or fungi has caused an infection in the blood. Blood cultures are collected separately from other blood tests and often they are taken more than once from different veins. It can take several days to get the results of a blood culture. Due to the *in vitro* culture conditions, only a third to a half of people with sepsis will have blood cultures that are positive, meaning that bacteria actually grow and proliferate in the *in vitro* conditions.

SUMMARY

[0004] Currently, detection of bacteria often requires culturing in order to (1) isolate bacteria for analysis and (2) reduce any contaminating background cells or other material that could make analysis difficult or impossible. For example, in patients with sepsis, blood must be cultured in order to isolate pathogenic bacteria. Similarly, in the monitoring of food, samples must be cultured in order to isolate contaminating microbes. Unfortunately, the standard culturing process can take several days. In the case of sepsis, this lag time can lead to the unnecessary administration of antibiotics or a misdiagnosis, leading to patient complications or death. In the food industry, this lag time delays information that would lead to recalls or other preventive measures. Thus, the rapid detection of active infections can enable measures that can reduce problems and save human lives.

[0005] While PCR detection methods may not require extended culturing, PCR, in contrast to unbiased sequencing approaches, requires *a priori* knowledge of the genome sequence of the organisms of interest. That is, a researcher has to know what they are looking for, and this likely will not be the case for rare or undiscovered organisms. Additionally, PCR-dependent methods only detect the presence of genetic material in the sample and cannot distinguish whether that material came from a live or dead organism. In many cases, the identification of active infections caused by live microorganisms is the most important consideration for treatment options, or even for identification of contaminants in foodstuffs or the environment.

[0006] In one example, the disclosure provides a method for the identification and analysis of viable and/or proliferating microorganisms in a sample, comprising:(a) obtaining a sample having or suspected of

having one or more types of microorganisms; (b) incubating the sample in the presence of one or more types of nucleoside or nucleotide analogs, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids; (c) labelling newly synthesized microbial nucleic acids by contacting the newly synthesized microbial nucleic acids with a labelling reagent that selectively binds to or with the one or more types of nucleoside or nucleotide analogs; (d) isolating or purifying the labelled newly synthesized microbial nucleic acids; and (e) determining the identity of the viable and/or proliferating microorganisms in the sample based upon sequencing or determining the identity of the isolated or purified newly synthesized microbial nucleic acids. In one aspect, the present disclosure refers to a method for the identification and analysis of viable and/or proliferating microorganisms in a sample, comprising: (a) obtaining a sample having or suspected of having one or more types of microorganisms; (b) incubating the sample in the presence of one or more types of nucleoside or nucleotide analogs, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids; (c) labelling newly synthesized microbial nucleic acids by contacting the newly synthesized microbial nucleic acids with an azide-linker-biotin labelling reagent that selectively binds to or with the one or more types of nucleoside or nucleotide analogs; (d) isolating or purifying the labelled newly synthesized microbial nucleic acids using a streptavidin or avidin solid support pull-down agent; (e) cleaving the linker to obtain isolated or purified microbial nucleic acids from the pull-down agent; and (f) determining the identity of the viable and/or proliferating microorganisms in the sample based upon sequencing or determining the identity of the isolated or purified newly synthesized microbial nucleic acids. In another example, the sample is obtained from a subject suspected of having or having a microbial infection. In yet another example, the subject is suspected of having or has sepsis. In a further example, for (a), the obtained sample is processed using a dehosting method prior to (b) in order to selectively remove non-microbial nucleic acids. In yet a further example, the dehosting method comprises: removing non-microbial nucleic acids by: (i) selectively cleaving non-microbial DNA by contacting the obtained sample with a recombinant protein comprising: a binding domain that selectively binds to non-microbial nucleic acids bound by histone(s) or to non-microbial nucleic acids comprising methylated CpG residues, and a nuclease domain having activity to cleave nucleic acids; or (ii) use of an affinity agent that is bound to a solid substrate that selectively binds to nucleic acids bound by histone(s) or selectively binds to methylated CpG residues of non-microbial nucleic acids. In a certain example, the sample is an environmental sample obtained from an environmental test site. In another example, the environmental site is being tested for microbial contamination. In yet another example, the sample is a sample obtained from a foodstuff suspected of microbial contamination. In a further example, the one or more types of microorganisms are bacteria, fungi, viruses, algae, archaea, and/or protozoa. In yet a further example, the bacteria are selected from *Actinomyces israelii*, *Bacillus anthracis*, *Bacillus cereus*, *Bartonella henselae*, *Bartonella quintana*, *Bordetella pertussis*,

or purified newly synthesized microbial nucleic acids to bead-linked transposomes, wherein the bead-linked transposomes mediate the simultaneous fragmentation of microbial nucleic acids and the addition of sequencing primers; (b) amplifying the microbial nucleic acid fragments with primers that comprise index and adapter sequences to form library of amplified products; (c) washing and pooling the library of amplified products; (d) sequencing the library of amplified products; and (e) determining the identity of the viable and/or proliferating microorganisms based upon correlating the sequences obtained from the library of amplified products with databases of known sequences of microorganisms using bioinformatic analysis. In another example, the newly synthesized microbial nucleic acids are RNA, wherein the microbial RNA is reversed transcribed into cDNA prior (e) described above, and wherein the gene expression of the viable and/or proliferating microorganisms can be determined based on analyzing the expression level of gene products from newly synthesized microbial RNA using a microarray and/or by sequencing.

[0007] In a particular example, the disclosure also provides a method for determining the effectiveness of an antimicrobial agent in modulating the growth and proliferation of microorganism(s) in a sample, comprising: (a) obtaining a sample having or suspected of having one or more types of microorganisms; (b) splitting the sample into two samples, a control sample and a treated sample; (c) incubating the control sample in the presence of one or more types of nucleoside or nucleotide analogs, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids; (c') incubating the treated sample in the presence of one or more types of nucleoside or nucleotide analogs and an antimicrobial agent, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids; (d) labelling newly synthesized microbial nucleic acids of the control sample and the treated sample by contacting the newly synthesized microbial nucleic acids with a labelling reagent that selectively binds to or with the one or more types of nucleoside or nucleotide analogs; (e) isolating or purifying the labelled newly synthesized microbial nucleic acids

