing no growth (51), and 3 samples exhibiting less than 0.4 divisions per hour (40, 51, and 81). Figure 1B shows that all samples tested produced a positive signal (relative light units; RLU) over background.

[0015] Figure 2 – Figure 2A summarizes the ratio of CFU counts of MRSA at the end of the assay and at the beginning of the assay where most samples exhibited minimal growth during the 4-hour assay (e.g., 1-2 divisions) and one sample (3037) showed a decrease in growth. Figure 2B shows that all samples produced a positive signal (RLU) during the assay over background.

DETAILED DESCRIPTION

[0016] Terms used in the claims and specification are defined as set forth below unless otherwise specified.

[0017] As used herein, “reporter nucleic acid molecule” or “reporter nucleic acid sequence” refers to a nucleotide sequence comprising a DNA or RNA molecule capable of producing a signal. The reporter nucleic acid molecule can be naturally occurring or an artificial or synthetic molecule. In some embodiments, the reporter nucleic acid molecule is exogenous to a host cell and can be introduced into a host cell as part of an exogenous nucleic acid molecule, such as a plasmid or vector. In certain embodiments, the reporter nucleic acid molecule can be complementary to a target gene in a cell. In other embodiments, the reporter nucleic acid molecule comprises a reporter gene encoding a reporter molecule (e.g., reporter enzyme, protein). In some embodiments, the reporter nucleic acid molecule is referred to as a “reporter construct” or “nucleic acid reporter construct.”

[0018] A “reporter molecule” or “reporter” refers to a molecule (e.g., nucleic acid or protein) that confers onto an organism a detectable or selectable phenotype. The detectable phenotype can be colorimetric, fluorescent or luminescent, for example. Reporter molecules can be expressed from reporter genes encoding enzymes mediating luminescence reactions (luxA, luxB, luxAB, luc, ruc, nluc), genes encoding enzymes mediating colorimetric reactions (lacZ, HRP), genes encoding fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent proteins), nucleic acid molecules encoding affinity peptides (His-tag, 3X-FLAG), and genes encoding selectable markers (ampC, tet(M), CAT, erm). The reporter molecule can be used as a marker for successful uptake of a nucleic acid molecule or exogenous sequence (plasmid) into a cell. The reporter molecule can also be used to indicate the presence of a target gene, target nucleic acid molecule, target intracellular molecule, or a

cell, as described herein. Alternatively, the reporter molecule can be a nucleic acid, such as an aptamer or ribozyme.

[0019] In some aspects of the invention, the reporter nucleic acid molecule is operatively linked to a promoter. In other aspects of the invention, the promoter can be chosen or designed to contribute to the reactivity and cross-reactivity of the reporter system based on the activity of the promoter in specific cells (*e.g.*, specific species) and not in others. In certain aspects, the reporter nucleic acid molecule comprises an origin of replication. In other aspects, the choice of origin of replication can similarly contribute to reactivity and cross-reactivity of the reporter system, when replication of the reporter nucleic acid molecule within the target cell contributes to or is required for reporter signal production based on the activity of the origin of replication in specific cells (*e.g.*, specific species) and not in others. In some embodiments, the reporter nucleic acid molecule forms a replicon capable of being packaged as concatameric DNA into a progeny virus during virus replication.

[0020] A “detectable indication of viability” refers to an indicator associated with a cell that can be observed and that demonstrates whether the cell is more or less viable or if its viability is affected, *e.g.*, relative to a control cell, where the control cell can be the same cell at a different time point or a separate cell. Examples include one or more signals, one or more reporters, one or more markers, growth or lack thereof, light (*e.g.*, light emitted by a luciferase) or lack thereof, etc.

[0021] As used herein, a “target transcript” refers to a portion of a nucleotide sequence of a DNA sequence or an mRNA molecule that is naturally formed by a target cell including that formed during the transcription of a target gene and mRNA that is a product of RNA processing of a primary transcription product. The target transcript can also be referred to as a cellular transcript or naturally occurring transcript.

[0022] As used herein, the term “transcript” refers to a length of nucleotide sequence (DNA or RNA) transcribed from a DNA or RNA template sequence or gene. The transcript can be a cDNA sequence transcribed from an RNA template or an mRNA sequence transcribed from a DNA template. The transcript can be protein coding or non-coding. The transcript can also be transcribed from an engineered nucleic acid construct.

[0023] A transcript derived from a reporter nucleic acid molecule can be referred to as a “reporter transcript.” The reporter transcript can include a reporter sequence and a cis-repressing sequence. The reporter transcript can have sequences that form regions of complementarity, such that the transcript includes two regions that form a duplex (*e.g.*, an intermolecular duplex region). One region can be referred to as a “cis-repressing sequence”

and has complementarity to a portion or all of a target transcript and/or a reporter sequence. A second region of the transcript is called a “reporter sequence” and can have complementarity to the cis-repressing sequence. Complementarity can be full complementarity or substantial complementarity. The presence and/or binding of the cis-repressing sequence with the reporter sequence can form a conformation in the reporter transcript, which can block further expression of the reporter molecule. The reporter transcript can form secondary structures, such as a hairpin structure, such that regions within the reporter transcript that are complementary to each other can hybridize to each other.

[0024] “Introducing into a cell,” when referring to a nucleic acid molecule or exogenous sequence (*e.g.*, plasmid, vector, construct), means facilitating uptake or absorption into the cell, as is understood by those skilled in the art. Absorption or uptake of nucleic acid constructs or transcripts can occur through unaided diffusive or active cellular processes, or by auxiliary agents or devices including via the use of bacteriophage, virus, and transduction particles. The meaning of this term is not limited to cells *in vitro*; a nucleic acid molecule may also be “introduced into a cell,” wherein the cell is part of a living organism. In such instance, introduction into the cell will include the delivery to the organism. For example, for *in vivo* delivery, nucleic acid molecules, constructs or vectors of the invention can be injected into a tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art, such as electroporation and lipofection. Further approaches are described herein or known in the art.

[0025] A “plasmid” is a small DNA molecule that is physically separate from, and can replicate independently of, chromosomal DNA within a cell. Most commonly found as small circular, double-stranded DNA molecules in bacteria, plasmids are sometimes present in archaea and eukaryotic organisms. Plasmids are considered replicons, capable of replicating autonomously within a suitable host.

[0026] A “vector” is a nucleic acid molecule used as a vehicle to artificially carry foreign genetic material into another cell, where it can be replicated and/or expressed.

[0027] A “virus” is a small infectious agent that replicates only inside the living cells of other organisms. Virus particles (known as virions) include two or three parts: i) the genetic material made from either DNA or RNA molecules that carry genetic information; ii) a protein coat that protects these genes; and in some cases, iii) an envelope of lipids that surrounds the protein coat.

[0028] “MRSA” refers to Methicillin-resistant *Staphylococcus aureus*.

[0029] “MSSA” refers to Methicillin-sensitive *Staphylococcus aureus*.

