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(54) Title: METHODS AND COMPOSITIONS FOR KILLING A TARGET BACTERIUM

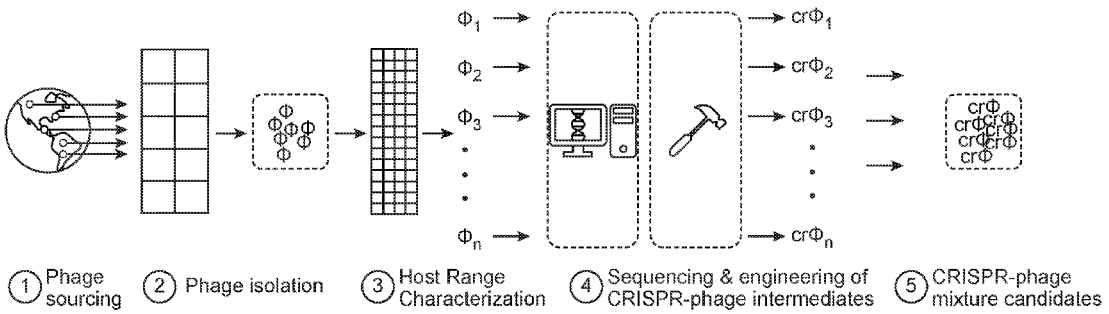

FIGURE 1

(57) Abstract: Provided herein are methods and compositions for killing a target bacterium. Also disclosed are engineered bacteriophages.

[Continued on next page]

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METHODS AND COMPOSITIONS FOR KILLING A TARGET BACTERIUM

CROSS REFERENCE

[0001] This application claims the benefit of U.S. Provisional Application No.62/667,400, filed May 4, 2018, U.S. Provisional Application No.62/743,740, filed October 10, 2018, and U.S. Provisional Application No.62/818,066, filed March 13, 2019, all of which are incorporated herein by reference.

SUMMARY

[0002] Disclosed herein, in certain embodiments, are methods for killing a target bacterium. In some embodiments, the method for killing a target bacterium comprises introducing into a target bacterium a bacteriophage comprising: a first nucleic acid encoding a spacer sequence or a crRNA transcribed therefrom, wherein the spacer sequence is complementary to a target nucleotide sequence from a target gene in the target bacterium; and a gene that is capable of inducing lysis of the target bacterium. In some embodiments, the target bacterium is killed by lytic activity of the bacteriophage or activity of a CRISPR-Cas system using the spacer sequence or the crRNA transcribed therefrom. In some embodiments, the first nucleic acid sequence is a CRISPR array further comprising at least one repeat sequence. In some embodiments, the bacteriophage further comprises a second nucleic acid encoding a transcriptional activator for the CRISPR-Cas system. In some embodiments, the gene is endogenous or exogenous. In some embodiments, the transcriptional activator is regulated by quorum sensing (QS) signals. In some embodiments, the transcriptional activator is a protein involved in sensing stress of a bacterium membrane. In some embodiments, the protein involved in sensing stress is response regulator BaeSR. In some embodiments, the transcriptional activator is a protein that stabilizes Cas. In some embodiments, the protein that stabilizes Cas is heat shock protein G (HtpG). In some embodiments, the transcriptional activator is a metabolic sensing protein. In some embodiments, the metabolic sensing protein is cAMP receptor protein (CRP). In some embodiments, the CRP is sensitive to cyclic AMP (cAMP). In some embodiments, the metabolic sensing protein is a sigma factor. In some embodiments, the sigma factor is RpoN (σ^{54}). In some embodiments, the transcriptional activator disrupts the activity of an inhibitory element. In some embodiments, the inhibitory element comprises heat-stable nucleoid-structuring protein (H-NS), leucine responsive regulatory protein (LRP), or CodY. In some embodiments, the inhibitory element is a transcriptional repressor. In some embodiments, the transcriptional repressor is a global transcriptional repressor. In some embodiments, the transcriptional activator comprises LeuO or a polypeptide having at least 75% sequence homology with SEQ ID NO: 1. In some embodiments, the transcriptional activator