from the control sample and the treated sample; (f) determining the gene expression level, and/or amounts or identity of the isolated or purified newly synthesized microbial nucleic acids in the control sample; (f') determining the gene expression level, and/or amounts and identity of the isolated or purified newly synthesized microbial nucleic acids in the treated sample; (g) comparing and determining any changes in the gene expression level and/or amounts and/or identity of the isolated or purified newly synthesized microbial nucleic acids in the control sample with the gene expression level and/or amounts or identity of the isolated or purified newly synthesized microbial nucleic acids in the treated sample, wherein if there is a decrease in the gene expression level of the newly synthesized microbial nucleic acids in the treated sample v. the control sample, or there is decrease in the amounts and/or identity of the newly synthesized microbial nucleic acids in the treated sample v. the control sample indicates that the antimicrobial agent is effective in modulating the growth and proliferation of the microorganism(s). In one aspect, the present disclosure refers to a method for determining the effectiveness of an antimicrobial agent in modulating the growth and proliferation of microorganism(s) in a sample, comprising: (a) obtaining a sample having or suspected of having one or more types of microorganisms; (b) splitting the sample into two samples, a control sample and a treated sample; (c) incubating the control sample in the presence of one or more types of nucleoside or nucleotide analogs, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids; (c') incubating the treated sample in the presence of one or more types of nucleoside or nucleotide analogs and an antimicrobial agent, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids; (d) labelling newly synthesized microbial nucleic acids of the control sample and the treated sample by contacting the newly synthesized microbial nucleic acids with an azide-linker-biotin labelling reagent that selectively binds to or with the one or more types of nucleoside or nucleotide analogs; (e) isolating or purifying the labelled newly synthesized microbial nucleic acids from the control sample and the treated sample using a streptavidin or avidin solid support pull-down agent;

(f) determining the gene expression level, and/or amounts or identity of the isolated or purified newly synthesized microbial nucleic acids in the control sample; (f') determining the gene expression level, and/or amounts and identity of the isolated or purified newly synthesized microbial nucleic acids in the treated sample; and (g) comparing and determining any changes in the gene expression level and/or amounts and/or identity of the isolated or purified newly synthesized microbial nucleic acids in the control sample with the gene expression level and/or amounts or identity of the isolated or purified newly synthesized microbial nucleic acids in the treated sample, wherein if there is a decrease in the gene expression level of the newly synthesized microbial nucleic acids in the treated sample v. the control sample, or there is decrease in the amounts and/or identity of the newly synthesized microbial nucleic acids in the treated sample v. the control sample indicates that the antimicrobial agent is effective in modulating the growth and proliferation of the microorganism(s). In another example, the antimicrobial agent is selected from an antibiotic, an antifungal, and an antiviral. In a further example, the antibiotic is selected from amoxicillin, ampicillin, bacampicillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, nafcillin, oxacillin, penicillin G, penicillin V, piperacillin, pivampicillin, pivmecillinam, ticarcillin, cefacetrile, cefadroxil, cefalexin, cefaloglycin, cefalonium, cefaloridine, cefalotin, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefradine, cefroxadine, ceftezole, cefaclor, cefamandole, cefmetazole, cefonicid, cefotetan, cefoxitin, cefprozil, cefuroxime, cefuzonam, cefcapene, cefdaloxime, cefdinir, cefditoren, cefetamet, cefixime, cefmenoxime, cefodizime, cefotaxime, cefpimizole, cefpodoxime, cefteram, ceftibuten, ceftiofur, ceftiolene, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefclidine, cefepime, cefluprenam, cefoselis, cefozopran, cefpirome, cefquinome, ceftobiprole, ceftaroline, cefaclomezine, cefaloram, cefaparole, cefcanel, cefedrolor, cefempidone, cefetrizole, cefivitril, cefmatilen, cefmepidium, cefovecin, cefoxazole, cefrotil, cefsumide, cefuracetime, ceftioxide, aztreonam, imipenem, doripenem, ertapenem,

WHAT IS CLAIMED IS:

1. A method for the identification and analysis of viable and/or proliferating microorganisms in a sample, comprising:

(a) obtaining a sample having or suspected of having one or more types of microorganisms;

(b) incubating the sample in the presence of one or more types of nucleoside or nucleotide analogs, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids;

(c) labelling newly synthesized microbial nucleic acids by contacting the newly synthesized microbial nucleic acids with an azide-linker-biotin labelling reagent that selectively binds to or with the one or more types of nucleoside or nucleotide analogs;

(d) isolating or purifying the labelled newly synthesized microbial nucleic acids using a streptavidin or avidin solid support pull-down agent;

(e) cleaving the linker to obtain isolated or purified microbial nucleic acids from the pull-down agent; and

(f) determining the identity of the viable and/or proliferating microorganisms in the sample based upon sequencing or determining the identity of the isolated or purified newly synthesized microbial nucleic acids.

2. The method of claim 1, wherein the sample is obtained from a subject suspected of having or having a microbial infection.

3. The method of claim 2, wherein the subject is suspected of having or has sepsis.

4. The method of any one of claims 1 to 3, wherein for (a), the obtained sample is processed using a dehosting method prior to (b) in order to selectively remove nonmicrobial nucleic acids.

5. The method of claim 4, wherein the dehosting method comprises:

removing nonmicrobial nucleic acids by:

(a) selectively cleaving nonmicrobial DNA by contacting the obtained sample with a recombinant protein comprising: a binding domain that selectively binds to nonmicrobial nucleic acids bound by histone(s) or to nonmicrobial nucleic acids comprising methylated CpG residues, and a nuclease domain having activity to cleave nucleic acids; or
(b) use of an affinity agent that is bound to a solid substrate that selectively binds to nucleic acids bound by histone(s) or selectively binds to methylated CpG residues of nonmicrobial nucleic acids.

6. The method of claim 1, wherein the sample is an environmental sample obtained from an environmental test site.

7. The method of claim 6, wherein the environmental site is being tested for microbial contamination.

8. The method of claim 1, wherein the sample is a sample obtained from a foodstuff suspected of microbial contamination.

9. The method of any one of the preceding claims, wherein the one or more types of microorganisms are bacteria, fungi, viruses, algae, archaea, and/or protozoa.