[0030] The term “ameliorating” refers to any therapeutically beneficial result in the treatment of a disease state, *e.g.*, a disease state, including prophylaxis, lessening in the severity or progression, remission, or cure thereof.

[0031] The term “in situ” refers to processes that occur in a living cell growing separate from a living organism, *e.g.*, growing in tissue culture.

[0032] The term “in vivo” refers to processes that occur in a living organism.

[0033] The term “mammal” as used herein includes both humans and non-humans and include but is not limited to humans, non-human primates, canines, felines, murines, bovines, equines, and porcines.

[0034] “G,” “C,” “A” and “U” each generally stand for a nucleotide that contains guanine, cytosine, adenine, and uracil as a base, respectively. “T” and “dT” are used interchangeably herein and refer to a deoxyribonucleotide wherein the nucleobase is thymine, *e.g.*, deoxyribothymine. However, it will be understood that the term “ribonucleotide” or “nucleotide” or “deoxyribonucleotide” can also refer to a modified nucleotide, as further detailed below, or a surrogate replacement moiety. The skilled person is well aware that guanine, cytosine, adenine, and uracil may be replaced by other moieties without substantially altering the base pairing properties of an oligonucleotide comprising a nucleotide bearing such replacement moiety. For example, without limitation, a nucleotide comprising inosine as its base may base pair with nucleotides containing adenine, cytosine, or uracil. Hence, nucleotides containing uracil, guanine, or adenine may be replaced in the nucleotide sequences of the invention by a nucleotide containing, for example, inosine. Sequences comprising such replacement moieties are embodiments of the invention.

[0035] As used herein, the term “complementary,” when used to describe a first nucleotide sequence in relation to a second nucleotide sequence, refers to the ability of an oligonucleotide or polynucleotide comprising the first nucleotide sequence to hybridize and form a duplex structure under certain conditions with an oligonucleotide or polynucleotide comprising the second nucleotide sequence, as will be understood by the skilled person. Complementary sequences are also described as binding to each other and characterized by binding affinities.

[0036] For example, a first nucleotide sequence can be described as complementary to a second nucleotide sequence when the two sequences hybridize (*e.g.*, anneal) under stringent hybridization conditions. Hybridization conditions include temperature, ionic strength, pH, and organic solvent concentration for the annealing and/or washing steps. The term stringent hybridization conditions refers to conditions under which a first nucleotide sequence will

hybridize preferentially to its target sequence, *e.g.*, a second nucleotide sequence, and to a lesser extent to, or not at all to, other sequences. Stringent hybridization conditions are sequence dependent, and are different under different environmental parameters. Generally, stringent hybridization conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the nucleotide sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the first nucleotide sequences hybridize to a perfectly matched target sequence. An extensive guide to the hybridization of nucleic acids is found in, *e.g.*, Tijssen (1993) *Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes* part I, chap. 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," Elsevier, N.Y. ("Tijssen"). Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

[0037] This includes base-pairing of the oligonucleotide or polynucleotide comprising the first nucleotide sequence to the oligonucleotide or polynucleotide comprising the second nucleotide sequence over the entire length of the first and second nucleotide sequence. Such sequences can be referred to as "fully complementary" with respect to each other herein. However, where a first sequence is referred to as "substantially complementary" with respect to a second sequence herein, the two sequences can be fully complementary, or they may form one or more, but generally not more than 4, 3 or 2 mismatched base pairs upon hybridization, while retaining the ability to hybridize under the conditions most relevant to their ultimate application. However, where two oligonucleotides are designed to form, upon hybridization, one or more single stranded overhangs, such overhangs shall not be regarded as mismatches with regard to the determination of complementarity. For example, a dsRNA comprising one oligonucleotide 21 nucleotides in length and another oligonucleotide 23 nucleotides in length, wherein the longer oligonucleotide comprises a sequence of 21 nucleotides that is fully complementary to the shorter oligonucleotide, may yet be referred to as "fully complementary" for the purposes described herein.

[0038] "Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, in as far as the above requirements with respect to their ability to hybridize are

fulfilled. Such non-Watson-Crick base pairs includes, but not limited to, G:U Wobble or Hoogsteen base pairing.

[0039] The terms “complementary,” “fully complementary” and “substantially complementary” herein may be used with respect to the base matching between two strands of a dsRNA, or between the antisense strand of a dsRNA and a target sequence, between complementary strands of a single stranded RNA sequence or a single stranded DNA sequence, as will be understood from the context of their use.

[0040] As used herein, a “duplex structure” comprises two anti-parallel and substantially complementary nucleic acid sequences. Complementary sequences in a nucleic acid construct, between two transcripts, between two regions within a transcript, or between a transcript and a target sequence can form a “duplex structure.” In general, the majority of nucleotides of each strand are ribonucleotides, but as described in detail herein, each or both strands can also include at least one non-ribonucleotide, *e.g.*, a deoxyribonucleotide and/or a modified nucleotide. The two strands forming the duplex structure may be different portions of one larger RNA molecule, or they may be separate RNA molecules. Where the two strands are part of one larger molecule, and therefore are connected by an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting RNA chain is referred to as a “hairpin loop.” Where the two strands are connected covalently by means other than an uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of the respective other strand forming the duplex structure, the connecting structure is referred to as a “linker.” The RNA strands may have the same or a different number of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the duplex minus any overhangs that are present in the duplex. Generally, the duplex structure is between 15 and 30 or between 25 and 30, or between 18 and 25, or between 19 and 24, or between 19 and 21, or 19, 20, or 21 base pairs in length. In one embodiment the duplex is 19 base pairs in length. In another embodiment the duplex is 21 base pairs in length. When two different siRNAs are used in combination, the duplex lengths can be identical or can differ.

[0041] As used herein, the term “region of complementarity” refers to the region on the antisense strand that is substantially complementary to a sequence, for example a target sequence, as defined herein. Where the region of complementarity is not fully complementary to the target sequence, the mismatches are most tolerated in the terminal regions and, if present, are generally in a terminal region or regions, *e.g.*, within 6, 5, 4, 3, or 2 nucleotides of the 5' and/or 3' terminus.

[0042] The term percent “identity,” in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (*e.g.*, BLASTP and BLASTN or other algorithms available to persons of skill) or by visual inspection. Depending on the application, the percent “identity” can exist over a region of the sequence being compared, *e.g.*, over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

[0043] For sequence comparison, typically one sequence acts as a reference sequence to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are input into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. The sequence comparison algorithm then calculates the percent sequence identity for the test sequence(s) relative to the reference sequence, based on the designated program parameters.

[0044] Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2:482 (1981), by the homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48:443 (1970), by the search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), or by visual inspection (see generally Ausubel *et al.*, *infra*).

[0045] One example of an algorithm that is suitable for determining percent sequence identity and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol. Biol.* 215:403-410 (1990). Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov/).

[0046] The term “sufficient amount” means an amount sufficient to produce a desired effect, *e.g.*, an amount sufficient to produce a detectable signal from a cell.

[0047] The term “therapeutically effective amount” is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a “prophylactically effective amount” as prophylaxis can be considered therapy.