comprises CD2983 or a polypeptide having at least 75% sequence homology with SEQ ID NO: 2. In some embodiments, the CRISPR-Cas system is endogenous to the target bacterium. In some embodiments, the CRISPR-Cas system is exogenous to the target bacterium. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system. In some embodiments, the CRISPR-Cas system comprises the type I CRISPR-Cas system. In some embodiments, the target nucleotide sequence comprises all or a part of a promoter sequence for the target gene. In some embodiments, the target nucleotide sequence comprises all or a part of a nucleotide sequence located on a coding strand of a transcribed region of the target gene. In some embodiments, the target nucleotide sequence is at least a portion of an essential gene that is needed for survival of the target bacterium. In some embodiments, the essential gene is *Tsf*, a*cpP*, *gapA*, *infA*, *secY*, *csrA*, *trmD*, *ftsA*, *fusA*, *glyQ*, *eno*, or *nusG*. In some embodiments, the at least one repeat sequence is operably linked to the at least one spacer sequence at either its 5' end or its 3' end. In some embodiments, the target bacterium is killed solely by the lytic activity of the bacteriophage. In some embodiments, the target bacterium is killed solely by the activity of the CRISPR-Cas system. In some embodiments, the target bacterium is killed by both the lytic activity of the bacteriophage and the activity of the CRISPR-Cas system in combination. In some embodiments, the target bacterium is killed by the activity of the CRISPR-Cas system independently of the lytic activity of the bacteriophage. In some embodiments, the activity of the CRISPR-Cas system supplements or enhances the lytic activity of the bacteriophage. In some embodiments, the spacer nucleotide sequence overlaps with a second spacer sequence. In some embodiments, the lytic activity of the bacteriophage and the activity of the CRISPR-Cas system are synergistic. In some embodiments, the lytic activity of the bacteriophage, the activity of the CRISPR-Cas system, or both is modulated by a concentration of the bacteriophage. In some embodiments, the bacteriophage infects multiple bacterial strains. In some embodiments, the bacteriophage is an obligate lytic bacteriophage. In some embodiments, the bacteriophage is a temperate bacteriophage that is rendered lytic. In some embodiments, the bacteriophage does not confer any new properties onto the target bacterium beyond cellular death caused by the lytic activity of the bacteriophage and/or the activity of the CRISPR-Cas array. In some embodiments, the target bacterium is *C. difficile*. In some embodiments, the bacteriophage is ϕCD146 or ϕCD24-2. In some embodiments, the target bacterium is *E. coli*. In some embodiments, the bacteriophage is T4, T7, or T7m. In some embodiments, the first nucleic acid encoding a spacer sequence or a crRNA is inserted into a non-essential bacteriophage gene. In some embodiments, the non-essential gene is *gp49*, *gp75,* or *hoc*. In some embodiments, the non-essential gene is *gp0.7, gp4.3, gp4.5,* or *gp4.7*. In some embodiments, the non-essential gene is *gp0.6, gp0.65, gp0.7, gp4.3,* or *gp4.5*.

[0003] In some embodiments, disclosed herein are methods for killing a plurality of target bacteria, such as in a mixed population of bacteria comprising the target bacteria and non-target bacteria (e.g., in therapeutic and/or environmental treatment processes, such as described herein). In specific embodiments, the target bacteria are treated according to any process described herein (e.g., for killing target bacterium), and a first population of the target bacteria is killed by lytic activity of the bacteriophage and a second population of the target bacteria is killed by activity of a CRISPR-Cas system using the spacer sequence or the crRNA transcribed therefrom (e.g., wherein the non-target bacteria is not killed (e.g., killed at a lesser rate than the target bacteria, such as at 50%, the rate, less than 25% the rate, less than 10% the rate, or less than 20% killed, less than 10% killed, less than 5% killed, or the like).

[0004] Disclosed herein, in certain embodiments, are methods for modulating the activity of a CRISPR-Cas system in a target bacterium. In some embodiments, the method comprises: introducing a bacteriophage comprising a nucleic acid encoding a transcriptional activator for the CRISPR-Cas system in the target bacterium. In some embodiments, the transcriptional activator is regulated by quorum sensing (QS) signals. In some embodiments, the transcriptional activator is a protein involved in sensing stress to a bacterium membrane. In some embodiments, the protein involved in sensing stress is response regulator BaeSR. In some embodiments, the transcriptional activator is a protein that stabilizes Cas. In some embodiments, the protein that stabilizes Cas is heat shock protein G (HtpG). In some embodiments, the transcriptional activator is a metabolic sensing protein. In some embodiments, the metabolic sensing protein is cAMP receptor protein (CRP). In some embodiments, the CRP is sensitive to cyclic AMP (cAMP). In some embodiments, the metabolic sensing protein is a sigma factor. In some embodiments, the sigma factor is RpoN (σ54). In some embodiments, the transcriptional activator disrupts the activity of an inhibitory element. In some embodiments, the inhibitory element is heat-stable nucleoid-structuring protein (H-NS), leucine responsive regulatory protein (LRP), or CodY. In some embodiments, the inhibitory element is a transcriptional repressor. In some embodiments, the transcriptional repressor is a global transcriptional repressor. In some embodiments, the transcriptional activator comprises LeuO or a polypeptide having at least 75% sequence homology with SEQ ID NO: 1. In some embodiments, the transcriptional activator comprises CD2983 or a polypeptide having at least 75% sequence homology with SEQ ID NO: 2. In some embodiments, the CRISPR-Cas system is endogenous. In some embodiments, the CRISPR-Cas system is exogenous. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system. In some embodiments, the bacteriophage infects multiple bacterial strains. In some

embodiments, the bacteriophage is an obligate lytic bacteriophage. In some embodiments, the bacteriophage is a temperate bacteriophage that is rendered lytic. In some embodiments, the target bacterium is *C. difficile*. In some embodiments, the bacteriophage is ϕCD146 or ϕCD24-2. In some embodiments, the target bacterium is *E. coli*. In some embodiments, the bacteriophage is T4, T7, or T7m. In some embodiments, the nucleic acid encoding a transcriptional activator is inserted into a non-essential bacteriophage gene. In some embodiments, the non-essential gene is *gp49*. In some embodiments, the non-essential gene is *gp75*. In some embodiments, the non-essential gene is *hoc*. In some embodiments, the non-essential gene is *gp0.7, gp4.3, gp4.5,* or *gp4.7*. In some embodiments, the non-essential gene is *gp0.6, gp0.65, gp0.7, gp4.3,* or *gp4.5*.