10. The method of claim 9, wherein the bacteria are selected from *Actinomyces israelii, Bacillus anthracis, Bacillus cereus, Bartonella henselae, Bartonella quintana, Bordetella pertussis, Borrelia burgdorferi, Borrelia garinii, Borrelia afzelii, Borrelia recurrentis, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydophila psittaci, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Corynebacterium diphtheriae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Leptospira interrogans, Leptospira santarosai, Leptospira weilii, Leptospira noguchii, Listeria monocytogenes, Mycobacterium leprae,*

Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pseudomonas aeruginosa, Rickettsia rickettsia, Salmonella typhi, Salmonella typhimurium, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Yersinia pestis, Yersinia enterocolitica, and/or *Yersinia pseudotuberculosis*.

11. The method of claim 9, wherein the fungi are selected from *Absidia corymbifera, Absidia ramose, Achorion gallinae, Actinomadura spp., Ajellomyces dermatididis, Aleurisma brasiliensis, Allersheria boydii, Arthroderma spp., Aspergillus flavus, Aspergillus fumigatu, Basidiobolus spp, Blastomyces spp, Cadophora spp, Candida albicans, Cercospora apii, Chrysosporium spp, Cladosporium spp, Cladothrix asteroids, Coccidioides immitis, Cryptococcus albidus, Cryptococcus gattii, Cryptococcus laurentii, Cryptococcus neoformans, Cunninghamella elegans, Dematium wernecke, Discomyces israelii, Emmonsia spp, Emmonsiella capsulate, Endomyces geotrichum, Entomophthora coronate, Epidermophyton floccosum, Filobasidiella neoformans, Fonsecaea spp., Geotrichum candidum, Glenospora khartoumensis, Gymnoascus gypseus, Haplosporangium parvum, Histoplasma, Histoplasma capsulatum, Hormiscium dermatididis, Hormodendrum spp., Keratinomyces spp, Langeronia soudanense, Leptosphaeria senegalensis, Lichtheimia corymbifera, Lobmyces loboi., Loboa loboi, Lobomycosis, Madurella spp., Malassezia furfur, Micrococcus pelletieri, Microsporum spp, Monilia spp., Mucor spp., Mycobacterium tuberculosis, Nannizzia spp., Neotestudina rosatii, Nocardia spp., Oidium albicans, Oospora lactis, Paracoccidioides brasiliensis, Petriellidium boydii, Phialophora spp., Piedraia hortae, Pityrosporum furfur, Pneumocystis jirovecii (or Pneumocystis carinii), Pullularia gougerotii, Pyrenochaeta romeroi, Rhinosporidium seeberi, Sabouraudites (Microsporum), Sartorya fumigate, Sepedonium, Sporotrichum spp., Stachybotrys, Stachybotrys chartarum, Streptomyce spp., Tinea spp., Torula spp, Trichophyton spp, Trichosporon spp,* and/or *Zopfia rosatii*.

12. The method of claim 9, wherein the viruses are selected from *Simplexvirus*, *Varicellovirus*, *Cytomegalovirus*, *Roseolovirus*, *Lympho-cryptovirus*, *Rhadinovirus*, *Mastadenovirus*, *α-Papillomavirus*, *β-Papillomavirus*, *X-Papillomavirus*, *γ-Papillomavirus*, *Mupapillomavirus*, *Nupapillomavirus*, *Alphapolyomavirus*, *Betapolyomavirus*, *γ-Polyomavirus*, *Deltapolyomavirus*, *Molluscipoxvirus*, *Orthopoxvirus*, *Parapoxvirus*, *α-Torquevirus*, *β-Torquevirus*, *γ-Torquevirus*, *Cyclovirus*, *Gemycircular*, *Gemykibivirus*, *Gemyvongvirus*, *Erythrovirus*, *Dependovirus*, *Bocavirus*, Orthohepadnavirus, *Gammaretrovirus*, *Deltaretrovirus*, *Lentivirus*, *Simiispumavirus*, *Coltivirus*, *Rotavirus*, *Seadornavirus*, *α-Coronavirus*, *β-Coronavirus*, *Torovirus*, *Mamastrovirus*, *Norovirus*, *Sapovirus*, *Flavivirus*, *Hepacivirus*, *Pegivirus*, *Orthohepevirus*, *Cardiovirus*, *Cosavirus*, *Enterovirus*, *Hepatovirus*, *Kobuvirus*, *Parechovirus*, *Rosavirus*, *Salivirus*, *Alphavirus*, *Rubivirus*, *Ebolavirus*, *Marburgvirus*, *Henipavirus*, *Morbilivirus*, *Respirovirus*, *Rubulavirus*, *Metapneumovirus*, *Orthopneumovirus*, *Ledantevirus*, *Lyssavirus*, *Vesiculovirus*, *Mammarenavirus*, *Orthohantavirus*, *Orthonairovirus*, *Orthobunyavirus*, *Phlebovirus*, *α-Influenzavirus*, *β-Influenzavirus*, *γ-Influenzavirus*, *Quaranjavirus*, *Thogotovirus*, and/or *Deltavirus*.

13. The method of any one of the preceding claims, wherein the one or more types of nucleoside or nucleotide analogs are selected from 2-ethynyl-adenosine, N6-propargyl-adenosine, 2'-(O-propargyl)-adenosine, 3'-(O-propargyl)-adenosine, 5-ethynyl-cytidine, 5-ethynyl-2'-deoxycytidine, 2'-(O-propargyl)-cytidine, 3'-(O-propargyl)-cytidine, 2'-(O-propargyl)-guanosine, 3'-(O-propargyl)-guanosine, 5-ethynyl-uridine, 5-ethynyl-2'-deoxyuridine, 2'-(O-propargyl)-uridine, 3'-(O-propargyl)-uridine, (2'S)-2'-deoxy-2'-fluoro-5-ethynyluridine, (2'S)-2'-fluoro-5-ethynyluridine, 2'(S)-2'-deoxy-2'-fluoro-5-ethynyluridine, (2'S)-2'-fluoro-5-ethynyluridine, 8-azido-adenosine, N^6-(6-azido)hexyl-2'deoxy-adenosine, 2'-azido-2'-deoxyadenosine, 5-azidomethyl-uridine, 5-(15-azido-4,7,10,13-tetraoxa-pentadecanoyl-aminoallyl)-2'-deoxyuridine, 5-(3-azidopropyl)-uridine, 5-azido-PEG_4-uridine, 5-azido-PEG_4-cytidine, 5-

azido-PEG_4-2'-deoxycytidine, 5-bromo-2'deoxyuridine, 5-bromouridine, 5-iodo-2'deoxyuridine, and 5-iodouridine.