[0048] It must be noted that, as used in the specification and the appended claims, the singular forms “a,” “an” and “the” include plural referents unless the context clearly dictates otherwise.

Lysogenic and Lytic Cycle of Viruses

[0049] Viruses undergo lysogenic and lytic cycles in a host cell. If the lysogenic cycle is adopted, the phage chromosome can be integrated into the bacterial chromosome, or it can establish itself as a stable plasmid in the host, where it can remain. If the lytic cycle of a lysogen is induced, the phage genome is excised from the bacterial chromosome and initiates the lytic cycle, which culminates in lysis of the cell and the release of phage particles. The lytic cycle leads to the production of new phage particles which are released by lysis of the host.

[0050] Certain temperate phage can exhibit lytic activity, and the propensity for this may vary with varying host bacteria. To illustrate this phenomenon, the lytic activity of two temperate *S. aureus* phages on ten MRSA clinical isolates was examined via plaque assay (Table 1). The phage $\phi 11$ exhibited lytic activity on 10 out of 10 clinical MRSA isolates and $\phi 80\alpha$ exhibited lytic activity on six of the 10 clinical MRSA isolates. Thus, reporter assays relying on the natural lysogenic cycle of phages can be expected to exhibit lytic activity sporadically.

Table 1: Lytic activity (denoted by the letter “x”) of the *S. aureus* temperate phages $\phi 11$ and $\phi 80\alpha$ on ten clinical MRSA isolates

MRSA isolate	$\phi 11$	$\phi 80\alpha$
1	x	
2	x	
3	x	x
4	x	x
5	x	x
6	x	
7	x	x
8	x	
9	x	x
10	x	x

[0051] In addition, virus-based reporter assays, such as phage-based reporters, can suffer from limited reactivity (*i.e.*, analytical inclusivity) due to limits in the phage host range caused by host-based and prophage-derived phage resistance mechanisms. These resistance mechanisms target native phage nucleic acid that can result in the degradation or otherwise

inhibition of the phage DNA and functions. Such resistance mechanisms include restriction systems that cleave phage DNA and CRISPR systems that target phage-derived sequences.

[0052] Both lytic activity and phage resistance can be inhibitory to assays based on reporter phages. Lytic activity can inhibit signal by destroying or otherwise inhibiting the cell in its ability to generate a detectable signal and thus affecting limits of detection by reducing the amount of detectable signal or preventing the generation of a detectable signal. Phage resistance mechanisms can limit the host range of the phage and limit the inclusivity of the phage-based reporter, similarly affecting limits of detection by reducing the amount of detectable signal or preventing the generation of a detectable signal. Both lytic activity and phage resistance caused by the incorporation of phage DNA in a reporter phage can lead to false-negative results in assays that incorporate these phage reporters.

Non-Replicative Transduction Particles (NRTPs), Methods for Producing Non-Replicative Transduction Particles (NRTP), and Related Assays

[0053] A “transduction particle” refers to a virus capable of delivering a non-viral nucleic acid molecule into a cell. The virus can be a bacteriophage, adenovirus, etc.

[0054] A “non-replicative transduction particle” refers to a virus capable of delivering a non-viral nucleic acid molecule into a cell, but does not package its own replicated viral genome into the transduction particle. The virus can be a bacteriophage, adenovirus, *etc.*

[0055] Various NRTPs, methods of making the various NRTPs, and methods of using the NRTPs are described in: PCT/US2014/026536, filed on March 13, 2014, which is hereby incorporated by reference, in its entirety, for all purposes. Examples of such methods of producing NRTPs include disruption/complementation systems employing lysogenized virus in which a sequence of DNA that is recognized by the viral packaging machinery is disrupted (e.g., via mutation, deletion, insertion, etc.), and the disruption is complemented by a reporter plasmid. In these systems, when the lytic cycle of the lysogenized virus is induced, the system produces virus particles but the particles carry plasmid DNA instead of virus DNA.

[0056] In some aspects, methods for the use of NRTPs as reporter molecules for use with endogenous or native inducers that target gene promoters within viable cells. In some embodiments, the method comprises employing a NRTP as a reporter, wherein the NRTP comprises a reporter gene that is operably linked to a promoter that controls the expression of a target gene within a target cell. When the NRTP that includes the reporter gene is introduced into the target cell, expression of the reporter gene is possible, e.g., via induction of the target gene promoter in the reporter nucleic acid molecule. In certain aspects, a

reporter nucleic acid sequence is operatively linked to a constitutive promoter. In some aspects the constitutive promoter is a *S. aureus clpB* promoter.

[0057] In some embodiments, constructs (including NRTPs) comprise a reporter nucleic acid sequence that can include a reporter gene. The reporter gene can encode a reporter molecule, and the reporter molecule can be a detectable or selectable marker. In certain embodiments, the reporter gene encodes a reporter molecule that produces a detectable signal when expressed in a cell.

[0058] In certain embodiments, a reporter nucleic acid sequence encodes a marker such as a detectable or selectable marker. The terms “marker” or “markers” encompass, without limitation, lipids, lipoproteins, proteins, cytokines, chemokines, growth factors, peptides, nucleic acids, genes, and oligonucleotides, together with their related complexes, metabolites, mutations, variants, polymorphisms, modifications, fragments, subunits, degradation products, elements, and other analytes or sample-derived measures. A marker can also include mutated proteins, mutated nucleic acids, variations in copy numbers, and/or transcript variants.

[0059] In certain embodiments, the reporter molecule can be a fluorescent reporter molecule, such as, but not limited to, a green fluorescent protein (GFP), enhanced GFP, yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), blue fluorescent protein (BFP), red fluorescent protein (RFP) or mCherry, as well as near-infrared fluorescent proteins.

[0060] In other embodiments, the reporter molecule can be an enzyme mediating luminescence reactions (*luxA*, *luxB*, *luxAB*, *luc*, *ruc*, *nluc*, *etc.*). Reporter molecules can include a bacterial luciferase, a eukaryotic luciferase, an enzyme suitable for colorimetric detection (*lacZ*, HRP), a protein suitable for immunodetection, such as affinity peptides (His-tag, 3X-FLAG), a nucleic acid that function as an aptamer or that exhibits enzymatic activity (ribozyme), or a selectable marker, such as an antibiotic resistance gene (*ampC*, *tet(M)*, *CAT*, *erm*). Other reporter molecules known in the art can be used for producing signals to detect target nucleic acids or cells.

[0061] In other aspects, the reporter molecule comprises a nucleic acid molecule. In some aspects, the reporter molecule is an aptamer with specific binding activity or that exhibits enzymatic activity (*e.g.*, aptazyme, DNazyme, ribozyme).

[0062] Delivery of cell reporter nucleic acid sequences can be accomplished by various means including electroporation, chemical, biolistic, and glass bead transformation, transduction, transfection, vectors, conjugation, including, but not limited to, delivery via

nucleic acid delivery vehicles including bacteriophage, virus, spheroplast, liposomes, virus-like particles, lipid-DNA complexes, lipoplexes, polymer-DNA complexes, polyplexes, etc.