[0005] Disclosed herein, in certain embodiments, are methods of killing a target bacterium. The method comprises introducing into a target bacterium a bacteriophage comprising: lytic activity, and a first nucleic acid sequence encoding an anti-CRISPR polypeptide. In specific embodiments, the anti-CRISPR polypeptide enhances the lytic activity of the bacteriophage (e.g., as determined by how fast the target bacterium is killed). In some embodiments, the anti-CRISPR polypeptide inactivates a CRISPR-Cas system. In some embodiments, the anti-CRISPR polypeptide inactivates the CRISPR-Cas system using a process comprising gene regulation interference. In some embodiments, the anti-CRISPR polypeptide inactivates the CRISPR-Cas system using a process comprising nuclease recruitment interference. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system. In some embodiments, the anti-CRISPR polypeptide binds directly or indirectly to a Cascade or Cascade-like complex. In some embodiments, the anti-CRISPR polypeptide is a truncated protein, a fusion protein, a dimer protein, or mutated protein. In some embodiments, the bacteriophage further comprises a second nucleic acid encoding a CRISPR array. In some embodiments, the CRISPR array comprises at least one repeat sequence and at least one spacer sequence that is complementary to a target nucleotide sequence from a target gene in the target bacterium. In specific embodiments, provided herein are methods of killing target bacteria (e.g., in a mixed population of bacteria comprising target bacteria and non-target bacteria). The method comprises introducing into target bacteria a bacteriophage having lytic activity and comprising a first nucleic acid sequence encoding an anti-CRISPR polypeptide. In specific embodiments, the anti-CRISPR polypeptide enhances the lytic activity of the bacteriophage (e.g., as measured by number of target bacteria killed in a given amount of time).

[0006] Disclosed herein, in certain embodiments, are bacteriophages comprising: a first nucleic acid encoding a spacer sequence or a crRNA transcribed therefrom, wherein the spacer sequence is

complementary to a (e.g., target) nucleotide sequence from a (e.g., target) gene in a (e.g., target) bacterium; and a gene that is capable of inducing lysis of the (e.g., target) bacterium. In specific embodiments, the target bacterium is killed by the lytic activity of the bacteriophage or activity of a CRISPR-Cas system using the spacer sequence or the crRNA transcribed therefrom. In some embodiments, the bacteriophage further comprises a second nucleic acid encoding a transcriptional activator for the CRISPR-Cas system. In some embodiments, the transcriptional activator is regulated by quorum sensing (QS) signals. In some embodiments, the transcriptional activator is a protein involved in sensing stress of a bacterium membrane. In some embodiments, the protein is response regulator BaeSR. In some embodiments, the transcriptional activator is a protein that stabilizes Cas. In some embodiments, the protein that stabilizes Cas is heat shock protein G (HtpG). In some embodiments, the transcriptional activator is a metabolic sensing protein. In some embodiments, the metabolic sensing protein is cAMP receptor protein (CRP). In some embodiments, the CRP is sensitive to cyclic AMP (cAMP). In some embodiments, the metabolic sensing protein is a sigma factor. In some embodiments, the sigma factor is RpoN (σ^{54}). In some embodiments, the transcriptional activator disrupts the activity of an inhibitory element of the target bacterium. In some embodiments, the inhibitory element is heat-stable nucleoid-structuring protein (H-NS), leucine responsive regulatory protein (LRP), or CodY. In some embodiments, the inhibitory element is a transcriptional repressor. In some embodiments, the transcriptional repressor is a global transcriptional repressor. In some embodiments, the transcriptional activator comprises LeuO or a polypeptide having at least 75% sequence homology with SEQ ID NO: 1. In some embodiments, the transcriptional activator comprises CD2983 or a polypeptide having at least 75% sequence homology with SEQ ID NO: 2. In some embodiments, the CRISPR-Cas system is endogenous. In some embodiments, the CRISPR-Cas system is exogenous. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system. In some embodiments, the target nucleotide sequence comprises all or a part of a promoter sequence for the target gene. In some embodiments, the target nucleotide sequence comprises all or a part of a nucleotide sequence located on a coding strand of a transcribed region of the target gene. In some embodiments, the target nucleotide sequence is essential. In some embodiments, the essential gene is *Tsf*, a*cpP*, *gapA*, *infA*, *secY*, *csrA*, *trmD*, *ftsA*, *fusA*, *glyQ*, *eno*, or *nusG*. In some embodiments, the target nucleotide sequence is a non-essential gene. In some embodiments, the first nucleic acid sequence is a CRISPR array comprising at least one repeat sequence. In some embodiments, the at least one repeat sequence is operably linked to the spacer sequence at either its 5' end or its 3' end. In some embodiments, the bacteriophage infects multiple bacterial strains. In