14. The method of any one of the preceding claims, wherein the one or more types of nucleoside or nucleotide analogs are selected from 2-ethynyl-adenosine, N6-propargyl-adenosine, 2'-(O-propargyl)-adenosine, 3'-(O-propargyl)-adenosine, 5-ethynyl-cytidine, 5-ethynyl-2'-deoxycytidine, 2'-(O-propargyl)-cytidine, 3'-(O-propargyl)-cytidine, 2'-(O-propargyl)-guanosine, 3'-(O-propargyl)-guanosine, 5-ethynyl-uridine, 5-ethynyl-2'-deoxyuridine, 2'-(O-propargyl)-uridine, 3'-(O-propargyl)-uridine, (2'S)-2'-deoxy-2'-fluoro-5-ethynyluridine, (2'S)-2'-fluoro-5-ethynyluridine, 2'(S)-2'-deoxy-2'-fluoro-5-ethynyluridine, and (2'S)-2'-fluoro-5-ethynyluridine.

15. The method of any one of the preceding claims, wherein the one or more types of nucleoside or nucleotide analogs are selected from 8-azido-adenosine, N^6-(6-azido)hexyl-2'deoxy-adenosine, wherein the one or more types of nucleoside or nucleotide analogs are selected from 2'-azido-2'-deoxyadenosine, 5-azidomethyl-uridine, 5-(15-azido-4,7,10,13-tetraoxa-pentadecanoyl-aminoallyl)-2'-deoxyuridine, 5-(3-azidopropyl)-uridine, 5-azido-PEG_4-uridine, 5-azido-PEG_4-cytidine, and 5-azido-PEG_4-2'-deoxycytidine.

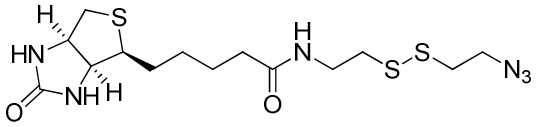
16. The method of any one of the preceding claims, wherein the one or more types of nucleoside or nucleotide analogs are selected from 5-bromo-2'deoxyuridine, 5-bromouridine, 5-iodo-2'deoxyuridine, and 5-iodouridine.

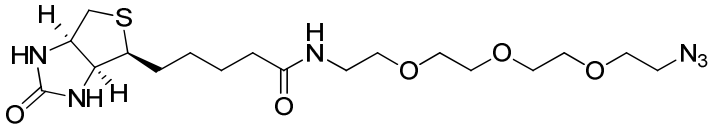
17. The method of any one of the preceding claims, wherein the sample is incubated in the presence of one or more types of nucleoside or nucleotide analogs for 5 min to 180 min.

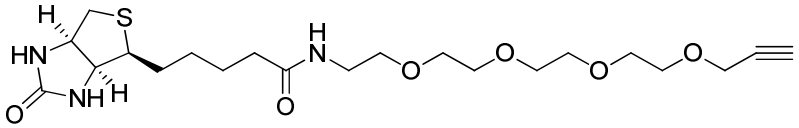
18. The method of claim 17, wherein the sample is incubated in the presence of one or more types of nucleoside or nucleotide analogs for 30 min to 120 min.

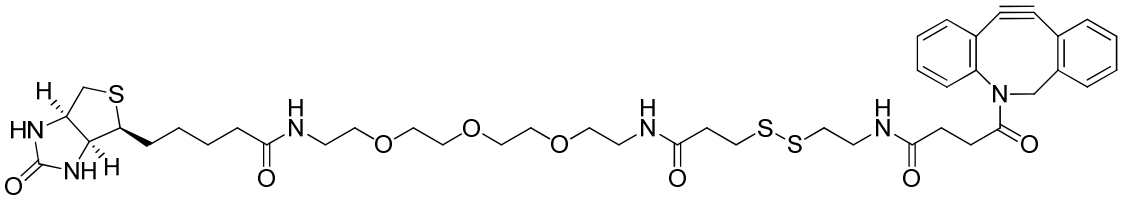
19. The method of any one of claims 1 to 18, wherein the labelling reagent binds to or with the one or more types of nucleoside or nucleotide analogs via click chemistry, a strained [3+2] cycloaddition reaction, or a Staudinger ligation.

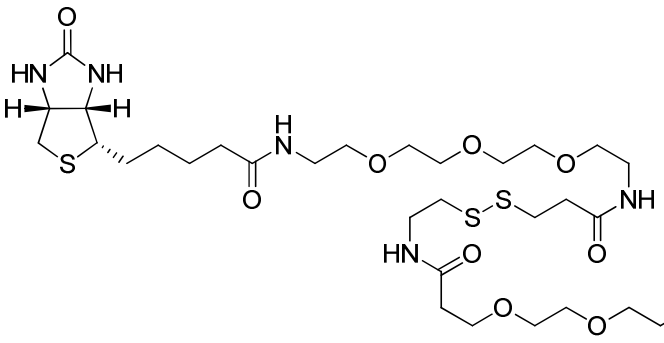
20. The method of claim 1, wherein the labelling reagent comprising a biotin group is selected from:


,


,


,




, and

21. The method of any one of the preceding claims, wherein the linker of the labelling reagent comprises a chemically cleavable linker or enzymatically cleavable linker.

22. The method of claim 21, wherein the cleavable linker is an acid-labile-based linker or a disulfide-based linker.

23. The method of claim 22, wherein the acid-labile-based linker comprises hydrazone or *cis*-aconityl groups.

24. The method of claim 21, wherein the enzymatically cleavable linker comprises a peptide-based linker or a β-glucuronide-based linker.

25. The method of claim 1, where the solid support pull-down agent is nano- or micro- materials, beads or a plate.

26. The method of claim 1, wherein the isolated or purified newly synthesized microbial nucleic acids are sequenced using a transposome-based sequencing method.

27. The method of claim 26, wherein sequencing of the newly synthesized microbial nucleic acids is by:

(a) applying the isolated or purified newly synthesized microbial nucleic acids to bead-linked transposomes, wherein the bead-linked transposomes mediate the simultaneous fragmentation of microbial nucleic acids and the addition of sequencing primers;

(b) amplifying the microbial nucleic acid fragments with primers that comprise index and adapter sequences to form library of amplified products;

(c) washing and pooling the library of amplified products;

(d) sequencing the library of amplified products; and

(e) determining the identity of the viable and/or proliferating microorganisms based upon correlating the sequences obtained from the library of amplified products with databases of known sequences of microorganisms using bioinformatic analysis.