[0063] In some aspects, the methods, systems, and compositions disclosed herein comprise a sample in contact with a fatty aldehyde bacterial luciferase substrate reagent to, e.g., produce a signal. Examples of fatty aldehyde bacterial luciferase substrate reagents can include tridecanal as well as other similar fatty aldehyde bacterial luciferase substrate reagents known in the art. Fatty aldehydes of various carbon chain lengths are suitable including hexanal, heptanal, octanal, nonanal, decanal, undecanal, dodecanal, and/or tetradecanal.

[0064] In some aspects a reporter nucleic acid sequence can produce a signal. In certain aspects a signal is a luminescence signal. In some aspects a signal can be measured in relative light units (RLU) emitted by the signal. Various devices are known in the art detecting a signal from a reporter nucleic acid sequence. Devices for detecting light emission include photomultiplier tubes, photo diodes, and/or avalanche photo diodes. Detection can be accomplished by simply collecting light signal from an area or volume and/or by imaging an area or volume.

[0065] In some aspects a signal is greater than a background threshold, e.g., where the background threshold is calculated from an average background signal plus 0x, 1x, 2x, or 3x the standard deviation of the average background signal.

[0066] In some aspects a signal can be detected at a limit of detection (LoD) of less than or equal to 10000-1, 1000-10, 1000-100, 100-1, 10,000, 1,000, 500, 400, 300, 200, 100, 90, 80, 70, 60, 50, 40, 30, 20, 10, 9, 8, 7, 6, 5, 4, or 3 colony forming units (CFU). In some aspects, a signal can be detected at a LoD of less than or equal to five CFU, or a signal can be detected at a LoD of less than or equal to three CFU, or a signal can be detected at a LoD of less than or equal to two CFU, or a signal can be detected at a LoD of less than or equal to one CFU.

[0067] In certain aspects, the sensitivity or specificity of a method of using a given NRTP to detect a cell can be determined, e.g., as described in PCT/US2014/026536, filed on March 13, 2014.

[0068] In some aspects, a method produces at least 80-100, 80-90, 90-100, 85-95, 90-95, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% specificity of detection with reference to a standard cell culture-based assay.

[0069] In some aspects, a method produces at least 80-100, 80-90, 90-100, 85-95, 90-95, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% sensitivity of detection with reference to a standard cell culture-based assay.

[0070] In some aspects, a method achieves at least 80-100, 80-90, 90-100, 85-95, 90-95, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% specificity of detection and at least 80-100, 80-90, 90-100, 85-95, 90-95, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or 100% sensitivity of detection with reference to a standard cell culture-based assay.

Cells and Samples

[0071] Cells disclosed herein can include prokaryotes and eukaryotes. In some aspects, a cell can be a microorganism. The term “microorganism” means prokaryotic and eukaryotic microbial species from the Domains *Archaea*, *Bacteria* and *Eucarya*, the latter including yeast and filamentous fungi, protozoa, algae, or higher Protista. The terms “microbial cells” and “microbes” are used interchangeably with the term microorganism. A microorganism can include a Methicillin Resistant *Staphylococcus aureus* (MRSA) cell, *Staphylococcus aureus*, *Staphylococcus spp.*, Enterobacteriaceae, *Enterococcus spp.*, *Streptococcus spp.*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Stenotrophomonas spp.*, or *Mycobacterium spp.*

[0072] The term “sample” can include a single cell or multiple cells or an aliquot of body fluid, taken from an environment or subject, by means including venipuncture, excretion, ejaculation, massage, biopsy, needle aspirate, lavage sample, scraping, surgical incision, swabbing, or intervention or other means known in the art. In some aspects, a sample can include a clinical sample such as a sample obtained from a subject in a clinical setting such as a hospital. In some aspects, a sample is a nasal swab sample, a rectal swab sample, a blood sample, a positive blood culture sample, a skin/soft tissue sample, a bronchoalveolar lavage sample, a sputum sample, a stool sample, a urine sample, and/or a sample of an isolated microorganism.

[0073] The term “subject” encompasses a cell, tissue, or organism, human or non-human, whether in vivo, ex vivo, or in vitro, male or female.

Antimicrobial Agents

[0074] An “antimicrobial agent” refers to a compound that can kill, inhibit the growth, or otherwise compromise the viability of one or more microorganisms. Antimicrobial agents include antibiotics, antifungals, antiprotozoals, antivirals, and other compounds.

[0075] An antimicrobial agent can include cefoxitin, a β -lactam, an extended-spectrum β -lactam, an Aminoglycoside, an Ansamycin, a Carbacephem, Carbapenems, any generation of Cephalosporin, a Glycopeptide, a Lincosamide, a Lipopeptide, a Macrolide, a Monobactam, a Nitrofurantoin, an Oxazolidinone, a Penicillin, a Polypeptide, a Quinolone, a Fluoroquinolone, a

Streptogramin, a Sulfonamide, a Tetracycline, a Rifampicin, a mycobacterial antibiotic, Chloramphenicol, and Mupirocin.

[0076] In some aspects, the methods, systems, and compositions disclosed herein can include an antimicrobial agent in contact with a sample and detection of a signal produced by a reporter nucleic acid sequence of an NRTP to determine whether one or more microorganisms of interest is susceptible or non-susceptible to the antimicrobial agent. In certain aspects, the antimicrobial agent is an antibiotic.

[0077] In some aspects, the methods, systems, and compositions disclosed herein can include varying pre-determined concentrations of antimicrobial agent in contact with a sample and detecting the amount of a signal to determine the minimum inhibitory concentration of the one or more microorganisms of interest to the antimicrobial agent. In certain aspects, the antimicrobial agent is an antibiotic.

Cell Growth

[0078] Methods, systems, and compositions disclosed herein are typically growth independent methods, systems, and compositions, e.g., for the detection of one or more cells in a sample derived from a subject. For example, methods described herein can include isolating or obtaining a sample from a subject of interest and directly contacting an NRTP described herein with the sample (or a culture comprising the sample) for detecting a cell or set of cells of interest that may or may not be present in the sample, regardless of growth.

[0079] Various methods for determining growth are known in the art, e.g., methods that detect bulk growth of cells, e.g., by measuring an increasing in optical density of a sample and/or methods that measure growth of discrete cells in a sample such as microscopy, automated microscopy, and/or traditional culture of organisms on solid media to detect the presence of a colony of bacteria on the solid media.

[0080] Various culture conditions can be used for detecting a cell in a growth-independent manner, e.g., one or more nutrient formulations that support a cell's ability to transcribe and translate regardless of cell replication rate. In some aspects, culture conditions include limited nutrient conditions, e.g., such as those provided by Roswell Park Memorial Institute (RPMI) media (Fisher Scientific Company, LLC). In some aspects, culture conditions are selected such that they mimic the metabolic state of cells in a natural environment. In some aspects, culture conditions are limited to or include use of a sample that is in a state similar to or identical to its state in its natural environment.