some embodiments, the bacteriophage is an obligate lytic bacteriophage. In some embodiments, the bacteriophage is a temperate bacteriophage that is rendered lytic. In some embodiments, the temperate bacteriophage is rendered lytic by the removal, replacement, or inactivation of one or more lysogeny genes. In some embodiments, the target bacterium is *C. difficile*. In some embodiments, the bacteriophage is ϕCD146 or ϕCD24-2. In some embodiments, the target bacterium is *E. coli*. In some embodiments, the bacteriophage is T4, T7, or T7m. In some embodiments, the first nucleic acid encoding a spacer sequence or a crRNA is inserted into a non-essential gene. In some embodiments, the non-essential gene is *gp49*. In some embodiments, the non-essential gene is *gp75*. In some embodiments, the non-essential gene is *hoc*. In some embodiments, the non-essential gene is *gp0.7, gp4.3, gp4.5*, or *gp4.7*. In some embodiments, non-essential gene is *gp0.6, gp0.65, gp0.7, gp4.3,* or *gp4.5*. Also disclosed herein, in some embodiments, are pharmaceutical compositions comprising the bacteriophage disclosed herein, and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical compositions is in a form of a tablet, a liquid, a syrup, an oral formulation, an intravenous formulation, an intranasal formulation, an ocular formulation, an otic formulation, a subcutaneous formulation, an inhalable respiratory formulation, a suppository, or any combination thereof. Further disclosed herein, in some embodiments, are methods of treating a disease in a subject comprising administering the bacteriophage disclosed herein to the subject. In some embodiments, the subject is a mammal. In some embodiments, the disease is a bacterial infection. In some embodiments, a bacteria causing the bacterial infection is an *Acinetobacter* species, an *Actinomyces* species, *Burkholderia cepacia* complex, a *Campylobacter* species, a *Candida* species, *Clostridium difficile*, *Corynebacterium minutissium, Corynebacterium pseudodiphtheriae, Corynebacterium stratium, Corynebacterium group G1, Corynebacterium group G2, Enterobacteriaceae*, an *Enterococcus* species, *Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae,* a *Moraxella* species, *Mycobacterium tuberculosis* complex, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, a non-tuberculous mycobacteria species, a *Porphyromonas* species, *Prevotella melaninogenicus,* a *Pseudomonas* species, *Salmonella typhimurium, Serratia marcescens Staphylococcus aureus*, *Streptococcus agalactiae*, *Staphylococcus epidermidis, Staphylococcus salivarius, Streptococcus mitis, Streptococcus sanguis, Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Vibrio cholerae*, a *Coccidioides* species, a *Cryptococcus* species, *Helicobacter felis, Helicobacter pylori*, *Clostridium bolteae* and any combination thereof. In some embodiments, the bacterium is a drug resistant bacterium that is resistant to at least one antibiotic. In some embodiments, the bacterium is a multi-drug resistant bacterium that is resistant to at least one antibiotic. In some embodiments, the bacterium is Pseudomonas. In some embodiments, the bacterium is staphylococcus. In some

embodiments, the bacterium is *Escherichia coli.* In some embodiments, the bacterium is *Clostridium difficile.* In some embodiments, the bacterium is methicillin resistant. In some embodiments, the bacterium is methicillin resistant staphylococcus aureus. In some embodiments, the bacterium is multidrug resistant Pseudomonas Aeruginosa. In some embodiments, the antibiotic comprises a cephalosporin, a fluoroquinolone, a carbapenem, a colistin, an aminoglycoside, vancomycin, streptomycin, or methicillin. In some embodiments, the administering is intra-arterial, intravenous, intramuscular, oral, subcutaneous, topical, inhalation, intravesical or any combination thereof.

[0007] Disclosed herein, in certain embodiments, are bacteriophages comprising a nucleic acid encoding a transcriptional activator for a CRISPR-Cas system in a (e.g.,target) bacterium. In some embodiments, the transcriptional activator is regulated by quorum sensing (QS) signals. In some embodiments, the transcriptional activator is a protein involved in sensing stress to a bacterium membrane. In some embodiments, the protein involved in sensing stress is response regulator BaeSR. In some embodiments, the transcriptional activator is a protein that stabilizes Cas. In some embodiments, the protein that stabilizes Cas is heat shock protein G (HtpG). In some embodiments, the transcriptional activator is a metabolic sensing protein. In some embodiments, the metabolic sensing protein is cAMP receptor protein (CRP). In some embodiments, the CRP is sensitive to cyclic AMP (cAMP). In some embodiments, the metabolic sensing protein is a sigma factor. In some embodiments, the sigma factor is RpoN (σ54). In some embodiments, the transcriptional activator disrupts the activity of an inhibitory element. In some embodiments, the inhibitory element is heat-stable nucleoid-structuring protein (H-NS), leucine responsive regulatory protein (LRP), or CodY. In some embodiments, the inhibitory element is a transcriptional repressor. In some embodiments, the transcriptional repressor is a global transcriptional repressor. In some embodiments, the transcriptional activator comprises LeuO or a polypeptide having at least 75% sequence homology with SEQ ID NO: 1. In some embodiments, the transcriptional activator comprises CD2983 or a polypeptide having at least 75% sequence homology with SEQ ID NO: 2. In some embodiments, the CRISPR-Cas system is endogenous. In some embodiments, the CRISPR-Cas system is exogenous. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system. In some embodiments, the bacteriophage infects multiple bacterial strains. In some embodiments, the bacteriophage is an obligate lytic bacteriophage. In some embodiments, the bacteriophage is a temperate bacteriophage that is rendered lytic. In some embodiments, the target bacterium is *C. difficile.* In some embodiments, the bacteriophage is ϕCD146 or ϕCD24-2. In some embodiments, the target