28. The method of any one of the preceding claims, wherein the newly synthesized microbial nucleic acids are RNA, wherein the microbial RNA is reversed transcribed into cDNA prior to (f) of claim 1, and wherein the gene expression of the viable and/or proliferating microorganisms can be determined based on analyzing

the expression level of gene products from newly synthesized microbial RNA using a microarray and/or by sequencing.

29. A method for determining the effectiveness of an antimicrobial agent in modulating the growth and proliferation of microorganism(s) in a sample, comprising:

(a) obtaining a sample having or suspected of having one or more types of microorganisms;

(b) splitting the sample into two samples, a control sample and a treated sample;

(c) incubating the control sample in the presence of one or more types of nucleoside or nucleotide analogs, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids;

(c') incubating the treated sample in the presence of one or more types of nucleoside or nucleotide analogs and an antimicrobial agent, wherein the one or more types of nucleoside or nucleotide analogs are incorporated into newly synthesized microbial nucleic acids;

(d) labelling newly synthesized microbial nucleic acids of the control sample and the treated sample by contacting the newly synthesized microbial nucleic acids with an azide-linker-biotin labelling reagent that selectively binds to or with the one or more types of nucleoside or nucleotide analogs;

(e) isolating or purifying the labelled newly synthesized microbial nucleic acids from the control sample and the treated sample using a streptavidin or avidin solid support pull-down agent;

(f) determining the gene expression level, and/or amounts or identity of the isolated or purified newly synthesized microbial nucleic acids in the control sample;

(f') determining the gene expression level, and/or amounts and identity of the isolated or purified newly synthesized microbial nucleic acids in the treated sample; and

(g) comparing and determining any changes in the gene expression level and/or amounts and/or identity of the isolated or purified newly synthesized microbial nucleic acids in the control sample with the gene expression level and/or amounts or identity of

the isolated or purified newly synthesized microbial nucleic acids in the treated sample,

wherein if there is a decrease in the gene expression level of the newly synthesized microbial nucleic acids in the treated sample v. the control sample, or there is decrease in the amounts and/or identity of the newly synthesized microbial nucleic acids in the treated sample v. the control sample indicates that the antimicrobial agent is effective in modulating the growth and proliferation of the microorganism(s).

30. The method of claim 29, wherein the antimicrobial agent is selected from an antibiotic, an antifungal, and an antiviral.

31. The method of claim 30, wherein the antibiotic is selected from amoxicillin, ampicillin, bacampicillin, carbenicillin, cloxacillin, dicloxacillin, flucloxacillin, mezlocillin, nafcillin, oxacillin, penicillin G, penicillin V, piperacillin, pivampicillin, pivmecillinam, ticarcillin, cefacetrile, cefadroxil, cefalexin, cefaloglycin, cefalonium, cefaloridine, cefalotin, cefapirin, cefatrizine, cefazaflur, cefazedone, cefazolin, cefradine, cefroxadine, ceftezole, cefaclor, cefamandole, cefmetazole, cefonicid, cefotetan, cefoxitin, cefprozil, cefuroxime, cefuzonam, cefcapene, cefdaloxime, cefdinir, cefditoren, cefetamet, cefixime, cefmenoxime, cefodizime, cefotaxime, cefpimizole, cefpodoxime, cefteram, ceftibuten, ceftiofur, ceftiolene, ceftizoxime, ceftriaxone, cefoperazone, ceftazidime, cefclidine, cefepime, cefluprenam, cefoselis, cefozopran, cefpirome, cefquinome, ceftobiprole, ceftaroline, cefaclomezine, cefaloram, cefaparole, cefcanel, cefedrolor, cefempidone, cefetrizole, cefivitril, cefmatilen, cefmepidium, cefovecin, cefoxazole, cefrotil, cefsumide, cefuracetime, ceftioxide, aztreonam, imipenem, doripenem, ertapenem, meropenem, azithromycin, erythromycin, clarithromycin, dirithromycin, roxithromycin, telithromycin, clindamycin, lincomycin, amikacin, gentamicin, kanamycin, neomycin, netilmicin, paromomycin, streptomycin, tobramycin, flumequine, nalidixic acid, oxolinic acid, piromidic acid, pipemidic acid, rosoxacin, ciprofloxacin, enoxacin, lomefloxacin, nadifloxacin, norfloxacin,

ofloxacin, pefloxacin, rufloxacin, balofloxacin, gatifloxacin, grepafloxacin, levofloxacin, moxifloxacin, pazufloxacin, sparfloxacin, temafloxacin, tosufloxacin, besifloxacin, delafloxacin, clinafloxacin, gemifloxacin, prulifloxacin, sitafloxacin, trovafloxacin, sulfamethizole, sulfamethoxazole, sulfisoxazole, trimethoprim-sulfamethoxazole, demeclocycline, doxycycline, minocycline, oxytetracycline, tetracycline, tigecycline, vancomycin, teicoplanin, telavancin, linezolid, cycloserine, rifampin, rifabutin, rifapentine, rifalazil, viomycin, capreomycin, bacitracin, polymyxin B, chloramphenicol, metronidazole, tinidazole, and nitrofurantoin.

32. The method of claim 30, wherein the antifungal is selected from amorolfine, butenafine, naftifine, terbinafine, bifonazole, butoconazole, clotrimazole, econazole, fenticonazole, ketoconazole, isoconazole, luliconazole, miconazole, omoconazole, oxiconazole, sertaconazole, sulconazole, tioconazole, terconazole, albaconazole, efinaconazole, fluconazole, isavuconazole, itraconazole, posaconazole, ravuconazole, terconazole, voriconazole, abafungin, amphotericin B, nystatin, natamycin, trichomycin, anidulafungin, caspofungin, micafungin, tolnaftate, flucytosine, butenafine, griseofulvin, ciclopirox, selenium sulfide, tavaborole.