[0081] In some aspects, the growth rate of a microorganism or population of microorganisms is less than logarithmic phase. In some aspects, the growth rate of a microorganism or population of microorganisms can be less than or equal to 0.05, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, or 3 divisions per hour. In some aspects, the growth rate of a microorganism or population of microorganisms can be less than or equal to 1 cell division per 4 hour period, less than or equal to 2 cell divisions per 4 hour period, less than or equal to 3 cell divisions per 4 hour period, less than or equal to 4 cell divisions per 4 hour period, less than or equal to 5 cell divisions per 4 hour period, less than or equal to 6 cell divisions per 4 hour period, less than or equal to 7 cell divisions per 4 hour period, less than or equal to 8 cell divisions per 4 hour period, less than or equal to 9 cell divisions per 4 hour period, or less than or equal to 10 cell divisions per 4 hour period. In some aspects, the growth rate of a microorganism or population of microorganisms can be less than or equal to 0.1 divisions per hour, in particular when the microorganism or population of microorganisms is or includes Methicillin Resistant *Staphylococcus aureus* (MRSA).

[0082] In some aspects, the growth of a microorganism or population of microorganisms can be characterized as stationary phase, less than stationary phase, or greater than stationary phase but less than log phase. In some aspects, a microorganism or population of microorganisms can be undergoing no growth or no detectable growth. In some aspects, the growth of a microorganism or population of microorganisms can be negative (e.g., greater cell death is occurring than cell division) or homeostatic (e.g., cell death and division are relatively equal).

EXAMPLES

[0083] Below are examples of specific embodiments for carrying out the present invention. The examples are offered for illustrative purposes only, and are not intended to limit the scope of the present invention in any way. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperatures, etc.), but some experimental error and deviation should, of course, be allowed for.

[0084] The practice of the present invention will employ, unless otherwise indicated, conventional methods of protein chemistry, biochemistry, recombinant DNA techniques and pharmacology, within the skill of the art. Such techniques are explained fully in the literature. See, e.g., T.E. Creighton, *Proteins: Structures and Molecular Properties* (W.H. Freeman and Company, 1993); A.L. Lehninger, *Biochemistry* (Worth Publishers, Inc., current addition); Sambrook, et al., *Molecular Cloning: A Laboratory Manual* (2nd Edition, 1989); *Methods In Enzymology* (S. Colowick and N. Kaplan eds., Academic Press, Inc.);

Remington's Pharmaceutical Sciences, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); Carey and Sundberg *Advanced Organic Chemistry 3rd Ed.* (Plenum Press) Vols A and B(1992).

Example 1: Deletion/Complementation Packaging System.

[0085] The following is an example of the design and construction of a deletion/complementation-based packaging system for producing non-replicative transduction particles.

[0086] The materials used for developing the packaging system are listed below:

[0087] Bacterial Strains:

[0088] RN4220 is a restriction defective *S. aureus* strain that is a non-lysogenic derivative of NCTC 8325 and is an efficient recipient for *E. coli* DNA. It was first described in Kreiswirth, B.N. *et al.*, *The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage*. *Nature*, 1983. 305(5936): p. 709-712.

[0089] RN10616 is derived by lysogenizing RN4220 with bacteriophage $\phi 80\alpha$. Ubeda, C. *et al.*, *Specificity of staphylococcal phage and SaPI DNA packaging as revealed by integrase and terminase mutations*. *Molecular Microbiology*, 2009. 72(1): p. 98-108.

[0090] ST24 is derived from deleting the small terminase gene *terS* from the lysogenized bacteriophage $\phi 80\alpha$ in RN10616. Ubeda, C. *et al.*, *Specificity of staphylococcal phage and SaPI DNA packaging as revealed by integrase and terminase mutations*. *Molecular Microbiology*, 2009. 72(1): p. 98-108.

[0091] Vectors:

[0092] Examples of plasmids that can be used as source plasmids for cassettes, in some embodiments of the invention are described in Charpentier, E., *et al.*, *Novel Cassette-Based Shuttle Vector System for Gram-Positive Bacteria*. *Appl. Environ. Microbiol.*, 2004. **70**(10): p. 6076-6085.

[0093] The following GenBank accession numbers can be used for cassette sequences:

- SEQ ID NO:1 (*S. aureus* pT181 plasmid origin or replication copy number variant pT181cop-623 repC)
- M21136 (*tetA*(M))
- SEQ ID NO:2 (P_{clpB} promoter sequence)
- SEQ ID NO:3 ($\phi 11$ small terminase (*terS*) gene sequence)
- L09137 (*amp* ColE1 ori)
- X06758 (*luxAB*)

- M62650 (Transcription Termination)

[0094] terS Deletion: The construction of the terS knockout strain ST24 can be accomplished via an allelic-exchange-based strategy resulting in an in-frame deletion removing most of the coding sequence of the $\phi 80\alpha$ small terminase gene. The details of this strategy are described in Ubeda, C. *et al.*, *Specificity of staphylococcal phage and SaPI DNA packaging as revealed by integrase and terminase mutations*. Molecular Microbiology, 2009. 72(1): p. 98-108.

[0095] An exemplary sequence of a terS knockout strain is shown in SEQ ID NO:4, (shown in the sequence listing below). SEQ ID NO:4 is a RN10616 genomic sequence loci showing the $\phi 80\alpha$ terS deletion and complementation.

[0096] Vector Construction: The GW80A0001 vector is an *E. coli*/*S. aureus* shuttle vector. The vector contains *S. aureus* (pT181cop-623 repC) and *E. coli* (ColE1ori) origins of replication, the selectable markers for ampicillin (amp) and tetracycline (tet(M)) resistance for selection in *E. coli* and *S. aureus*, respectively, the $\phi 11$ small terminase (terS) gene sequence that includes its own promoter, the luxA and luxB genes are from *Vibrio harveyi* operatively linked to the constitutive *S. aureus* P_{clpB} promoter, and a transcription termination sequence (TT).

[0097] The resulting vector (pGW80A0001, SEQ ID NO:5) can be constructed in a variety of manners that are known to one of skill in the art. In one example, the tet(M) cassette and luxAB genes can be obtained via PCR amplification from the publically available pCN36 and pCN58 vectors (Charpentier, E., *et al.*). P_{clpB} can be obtained from PCR amplification from *S. aureus* RN4220 and terS can be obtained via PCR amplification from RN10616. A vector backbone can be obtained by removing the ermC gene from the publically available vector pCN48 (Charpentier, E., *et al.*), and the various components of the final vector pGW80A0001 can be assembled onto this vector backbone via appropriately designed restriction enzyme-based cloning.

[0098] Deletion/Complementation Packaging System: The packaging system can include the terS knockout strain ST24 complemented with the vector pGW80A0001 to generate strain GW24. As known to one of skill in the art, the manner of constructing this system can be accomplished by transformation ST24 with vector pGW80A0001. The vector pGW80A0001 can be maintained in cultures of the transformed ST24 by growing the transformant in the presence of 5 ug/mL of tetracycline.