bacterium is *E. coli*. In some embodiments, the bacteriophage is T4, T7, or T7m. In some embodiments, the nucleic acid encoding a transcriptional activator is inserted into a non-essential bacteriophage gene. In some embodiments, the non-essential gene is *gp49*. In some embodiments, the non-essential gene is *gp75*. In some embodiments, the non-essential gene is *hoc*. In some embodiments, the non-essential gene is *gp0.7, gp4.3, gp4.5*, or *gp4.7*. In some embodiments, the non-essential gene is *gp0.6, gp0.65, gp0.7, gp4.3*, or *gp4.5*. Also disclosed herein, in some embodiments, are pharmaceutical compositions comprising the bacteriophage disclosed herein, and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is in a form of a tablet, a liquid, a syrup, an oral formulation, an intravenous formulation, an intranasal formulation, an ocular formulation, an otic formulation, a subcutaneous formulation, an inhalable respiratory formulation, a suppository, and any combination thereof. Further disclosed herein are methods of treating a disease in a subject comprising administering the bacteriophage to the subject. In some embodiments, the subject is a mammal. In some embodiments, the disease is a bacterial infection. In some embodiments, a bacteria causing the bacterial infection is an *Acinetobacter* species, an *Actinomyces* species, *Burkholderia cepacia* complex, a *Campylobacter* species, a *Candida* species, *Clostridium difficile*, *Corynebacterium minutissium, Corynebacterium pseudodiphtheriae, Corynebacterium stratium, Corynebacterium group G1, Corynebacterium group G2, Enterobacteriaceae*, an *Enterococcus* species, *Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae,* a *Moraxella* species, *Mycobacterium tuberculosis* complex, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, a non-tuberculous mycobacteria species, a *Porphyromonas* species, *Prevotella melaninogenicus,* a *Pseudomonas* species, *Salmonella typhimurium, Serratia marcescens Staphylococcus aureus*, *Streptococcus agalactiae*, *Staphylococcus epidermidis, Staphylococcus salivarius, Streptococcus mitis, Streptococcus sanguis, Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Vibrio cholerae*, a *Coccidioides* species, a *Cryptococcus* species, *Helicobacter felis, Helicobacter pylori*, *Clostridium bolteae* and any combination thereof. In some embodiments, the bacterium is a drug resistant bacterium that is resistant to at least one antibiotic. In some embodiments, the bacterium is a multi-drug resistant bacterium that is resistant to at least one antibiotic. In some embodiments, the bacterium is Pseudomonas. In some embodiments, the bacterium is staphylococcus. In some embodiments, the bacterium is *Escherichia coli*. In some embodiments, the bacterium is *Clostridium difficile*. In some embodiments, the bacterium is methicillin resistant. In some embodiments, the bacterium is methicillin resistant staphylococcus aureus. In some embodiments, the bacterium is multidrug resistant Pseudomonas Aeruginosa. In some embodiments, the antibiotic comprises a cephalosporin, a fluoroquinolone, a carbapenem, a colistin, an aminoglycoside, vancomycin, streptomycin, or methicillin. In some embodiments, the

administering is intra-arterial, intravenous, intramuscular, oral, subcutaneous, topical, inhalation, or any combination thereof.