33. The method of claim 30, wherein the antiviral is selected from acyclovir, brivudine, docosanol, famciclovir, foscarnet, idoxuridine, penciclovir, trifluridine, vidarabine, cytarabine, valacyclovir, tromatandine, pritelivir, amantadine, rimantadine, oseltamivir, peramivir, zanamivir, asunaprevir, boceprevir, ciluprevir, danoprevir, faldaprevir, glecaprevir, grazoprevir, narlaprevir, paritaprevir, simeprevir, sovaprevir, telaprevir, vaniprevir, vedroprevir, voxilaprevir, daclatasvir, elbasvir, ledipasvir, odalasvir, ombitasvir, pibrentasvir, ravidasvir, ruzasvir, samatasvir, velpatasvir, beclabuvir, dasabuvir, deleobuvir, filibuvir, setrobuvir, sofosbuvir, radalbuvir, uprifosbuvir, lamivudine, telbivudine, clevudine, adefovir, tenofvir disoproxil, tenofovir alafenamide, enfuvirtide, maraviroc, vicriviroc, cenicriviroc, PRO 140, ibalizumab, fostemsavir,

didanosine, emtricitabine, lamivudine, stavudine, zidovudine, amdoxovir, apricitabine, censavudine, elvucitabine, racivir, stampidine, 4'-ethynyl-2-fluoro-2'-deoxyadenosine, zalcitabine, efavirenz, nevirapine, delavirdine, etravirine, rilpivirine, doravirine, dolutegravir, elvitegravir, raltegravir, BI 224436, cabotegravir, bictegravir, MK-2048, bevirimat, BMS-955176, amprenavir, fosamprenavir, indinavir, lopinavir, nelfinavir, ritonavir, saquinavir, atazanavir, darunavir, tipranavir, dolutegravir, elvitegravir, raltegravir, BI 224436, cabotegravir, bictegravir, MK-2048, cobicistat, ritonavir, interferon-α, peginterferon-α, methisazone, rifampicin, imiquimod, resiquimod, podophyllotoxin, fomivirsen, cidofovir, pleconaril, favipiravir, galidesivir, remdesivir, mericitabine, MK-608, NITD008, moroxydine, tromantadine, and triazavirin.

34. The method of any one of claim 29 to 33, wherein the sample is obtained from a subject suspected of having or having a microbial infection.

35. The method of claim 34, wherein the subject is suspected of having or has sepsis.

36. The method of any one of claims 29 to 35, wherein the one or more types of microorganisms are bacteria, fungi, and/or viruses.

37. The method of claim 36, wherein the bacteria are selected from *Actinomyces israelii, Bacillus anthracis, Bacillus cereus, Bartonella henselae, Bartonella quintana, Bordetella pertussis, Borrelia burgdorferi, Borrelia garinii, Borrelia afzelii, Borrelia recurrentis, Brucella abortus, Brucella canis, Brucella melitensis, Brucella suis, Campylobacter jejuni, Chlamydia pneumoniae, Chlamydia trachomatis, Chlamydophila psittaci, Clostridium botulinum, Clostridium difficile, Clostridium perfringens, Clostridium tetani, Corynebacterium diphtheriae, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Francisella tularensis, Haemophilus influenzae, Helicobacter pylori, Legionella pneumophila, Leptospira interrogans, Leptospira santarosai, Leptospira weilii, Leptospira*

noguchii, Listeria monocytogenes, Mycobacterium leprae, Mycobacterium tuberculosis, Mycobacterium ulcerans, Mycoplasma pneumoniae, Neisseria gonorrhoeae, Neisseria meningitidis, Pseudomonas aeruginosa, Rickettsia rickettsia, Salmonella typhi, Salmonella typhimurium, Shigella sonnei, Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus saprophyticus, Streptococcus agalactiae, Streptococcus pneumoniae, Streptococcus pyogenes, Treponema pallidum, Ureaplasma urealyticum, Vibrio cholerae, Yersinia pestis, Yersinia enterocolitica, and/or *Yersinia pseudotuberculosis*.

38. The method of claim 36, wherein the fungi are selected from *Absidia corymbifera, Absidia ramose, Achorion gallinae, Actinomadura spp., Ajellomyces dermatididis, Aleurisma brasiliensis, Allersheria boydii, Arthroderma spp., Aspergillus flavus, Aspergillus fumigatu, Basidiobolus spp., Blastomyces spp., Cadophora spp., Candida albicans, Cercospora apii, Chrysosporium spp., Cladosporium spp., Cladothrix asteroids, Coccidioides immitis, Cryptococcus albidus, Cryptococcus gattii, Cryptococcus laurentii, Cryptococcus neoformans, Cunninghamella elegans, Dematium wernecke, Discomyces israelii, Emmonsia spp, Emmonsiella capsulate, Endomyces geotrichum, Entomophthora coronate, Epidermophyton floccosum, Filobasidiella neoformans, Fonsecaea spp., Geotrichum candidum, Glenospora khartoumensis, Gymnoascus gypseus, Haplosporangium parvum, Histoplasma, Histoplasma capsulatum, Hormiscium dermatididis, Hormodendrum spp., Keratinomyces spp, Langeronia soudanense, Leptosphaeria senegalensis, Lichtheimia corymbifera, Lobmyces loboi., Loboa loboi, Lobomycosis, Madurella spp., Malassezia furfur, Micrococcus pelletieri, Microsporum spp, Monilia spp., Mucor spp., Mycobacterium tuberculosis, Nannizzia spp., Neotestudina rosatii, Nocardia spp., Oidium albicans, Oospora lactis, Paracoccidioides brasiliensis, Petriellidium boydii, Phialophora spp., Piedraia hortae, Pityrosporum furfur, Pneumocystis jirovecii (or Pneumocystis carinii), Pullularia gougerotii, Pyrenochaeta romeroi, Rhinosporidium seeberi, Sabouraudites (Microsporum), Sartorya fumigate, Sepedonium, Sporotrichum spp., Stachybotrys, Stachybotrys*

chartarum, Streptomyce spp., Tinea spp., Torula spp, Trichophyton spp., Trichosporon spp., and/*or Zopfia rosatii*.