[0099] Production of Transduction Particles Carrying Plasmid DNA: Non-replicative transduction particles carrying vector pGW80A0001 can be produced from GW24 via a

Mitomycin C-induction method that was first demonstrated in *E. coli* and is now a standard technique for obtaining prophages from lysogenized bacteria. Otsuji, N. *et al.*, *Induction of Phage Formation in the Lysogenic Escherichia coli K-12 by Mitomycin C*. *Nature*, 1959. 184(4692): p. 1079-1080. This prophage induction method results in induction of the $\phi 80\alpha$ lytic cycle in which the prophage excises from the GW24 genome, produces phage structural elements, and packages pGW80A0001 concatameric DNA in progeny phage particles. The resulting cell lysate is then collected and contains non-replicative transduction particles, each consisting of bacteriophage $\phi 80\alpha$ particles carrying a linear concatamer of pGW80A0001 DNA.

Example 2: Non-Replicative Transduction Particle-Based Reporter System.

[00100] The non-replicative transduction particles described above can be used in a reporter system for detecting the presence of viable bacteria via the expression of a reporter molecule (*e.g.* luxAB). When this transduction particle introduces a reporter vector (*e.g.* pGW80A0001) into a cell within the host range of the transduction particle, cells in which the promoter (*e.g.* P_{clpB}) is recognized by the cells transcription machinery are able to drive the expression of the reporter molecule within that cell.

[00101] To test the functionality of non-replicative transduction particles as reporters for detecting the presence of *S. aureus* cells, various MSSA/MRSA reporter assays were developed. In an embodiment, a non-replicative transduction particle was developed from a *S. aureus*-specific bacteriophage, and the bacterial luciferase genes luxAB under the control of a constitutive promoter were incorporated. When the non-replicative transduction particle delivered the reporter nucleic acid into *S. aureus*, the constitutive promoter expressed luxAB suitable for reporting on the presence of a viable *S. aureus*.

[00102] In addition, the antibiotic cefoxitin was added prior to, simultaneously with, or after the addition of the transduction particles to a sample containing *S. aureus* cells. If the cells were not phenotypically resistant to cefoxitin (*i.e.*, were not MRSA), luminescence was decreased or eliminated, indicating that the cells were MSSA. If, however, the cells were phenotypically resistant to cefoxitin (*i.e.*, were MRSA), increased or detectable luminescence was observed, indicating that the cells were MRSA.

Example 3: Growth-Independent Detection of Cells.

[00103] As an example, a test was conducted to evaluate the impact of cell replication on the ability to detect a target cell.

[00104] A *S. aureus* transduction particle and assay as described in Example 1 (see also PCT/US2014/026536, Example 2) was employed in an assay for detecting MRSA. The transduction particle causes viable *S. aureus* cells to produce bacterial luciferase that is capable of mediating a luminescence reaction that is monitored using a photomultiplier tube that measures relative light units (RLU) emitted by the luminescence reaction. When testing for MRSA, the assay employs cefoxitin such that MSSA does not produce a luminescence signal while MRSA does produce a luminescence signal in the assay. Briefly, cultures of clinical isolates of MRSA obtained from the Network for Antimicrobial Resistance in *Staphylococcus aureus* (NARSA) were prepared under limited nutrient conditions of culturing in Roswell Park Memorial Institute (RPMI) media (Fisher Scientific Company, LLC) in order to produce cell cultures with cells exhibiting limited metabolic activity – i.e. mimicking the metabolic state of cells in a natural environment. Cell cultures were normalized to an OD600 = 0.1 and used to inoculate samples. Samples were mixed with transduction particle reagent described in Example 2 (see also PCT/US2014/026536, Example 7) and cefoxitin and incubated for 4 hr at 37 °C then tested for the production of luminescence after the addition of a fatty aldehyde bacterial luciferase substrate reagent (tridecanal). In addition to running the luminescence assay, the amount of bacteria in each sample was quantified before the addition of transduction particles/incubation and after incubation/before the addition of substrate by plating an aliquot of each sample of TSB agar and enumerating the number of resulting colonies after 18-24 hours of incubation with each sample enumerated as total CFU in the sample at each time point.

[00105] FIG. 1 summarizes the data obtained from the test. FIG. 1a summarizes the ratio of CFU after (t=4h) and before (t=0h) the assay. FIG. 1b summarizes the signal produced from the samples summarized in FIG. 1a, where the dotted line at ~200 RLU is the background threshold calculated from the average background signal plus 3 times its standard deviation.

[00106] The results indicate that the assay did not require bacterial growth to detect the target cells. The analysis of bacterial growth during the assay revealed little to no growth during the assay despite a continuous increase of signal product in MRSA. As shown, FIG. 1a summarizes the ratio of CFU counts of MRSA at the end of the assay and at the beginning of the assay where most samples exhibited minimal growth during the 4-hour assay (e.g., 1-2 divisions) with two samples showing a decrease in growth (48 and 60), one sample showing no growth (51), and 3 samples exhibiting less than 0.4 divisions per hour (40, 51, and 81). As illustrated in FIG. 1b, all samples produced a positive signal during the assay. Despite

exhibiting little to no growth, all samples tested produced a positive signal (RLU) over background.

Example 4: Growth-Independent Detection of Cells from Clinical Samples.

[00107] As an example, a test was conducted to evaluate the impact of cell replication on the ability to detect a target cell directly from clinical samples.

[00108] A *S. aureus* transduction particle and assay as described in Example 1 (see also PCT/US2014/026536, Example 2) was employed in an assay for detecting MRSA. The transduction particle causes viable *S. aureus* cells to produce bacterial luciferase that is capable of mediating a luminescence reaction that is monitored using a photomultiplier tube that measures relative light units (RLU) emitted by the luminescence reaction. When testing for MRSA, the assay employs cefoxitin such that MSSA does not produce a luminescence signal while MRSA does produce a luminescence signal in the assay. Briefly, remnant nasal swab samples collected from patients by a hospital institution for the purpose of MRSA surveillance were tested for the presence of MRSA using the transduction particle assay. Samples were mixed with transduction particle reagent as described in Example 2 (see also PCT/US2014/026536, Example 7) and cefoxitin and incubated for 4 hr at 37 °C then tested for the production of luminescence after the addition of a fatty aldehyde bacterial luciferase substrate reagent (tridecanal). In addition to running the luminescence assay, the amount of bacteria in each sample was quantified before the addition of transduction particles/incubation and after incubation/before addition of substrate by plating an aliquot of each sample of TSB agar and enumerating the number of resulting colonies after 18-24 hours of incubation with each sample enumerated as total CFU in the sample at each time point.

[00109] FIG. 2 summarizes the data obtained from four clinical samples that were positive for MRSA. FIG. 2a summarizes the ratio of CFU after (t=4h) and before (t=0h) the assay. FIG. 2b summarizes the signal produced from the four clinical samples summarized in FIG. 2a, where the dotted line at ~200 RLU is the background threshold calculated from the average background signal plus 3 times its standard deviation.