[0008] Disclosed herein, in certain embodiments, are bacteriophages comprising: lytic activity, and a first nucleic acid sequence encoding an anti-CRISPR polypeptide. In specific embodiments, the anti-CRISPR polypeptide enhances the lytic activity of the bacteriophage. In some embodiments, the anti-CRISPR polypeptide inactivates a CRISPR-Cas system. In some embodiments, the anti-CRISPR polypeptide inactivates the CRISPR-Cas system using a process comprising gene regulation interference. In some embodiments, the anti-CRISPR polypeptide inactivates the CRISPR-Cas system using a process comprising nuclease recruitment interference. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system. In some embodiments, the anti-CRISPR polypeptide binds directly or indirectly to a Cascade or Cascade-like complex. In some embodiments, the anti-CRISPR polypeptide is a truncated protein, a fusion protein, a dimer protein, or mutated protein. In some embodiments, the bacteriophage further comprises a second nucleic acid encoding a CRISPR array. In some embodiments, CRISPR array comprises at least one repeat sequence and at least one spacer sequence that is complementary to a target nucleotide sequence from a target gene in the target bacterium. Also disclosed herein, in some embodiments, are pharmaceutical compositions comprising the bacteriophage disclosed herein, and a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is in a form of a tablet, a liquid, a syrup, an oral formulation, an intravenous formulation, an intranasal formulation, an ocular formulation, an otic formulation, a subcutaneous formulation, an inhalable respiratory formulation, a suppository, and any combination thereof. Further disclosed herein, in some embodiments, are methods of treating a disease in a subject comprising administering the bacteriophage disclosed herein to the subject. In some embodiments, the subject is a mammal. In some embodiments, the disease is a bacterial infection. In some embodiments, a bacterium causing the bacterial infection is an *Acinetobacter* species, an *Actinomyces* species, *Burkholderia cepacia* complex, a *Campylobacter* species, a *Candida* species, *Clostridium difficile*, *Corynebacterium minutissium, Corynebacterium pseudodiphtheriae, Corynebacterium stratium, Corynebacterium group G1, Corynebacterium group G2, Enterobacteriaceae*, an *Enterococcus* species, *Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae,* a *Moraxella* species, *Mycobacterium tuberculosis* complex, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, a non-tuberculous mycobacteria species, a *Porphyromonas* species, *Prevotella melaninogenicus,* a *Pseudomonas* species, *Salmonella typhimurium, Serratia marcescens Staphylococcus aureus*, *Streptococcus agalactiae*, *Staphylococcus epidermidis,*

Staphylococcus salivarius, Streptococcus mitis, Streptococcus sanguis, Streptococcus pneumoniae, Streptococcus pyogenes, Vibrio cholerae, a *Coccidioides* species, a *Cryptococcus* species, *Helicobacter felis, Helicobacter pylori, Clostridium bolteae* and any combination thereof. In some embodiments, the bacterium is a drug resistant bacterium that is resistant to at least one antibiotic. In some embodiments, the bacterium is a multi-drug resistant bacterium that is resistant to at least one antibiotic. In some embodiments, the bacterium is Pseudomonas. In some embodiments, the bacterium is staphylococcus. In some embodiments, the bacterium is *Escherichia coli.* In some embodiments, the bacterium is *Clostridium difficile.* In some embodiments, the bacterium is methicillin resistant. In some embodiments, the bacterium is methicillin resistant staphylococcus aureus. In some embodiments, the bacterium is multidrug resistant Pseudomonas Aeruginosa. In some embodiments, the antibiotic comprises a cephalosporin, a fluoroquinolone, a carbapenem, a colistin, an aminoglycoside, vancomycin, streptomycin, or methicillin. In some embodiments, the administering is intra-arterial, intravenous, intramuscular, oral, subcutaneous, topical, inhalation, or any combination thereof.

[0009] Disclosed herein, in certain embodiments, are methods of killing a target bacterium, comprising introducing into a target bacterium a temperate bacteriophage comprising a removal, replacement, or inactivation of at least one lysogeny gene, wherein the temperate bacteriophage is rendered lytic thereby killing the target bacterium. In some embodiments, the lysogeny gene is a repressor gene. In some embodiments, the lysogeny gene is *cI* phage repressor gene. In some embodiments, the bacteriophage infects multiple bacterial strains. In some embodiments, the target bacterium is *C. difficile.* In some embodiments, the bacteriophage is ϕCD146 or ϕCD24-2. In some embodiments, the bacteriophage further comprises a first nucleic acid encoding a spacer sequence or a crRNA transcribed therefrom. In some embodiments, the spacer sequence is complementary to a target nucleotide sequence from a target gene in the target bacterium. In some embodiments, the first nucleic acid sequence is a CRISPR array further comprising at least one repeat sequence. In some embodiments, the at least one repeat sequence is operably linked to the spacer sequence at either its 5' end or its 3' end. In some embodiments, the bacteriophage further comprises a second nucleic acid encoding a transcriptional activator for a CRISPR-Cas system. In some embodiments, the CRISPR-Cas system is endogenous to the target bacterium. In some embodiments, the CRISPR-Cas system is exogenous to the target bacterium. In some embodiments, the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system. In some embodiments, the CRISPR-Cas system comprises a type I CRISPR-Cas system. In some embodiments, the target nucleotide sequence comprises all or a part of a promoter sequence for the target gene. In some embodiments, the target nucleotide sequence comprises all or a part of

a nucleotide sequence located on a coding strand of a transcribed region of the target gene. In some embodiments, the target nucleotide sequence comprises at least a portion of an essential gene that is needed for survival of the target bacterium. In some embodiments, the target bacterium is killed by both lytic activity of the bacteriophage and activity of the CRISPR-Cas system in combination. In some embodiments, activity of the CRISPR-Cas system supplements or enhances lytic activity of the bacteriophage. In some embodiments, the target bacterium is killed by activity of a CRISPR-Cas system independently of lytic activity of the bacteriophage. In some embodiments, lytic activity of the bacteriophage and activity of a CRISPR-Cas system are synergistic. In some embodiments, lytic activity of the bacteriophage, activity of a CRISPR-Cas system, or both is modulated by a concentration of the bacteriophage.