39. The method of claim 36, wherein the viruses are selected from *Simplexvirus*, *Varicellovirus*, *Cytomegalovirus*, *Roseolovirus*, *Lympho-cryptovirus*, *Rhadinovirus*, *Mastadenovirus*, *α-Papillomavirus*, *β-Papillomavirus*, *X-Papillomavirus*, *γ-Papillomavirus*, *Mupapillomavirus*, *Nupapillomavirus*, *Alphapolyomavirus*, *Betapolyomavirus*, *γ-Polyomavirus*, *Deltapolyomavirus*, *Molluscipoxvirus*, *Orthopoxvirus*, *Parapoxvirus*, *α-Torquevirus*, *β-Torquevirus*, *γ-Torquevirus*, *Cyclovirus*, *Gemycircular*, *Gemykibivirus*, *Gemyvongvirus*, *Erythrovirus*, *Dependovirus*, *Bocavirus*, Orthohepadnavirus, *Gammaretrovirus*, *Deltaretrovirus*, *Lentivirus*, *Simiispumavirus*, *Coltivirus*, *Rotavirus*, *Seadornavirus*, *α-Coronavirus*, *β-Coronavirus*, *Torovirus*, *Mamastrovirus*, *Norovirus*, *Sapovirus*, *Flavivirus*, *Hepacivirus*, *Pegivirus*, *Orthohepevirus*, *Cardiovirus*, *Cosavirus*, *Enterovirus*, *Hepatovirus*, *Kobuvirus*, *Parechovirus*, *Rosavirus*, *Salivirus*, *Alphavirus*, *Rubivirus*, *Ebolavirus*, *Marburgvirus*, *Henipavirus*, *Morbilivirus*, *Respirovirus*, *Rubulavirus*, *Metapneumovirus*, *Orthopneumovirus*, *Ledantevirus*, *Lyssavirus*, *Vesiculovirus*, *Mammarenavirus*, *Orthohantavirus*, *Orthonairovirus*, *Orthobunyavirus*, *Phlebovirus*, *α-Influenzavirus*, *β-Influenzavirus*, *γ-Influenzavirus*, *Quaranjavirus*, *Thogotovirus*, and/or *Deltavirus*.

40. The method of any one of claims 29 to 39, wherein the one or more types of nucleoside or nucleotide analogs are selected from 2-ethynyl-adenosine, N6-propargyl-adenosine, 2'-(O-propargyl)-adenosine, 3'-(O-propargyl)-adenosine, 5-ethynyl-cytidine, 5-ethynyl-2'-deoxycytidine, 2'-(O-propargyl)-cytidine, 3'-(O-propargyl)-cytidine, 2'-(O-propargyl)-guanosine, 3'-(O-propargyl)-guanosine, 5-ethynyl-uridine, 5-ethynyl-2'-deoxyuridine, 2'-(O-propargyl)-uridine, 3'-(O-propargyl)-uridine, (2'S)-2'-deoxy-2'-fluoro-5-ethynyluridine, (2'S)-2'-fluoro-5-ethynyluridine, 2'(S)-2'-deoxy-2'-fluoro-5-ethynyluridine, (2'S)-2'-fluoro-5-ethynyluridine, 8-azido-adenosine, N^6-(6-azido)hexyl-2'deoxy-adenosine, 2'-azido-2'-deoxyadenosine, 5-azidomethyl-uridine, 5-(15-azido-4,7,10,13-

tetraoxa-pentadecanoyl-aminoallyl)-2'-deoxyuridine, 5-(3-azidopropyl)-uridine, 5-azido-PEG_4-uridine, 5-azido-PEG_4-cytidine, 5-azido-PEG_4-2'-deoxycytidine, 5-bromo-2'deoxyuridine, 5-bromouridine, 5-iodo-2'deoxyuridine, and 5-iodouridine.

41. The method of any one of claims 29 to 40, wherein the one or more types of nucleoside or nucleotide analogs are selected from 2-ethynyl-adenosine, N6-propargyl-adenosine, 2'-(O-propargyl)-adenosine, 3'-(O-propargyl)-adenosine, 5-ethynyl-cytidine, 5-ethynyl-2'-deoxycytidine, 2'-(O-propargyl)-cytidine, 3'-(O-propargyl)-cytidine, 2'-(O-propargyl)-guanosine, 3'-(O-propargyl)-guanosine, 5-ethynyl-uridine, 5-ethynyl-2'-deoxyuridine, 2'-(O-propargyl)-uridine, 3'-(O-propargyl)-uridine, (2'S)-2'-deoxy-2'-fluoro-5-ethynyluridine, (2'S)-2'-fluoro-5-ethynyluridine, 2'(S)-2'-deoxy-2'-fluoro-5-ethynyluridine, and (2'S)-2'-fluoro-5-ethynyluridine.

42. The method of any one of claims 29 to 41, wherein the one or more types of nucleoside or nucleotide analogs are selected from 8-azido-adenosine, N^6-(6-azido)hexyl-2'deoxy-adenosine, wherein the one or more types of nucleoside or nucleotide analogs are selected from 2'-azido-2'-deoxyadenosine, 5-azidomethyl-uridine, 5-(15-azido-4,7,10,13-tetraoxa-pentadecanoyl-aminoallyl)-2'-deoxyuridine, 5-(3-azidopropyl)-uridine, 5-azido-PEG_4-uridine, 5-azido-PEG_4-cytidine, and 5-azido-PEG_4-2'-deoxycytidine.

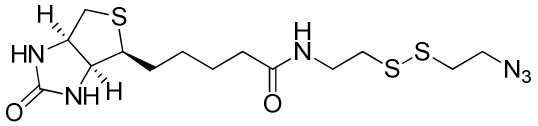
43. The method of any one of claims 29 to 42, wherein the one or more types of nucleoside or nucleotide analogs are selected from 5-bromo-2'deoxyuridine, 5-bromouridine, 5-iodo-2'deoxyuridine, and 5-iodouridine.

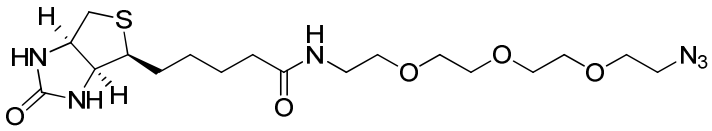
44. The method of any one of claims 29 to 43, wherein the control sample and the treated sample are both incubated for the same period time in the presence of one or more types of nucleoside or nucleotide analogs for 5 min to 180 min.

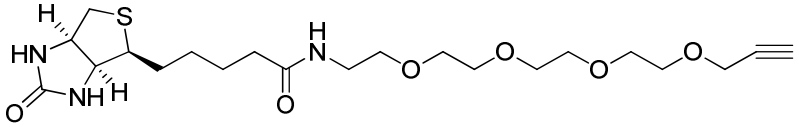
45. The method of claim 44, wherein the control sample and the treated sample are both incubated for the same period time in the presence of one or more types of nucleoside or nucleotide analogs for 30 min to 120 min.