[00110] The results indicate that the assay did not require bacterial growth to detect the target cells. The analysis of bacterial growth during the assay revealed little to no growth during the assay despite a continuous increase of signal product in MRSA. As shown, FIG. 2a summarizes the ratio of CFU counts of MRSA at the end of the assay and at the beginning of the assay where most samples exhibited minimal growth during the 4-hour assay (e.g., 1-2 divisions) and one sample (3037) showed a decrease in growth. As illustrated in FIG. 2b, all

samples produced a positive signal during the assay. Despite exhibiting little to no growth, all samples tested produced a positive signal (RLU) over background.

[00111] While the invention has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made therein without departing from the spirit and scope of the invention.

[00112] All references, issued patents and patent applications cited within the body of the instant specification are hereby incorporated by reference in their entirety, for all purposes.

SEQUENCES

SEQ ID NO: 1

S. aureus pT181 plasmid origin or replication copy number variant pT181cop-623 repC

TTTGCGGAAAGAGTTAGTAAGTTAACAGAAGACGAGCCAAACCTAAATGGTTTAGCAGGAACTTAGATAAAAAA
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 TGAATTTTACTGACTAGCCTTCAATATTTCC

SEQ ID NO:2

S. aureus P_{clpB} Promoter Sequence

GTCTAGTTAATGTGTAACGTAACATTAGCTAGATTTTTTTTATTCAAAAAATATTTACAAATATTAGGAAATTTA
 AGTGTAAGAGAGTTGATAAATGATTATATTGGGACTATAATATAATTAAGGTC

SEQ ID NO:3

Sequence containing native *terS* gene

AATTGGCAGTAAAGTGGCAGTTTTTGGATACCTAAAAATGAGATATTATGATAGTGTAGGATATTGACTATCTTACT
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 AAAAAGCGTATAGCGCGAGAGTTGGTGGTAAATGAAATGAACGAAAAACAAAAGAGATTTCGCAGATGAATATATA
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 ATTGGTGAGTACGATGACGAAAGTTAAATTTAACTTTAACAAACCATCTAATGTTTTCAACAG

SEQ ID NO:4

RN10616 genomic sequence loci showing the $\phi 80\alpha$ *terS* deletion and complementation. *terS*=Bracketed Text,
 Deletion=Underlined, Complement=Bold

ATTAGACAACAAACAAGTCATTGAAAATTCCGACTTATTATTCAAAAAGAAATTTGATAGCGCAGATATACAAGC
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SEQ ID NO:5

pGW80A0001 Full Sequence

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CLAIMS

What is claimed:

1. A growth-independent method for detecting a microorganism of interest in a sample, comprising:
 - contacting the sample with a plurality of non-replicative transduction particles (NRTPs) such that the plurality of NRTPs transduces one or more microorganisms of interest in the sample, wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase;
 - providing conditions for activation of the reporter nucleic acid sequence; and
 - detecting a signal produced by the reporter nucleic acid sequence, wherein the presence of the signal indicates the presence of the one or more microorganisms of interest, and wherein the absence of the signal indicates the absence of the one or more microorganisms of interest.
2. The method of claim 1, wherein the growth rate of the one or more microorganisms of interest is less than 0.1 divisions per hour, and wherein the one or more microorganisms of interest is Methicillin Resistant *Staphylococcus aureus* (MRSA).
3. The method of any preceding method claim, further comprising providing an antimicrobial agent to the sample and detecting for the signal produced by the reporter nucleic acid sequence to determine whether the one or more microorganisms of interest is susceptible or non-susceptible to the antimicrobial agent.
4. The method of any preceding method claim, wherein the one or more microorganisms of interest is in stationary phase; or wherein the one or more microorganisms of interest is undergoing no growth.
5. The method of any preceding method claim except claim 2, wherein the growth rate of the one or more microorganisms of interest is less than or equal to 0.1-5, 0.1-3, 0.1-1, 0.5-1, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, or 3 divisions per hour.
6. The method of any preceding method claim, wherein the growth rate of the one or more microorganisms of interest is less than or equal to 0-10, 1-9, 2-8, 3-7, 4-6, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cell division per 4 hour period.
7. The method of any preceding method claim, wherein the growth rate of the one or more microorganisms of interest is negative or homeostatic.
8. The method of any preceding method claim, wherein the one or more microorganisms of interest comprises a Methicillin Resistant *Staphylococcus aureus* (MRSA) cell,

Staphylococcus aureus, *Staphylococcus spp.*, Enterobacteriaceae, *Enterococcus spp.*, *Streptococcus spp.*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Stenotrophomonas spp.*, or *Mycobacterium spp.*.

9. The method of any preceding method claim, wherein the sample is a clinical sample.
10. The method of any preceding method claim, wherein the sample is a nasal swab sample, a rectal swab sample, a blood sample, a positive blood culture sample, a skin/soft tissue sample, a bronchoalveolar lavage sample, a sputum sample, a stool sample, a urine sample, and/or a sample of an isolated microorganism.
11. The method of any preceding method claim, wherein the signal is a luminescence signal; or wherein the detecting step comprises measuring the relative light units (RLU) emitted by the signal.
12. The method of any preceding method claim, wherein the providing step comprises contacting the sample with a fatty aldehyde bacterial luciferase substrate reagent, optionally wherein the reagent is tridecanal.
13. The method of any preceding method claim, wherein the signal is greater than a background threshold calculated from an average background signal plus 0-3x, 0x, 1x, 2x, or 3x the standard deviation of the average background signal.
14. The method of claim 3, wherein the antimicrobial agent is cefoxitin, a β -lactam, an extended-spectrum β -lactam, an Aminoglycoside, an Ansamycin, a Carbacephem, Carbapenems, any generation of Cephalosporin, a Glycopeptide, a Lincosamide, a Lipopeptide, a Macrolide, a Monobactam, a Nitrofurantoin, an Oxazolidinone, a Penicillin, a Polypeptide, a Quinolone, a Fluoroquinolone, a Streptogramin, a Sulfonamide, a Tetracycline, a Rifampicin, a mycobacterial antibiotic, Chloramphenicol, and/or Mupirocin.
15. The method of any preceding method claim, wherein the reporter nucleic acid sequence is a luciferase gene, optionally wherein the luciferase gene is *luxAB*, *luc*, *ruc*, or *nluc*.
16. The method of any preceding method claim, wherein the reporter nucleic acid sequence is operatively linked to a constitutive promoter, optionally wherein the constitutive promoter is a *S. aureus clpB* promoter.
17. The method of any preceding method claim, wherein said method produces at least 80-100, 80-90, 80-95, 90-95, 80, 85, 90, 95, 96, 97, 98, 99% specificity of detection with reference to a standard cell culture-based assay.