[0010] Disclosed herein, in certain embodiments, are pharmaceutical compositions comprising: (a) a temperate bacteriophage comprising a removal, replacement, or inactivation of at least one lysogeny gene; and (B) a pharmaceutically acceptable excipient. In some embodiments, the pharmaceutical composition is in a form of a tablet, a liquid, a syrup, an oral formulation, an intravenous formulation, an intranasal formulation, an ocular formulation, an otic formulation, a subcutaneous formulation, an inhalable respiratory formulation, a suppository, and any combination thereof.

[0011] Disclosed herein, in certain embodiments, are methods of tuning the microbiome of a subject, the method comprising: administering to the subject a pharmaceutical composition disclosed herein. In some embodiments, a pharmaceutical composition disclosed herein treats microbiome imbalance.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] The features of the disclosure are set forth with particularity in the appended claims. A better understanding of the features and advantages of the present disclosure will be obtained by reference to the following detailed description that sets forth illustrative embodiments, in which the principles of the disclosure are utilized, and the accompanying drawings of which:

[0013] Figure 1 illustrates the workflow process for engineering a CRISPR-enhanced bacteriophage.

[0014] Figure 2A exemplifies a schematic diagram of the linear alignment of CRISPR-Cas systems from within five strains of *C. difficile* with identification of the various CRISPR-Cas constituent components.

[0015] Figure 2B further exemplifies a schematic diagram of the CRISPR-Cas system operon structures for *C. difficile* strains 630 and R20291.

[0016] Figure 3A exemplifies the reduction in cell population for *E. coli* strain BW25113 when treated with a native wild-type bacteriophage T7m or a corresponding engineered crPhage T7m. Native wild-type bacteriophage shows bacteria killing by lytic activity. Corresponding crT7m comprising LeuO and a CRISPR array for *ftsA* shows an additional 5-log improvement in bacterial killing activity over the wild-type bacteriophage T7m.

[0017] Figure 3B exemplifies the lethality of the CRISPR array for *ftsA* (a 7-log reduction) when administered directly to the bacteria independent of phage delivery.

[0018] Figure 4A exemplifies the reduction in cell population for *C. difficile* strain R20291when treated with a native wild-type bacteriophage ϕCD146 or the corresponding engineered crPhage ϕCD146. Native wild-type bacteriophage shows bacteria killing by lytic activity. However, the corresponding crPhage ϕCD146 shows an additional 1-log improvement in bacterial killing activity over the wild-type bacteriophage.

[0019] Figure 4B exemplifies the lethality of the CRISPR array for *R20291-3* (a 3.5-log reduction) when administered directly to the bacteria independent of phage delivery.

[0020] Figure 5 exemplifies a bacterial lawn of *C. difficile* strain 069 which are insensitive to lysis by wild-type phage ϕCD146. The lack of phage plaques on the left of the image indicates lack of killing by the wild-type phage ϕCD146. The presence of plaques due to crPhage ϕCD146 on the right of the image is indicative of bacterial death due to the activity of the CRISPR array targeting *R20291-3*.

[0021] Figure 6A- Figure 6V exemplify the enhanced lethality of crPhage ϕCD146 as compared to the wild-type bacteriophage against different strains of *C. difficile*. **Figure 6A** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 043. Population growth of the various strains of *C. difficile* was monitored by optical density at 600nm for up to 6 hours following treatment with either the wild-type or CRISPR-array containing crPhage ϕCD146. **Figure 6B** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 051. **Figure 6C** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 073. **Figure 6D** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 093. **Figure 6E** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 0180. **Figure 6F** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 106. **Figure 6G** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 128. **Figure 6H** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 199. **Figure 6I** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 111. **Figure 6J** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 108. **Figure 6K** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 25. **Figure 6L** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 148. **Figure 6M** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 154. **Figure 6N** exemplifies

crPhage ϕCD146 lethality against *C. difficile* strain 195. **Figure 6O** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain FOBT195. **Figure 6P** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 038. **Figure 6Q** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 112. **Figure 6R** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 196. **Figure 6S** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 105. **Figure 6T** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain UK1. **Figure 6U** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain UK6. **Figure 6V** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain BI-9.

[0022] Figure 7A- Figure 7C exemplify the enhanced lethality of crPhage ϕCD24-2 as compared to the wild-type bacteriophage against different strains of *C. difficile*. Population growth of the various strains of *C. difficile* was monitored by optical density at 600nm for up to 6 hours following treatment with either the wild-type or CRISPR-array containing crPhage ϕCD24-2. **Figure 7A** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 041. **Figure 7B** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 042. **Figure 7C** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 046.