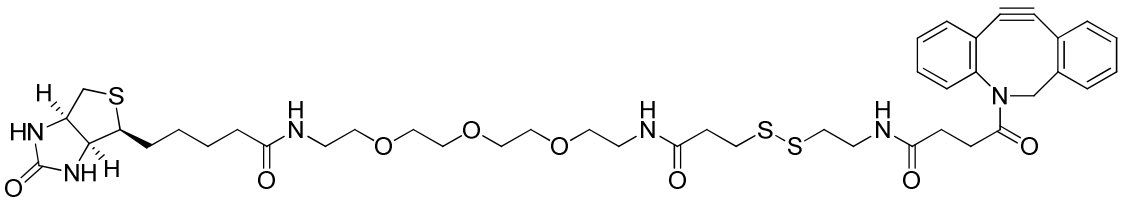
46. The method of any one of claims 29 to 45, wherein the labelling reagent binds to or with the one or more types of nucleoside or nucleotide analogs via click chemistry, a strained [3+2] cycloaddition reaction, or a Staudinger ligation.

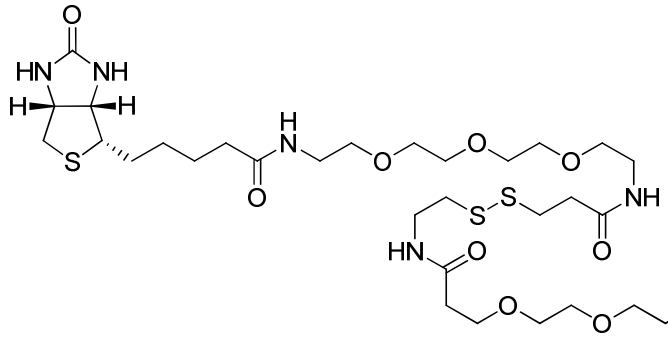
47. The method of claim 29, wherein the labelling reagent comprising the biotin group is selected from:


,


,


,





, and .

48. The method of any one of claims 29 to 47, wherein the labelling reagent further comprises a chemically cleavable linker or enzymatically cleavable linker.

49. The method of claim 48, wherein the cleavable linker is an acid-labile-based linker or a disulfide-based linker.

50. The method of claim 49, wherein the acid-labile-based linker comprises hydrazone or *cis*-aconityl groups.

51. The method of claim 48, wherein the enzymatically cleavable linker comprises a peptide-based linker or a β-glucuronide-based linker.

52. The method of claim 29, wherein the solid support is nano- or micro- materials, beads or a plate.

53. The method of claim 29, wherein the labelling reagent or label is removed or cleaved from the isolated or purified newly synthesized microbial nucleic acids prior to (f)(f') and (g) of claim 42.

54. The method of any one of claims 29 to 53, wherein determining the gene expression level and/or amounts and/or identity of the isolated or purified newly synthesized microbial nucleic acids in the control sample and the treated sample is determined by using a microarray comprising probes to nucleic acids from different microorganisms.

55. The method of claim 54, wherein determining the gene expression level and/or amounts and/or identity of the isolated or purified newly synthesized microbial nucleic acids in the control sample and the treated sample is by:

(i) amplifying the isolated or purified newly synthesized microbial nucleic acids from the control sample using a first PCR based method using primers containing a fluorescent dye to form

labelled products, wherein the primers comprise a sequence that is specific to a conserved microbial 16S rRNA gene region;

(i') amplifying the isolated or purified newly synthesized microbial nucleic acids from the treated sample using the first PCR based method using primers containing the fluorescent dye to form labelled products, wherein the primers comprise a sequence that is specific to a conserved microbial 16S rRNA gene region;

(ii) applying the labelled products from the control sample to a first microarray comprising probes that comprise unique 16s rRNA variable region sequences from 20 or more microorganisms;

(ii') applying the labelled products from the treated sample to a second microarray, wherein the second microarray is a duplicate of the first microarray; and

(iii) determining the effectiveness of an antimicrobial agent in modulating the growth and proliferation of microorganism(s) in a sample based upon imaging the first microarray and imaging the second microarray for fluorescent hybridization products and determining if there are any changes in regards to the intensity, location, or absence of the fluorescent hybridization products between the microarrays,

wherein if there is a decrease in the intensity of the fluorescent hybridization products between the first and second microarray, or if there are changes as to the location or an absence of fluorescent hybridization products between first and second microarray indicates that the antimicrobial agent is effective in modulating the growth and proliferation of the microorganism(s).

56. The method of any one of claims 29 to 55, wherein the effectiveness of an antimicrobial agent in modulating the growth and proliferation of microorganism(s) in a sample is determined or confirmed by sequencing the isolated or purified newly synthesized microbial nucleic acids from the control sample and from the treated sample,

wherein a decrease in the gene expression level of the newly synthesized microbial nucleic acids in the treated sample v. the control sample, or there is decrease in the amounts and/or identity of the newly synthesized microbial nucleic acids in the treated

sample v. the control sample indicates that the antimicrobial agent is effective in modulating the growth and proliferation of the microorganism(s).

57. The method of claim 56, wherein the isolated or purified newly synthesized microbial nucleic acids from the control and treated samples are sequenced using a transposome-based sequencing method.

58. The method of claim 57, wherein sequencing of the newly synthesized microbial nucleic acids from the control and treated samples are by:

(a) applying the isolated or purified newly synthesized microbial nucleic acids from the control and treated samples to bead-linked transposomes, wherein the bead-linked transposomes mediate the simultaneous fragmentation of microbial nucleic acids and the addition of sequencing primers;

(b) amplifying the microbial nucleic acid fragments with primers that comprise index and adapter sequences to form library of amplified products;

(c) washing and pooling the library of amplified products from the control sample;

(c') washing and pooling the library of amplified products from the treated sample;

(d) sequencing the libraries of amplified products from the control sample;

(d') sequencing the libraries of amplified products from the treated sample; and

(e) determining any changes in the gene expression level and/or amounts and/or identity of the isolated or purified newly synthesized microbial nucleic acids from the control and treated samples based using bioinformatic analysis.

59. The method of any one of claims 29 to 58, wherein the newly synthesized microbial nucleic acids are RNA, wherein the microbial RNA is reversed transcribed into cDNA prior to (f), (f') and (g) of claim 41, and wherein the effectiveness of an antimicrobial agent in modulating the growth and proliferation of microorganism(s) can be

determined based upon determining changes in the gene expression levels of newly synthesized microbial nucleic acids from the control and treated samples by using a microarray and/or by sequencing.