18. The method of any preceding method claim, wherein said method achieves at least 80-100, 80-90, 80-95, 90-95, 80, 85, 90, 95, 96, 97, 98, 99% sensitivity of detection with reference to a standard cell culture-based assay.
19. The method of any preceding method claim, wherein said method achieves at least 95% specificity of detection and at least 90% sensitivity of detection with reference to a standard cell culture-based assay.
20. The method of any one of claims 17-19, wherein the cells of the standard cell culture-based assay are in logarithmic phase.
21. The method of any preceding method claim, wherein the one or more microorganisms of interest is a Methicillin Resistant *Staphylococcus aureus* (MRSA) cell.
22. The method of any preceding method claim except claim 2, wherein the one or more microorganisms of interest is a Methicillin Sensitive *Staphylococcus aureus* (MSSA) cell.
23. The method of any preceding method claim, wherein the reporter nucleic acid sequence encodes a detectable or selectable marker.
24. The method of any preceding method claim, wherein the reporter nucleic acid sequence is selected from the group consisting of genes encoding enzymes mediating luminescence reactions (luxA, luxB, luxAB, luc, ruc, nluc), genes encoding enzymes mediating colorimetric reactions (lacZ, HRP), genes encoding fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent proteins), nucleic acid molecules encoding affinity peptides (His-tag, 3X-FLAG), and genes encoding selectable markers (ampC, tet(M), CAT, erm).
25. The method of any preceding method claim, wherein the reporter nucleic acid sequence is operatively linked to a constitutive promoter.
26. The method of any preceding method claim, wherein the signal can be detected at a limit of detection (LoD) of less than 1-1000, 10-100, 100-1000, 10, 100, 1,000 colony forming units (CFU).
27. The method of claim 26, wherein the signal can be detected at a LoD of less than or equal to one to five, two to four, three to five or five CFU, or wherein the signal can be detected at a LoD of less than or equal to three CFU, or wherein the signal can be detected at a LoD of less than or equal to two CFU, or wherein the signal can be detected at a LoD of less than or equal to one CFU.
28. The method of any preceding method claim, further comprising providing an antibiotic to the sample at a pre-determined concentration and detecting a presence or absence of the

signal to determine whether the one or more microorganisms of interest is resistant or sensitive to the antibiotic.

29. The method of any preceding method claim, further comprising providing varying pre-determined concentrations of antibiotic to the sample and detecting the amount of the signal to determine the minimum inhibitory concentration of the one or more microorganisms of interest to the antibiotic.
30. A sample or cell culture comprising a plurality of non-replicative transduction particles (NRTPs) and one or more microorganisms of interest, optionally wherein the plurality of NRTPs comprises a reporter nucleic acid sequence, and wherein the growth rate of the one or more microorganisms of interest is less than logarithmic phase.
31. The sample or cell culture of any preceding sample or cell culture claim, wherein the growth rate of the one or more microorganisms of interest is less than or equal to 0.1 divisions per hour, and wherein the one or more microorganisms of interest is Methicillin Resistant *Staphylococcus aureus* (MRSA).
32. The sample or cell culture of any preceding sample or cell culture claim, further comprising an antimicrobial agent.
33. The sample or cell culture of any preceding sample or cell culture claim, wherein the one or more microorganisms of interest is in stationary phase; or wherein the one or more microorganisms of interest is undergoing no growth.
34. The sample or cell culture of any preceding sample or cell culture claim, wherein the growth rate of the one or more microorganisms of interest is less than or equal to 0.1-5, 0.1-3, 0.1-1, 0.5-1, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, or 3 divisions per hour.
35. The sample or cell culture of any preceding sample or cell culture claim, wherein the growth rate of the one or more microorganisms of interest is less than or equal to 0-10, 1-9, 2-8, 3-7, 4-6, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 cell division per 4 hour period.
36. The sample or cell culture of any preceding sample or cell culture claim, wherein the growth rate of the one or more microorganisms of interest is negative or homeostatic.
37. The sample or cell culture of any preceding sample or cell culture claim, wherein the one or more microorganisms of interest comprises a Methicillin Resistant *Staphylococcus aureus* (MRSA) cell, *Staphylococcus aureus*, *Staphylococcus spp.*, Enterobacteriaceae, *Enterococcus spp.*, *Streptococcus spp.*, *Acinetobacter spp.*, *Pseudomonas spp.*, *Stenotrophomonas spp.*, or *Mycobacterium spp.*.
38. The sample or cell culture of any preceding sample or cell culture claim, wherein the sample is a clinical sample.

39. The sample or cell culture of any preceding sample or cell culture claim, wherein the sample is a nasal swab sample, a rectal swab sample, a blood sample, a positive blood culture sample, a skin/soft tissue sample, a bronchoalveolar lavage sample, a sputum sample, a stool sample, a urine sample, and/or a sample of an isolated microorganism.
40. The sample or cell culture of any preceding sample or cell culture claim, further comprising a fatty aldehyde bacterial luciferase substrate reagent, optionally wherein the reagent is tridecanal.
41. The sample or cell culture of claim 32, wherein the antimicrobial agent is cefoxitin, a β -lactam, an extended-spectrum β -lactam, an Aminoglycoside, an Ansamycin, a Carbacephem, Carbapenems, any generation of Cephalosporin, a Glycopeptide, a Lincosamide, a Lipopeptide, a Macrolide, a Monobactam, a Nitrofurantoin, an Oxazolidinone, a Penicillin, a Polypeptide, a Quinolone, a Fluoroquinolone, a Streptogramin, a Sulfonamide, a Tetracycline, a Rifampicin, a mycobacterial antibiotic, Chloramphenicol, and/or Mupirocin.
42. The sample or cell culture of any preceding sample or cell culture claim, wherein the reporter nucleic acid sequence is a luciferase gene, optionally wherein the luciferase gene is *luxAB*, *luc*, *ruc*, or *nluc*.
43. The sample or cell culture of any preceding sample or cell culture claim, wherein the reporter nucleic acid sequence is operatively linked to a constitutive promoter, optionally wherein the constitutive promoter is a *S. aureus clpB* promoter.
44. The sample or cell culture of any preceding sample or cell culture claim, wherein the one or more microorganisms of interest is a Methicillin Resistant *Staphylococcus aureus* (MRSA) cell.
45. The sample or cell culture of any preceding sample or cell culture claim, wherein the one or more microorganisms of interest is a Methicillin Sensitive *Staphylococcus aureus* (MSSA) cell.
46. The sample or cell culture of any preceding sample or cell culture claim, wherein the reporter nucleic acid sequence encodes a detectable or selectable marker.
47. The sample or cell culture of any preceding sample or cell culture claim, wherein the reporter nucleic acid sequence is selected from the group consisting of genes encoding enzymes mediating luminescence reactions (*luxA*, *luxB*, *luxAB*, *luc*, *ruc*, *nluc*), genes encoding enzymes mediating colorimetric reactions (*lacZ*, HRP), genes encoding fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared

fluorescent proteins), nucleic acid molecules encoding affinity peptides (His-tag, 3X-FLAG), and genes encoding selectable markers (ampC, tet(M), CAT, erm).

48. The sample or cell culture of any preceding sample or cell culture claim, wherein the reporter nucleic acid sequence is operatively linked to a constitutive promoter.