[0023] Figure 8A- Figure 8E exemplify the enhanced lethality of crPhage ϕCD146 as compared to the wild-type bacteriophage against different strains of *C. difficile*. Population growth of the various strains of *C. difficile* was monitored using CFU reduction assay for up to 6 hours following treatment with either the wild-type or CRISPR-array containing crPhage ϕCD146. CFU reduction assays provide enhanced quantitative sensitivity over optical density measurements. **Figure 8A** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 043. **Figure 8B** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 051. **Figure 8C** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 073. **Figure 8D** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain 093. **Figure 8E** exemplifies crPhage ϕCD146 lethality against *C. difficile* strain R20291.

[0024] Figure 9 exemplifies the enhanced lethality of crPhage ϕCD24-2 as compared to the wild-type bacteriophage against *C. difficile* strain F19. Population growth of *C. difficile* strain CD19 was monitored using CFU reduction assay for up to 6 hours following treatment with either the wild-type or CRISPR-array containing crPhage ϕCD24-2. The use of CFU reduction assay provides enhanced quantitative sensitivity over optical density measurements.

[0025] Figure 10 exemplifies a combinatorial comparison of crPhage ϕCD146 and crPhage ϕCD24-2 tested against *C. difficile* strain 043. A CFU assay of crPhage ϕCD146 and crPhage ϕCD24-2 anti-bacterial activity was conducted for each crPhage individually as well as the when

administered together. Co-administration showed improved killing efficacy as compared to treatment with a combination of both wild-type phages together.

[0026] Figure 11 exemplifies that the killing activity of the crPhage ϕCD146 consistently outperforms the lethality of the wild-type phage over a wide range of viral titer MOIs with a CRISPR array targeting *R20291-3*.

[0027] Figure 12A- Figure 12B exemplify an in-silico model predicting the number of resistant clones that emerge over time due to target site mutation as a function of the number of independent genes targeted by crRNAs. These models assume highly conservative assumptions that (1) mutational rate is independent of gene target and (2) that all 32 bases of crRNA match for activity. Two types of infection were modeled: an acute infection rising to a total burden of 10^{10} CFU by doubling every 6 hours as seen in **Figure 12A** or an aggressive infection to a total burden of 10^{14} CFU by doubling every 20 minutes as seen in **Figure 12B**. Both models show that 3 independent gene targets are sufficient to prevent mutational escape up to 28 days of infection length.

[0028] Figure 13 exemplifies the strain coverage for a series of individual Type I-E crRNAs targeted to conserved regions of the *E. coli* genome. crRNA array targets include the following genes in order of highest to lowest percentage of strain coverage: *Tsf* (100%), a*cpP* (99%), *gapA* (99%), *infA* (99%), *secY* (99%), *secY'2* (99%), *csrA* (99%), *trmD* (99%), *ftsA* (99%), *nusG* (99%), *fusA'2* (99%), *fusA* (98%), *glyQ* (98%), *eno* (95%), *gapA'2* (91%), *eno'2* (89%), and *nusG'2* (73%).

[0029] Figure 14 exemplifies the functional lethality assessment for a series of individual type I-E crRNAs with spacers targeted to conserved regions of the *E. coli* genome. crRNA targets include the following genes: *acpP, csrA, eno, fusA, gapA, glyQ, infA, musG, secY, , trmD,* and *Tsf.*

[0030] Figure 15 illustrates a schematic overview of three engineered CRISPR-enhanced bacteriophages against *E. coli* developed as a cocktail of three distinct obligate lytic bacteriophages that contain an identical DNA sequence encoding the transcriptional activator LeuO and a CRISPR-array. The three engineered LeuO enhanced bacteriophages include crT4, crT7, and crT7m.

[0031] Figure 16A exemplifies the relative prevalence and distribution of canonical Type I-E or Type I-F CRISPR-Cas systems in *E. coli*. Six hundred and twenty-five publicly available *E. coli* genomes were analyzed, spanning a diversity of strains including: uropathogenic *E. coli* (UPEC), Shiga toxin producing *E. coli*, (STEC), O157:H7 serotype *E. coli*, diarrheagenic *E. coli* (DEC), non-157 O antigen type *E. coli*, and enteropathogenic *E. coli* (EPEC).

[0032] Figure 16B shows that approximately 78% (487/625) of all tested strains in **Figure 16A** have a complete CRISPR-Cas3 system, either type I-E or type I-F.

[0033] Figure 17 exemplifies non-lytic M13-derived phagemid delivery of LeuO enhanced CRISPR array constructs using a validated *ftsA* spacer sequence designed to test the dependence on LeuO expression for CRISPR-mediated lethality. Lethality of LeuO enhanced phagemid vectors was tested via transduction of M13 bacteriophages into a range of strains including a parent EMG2 containing a wild-type H-NS repressed *E. coli* Type I-E CRISPR-Cas3 operon, a BW25113-derivative lacking the H-NS repression motifs in the CRISPR-Cas3 operon (Δhns), a BW25113-derivative containing an overexpressed CRISPR-Cas3 operon (BW+Cas) and a BW25113-derivative lacking Cas3 genes (BWΔCas).

[0034] Figure 18A-Figure 18C exemplify the improved lethality kinetics for the three LeuO enhanced crPhages (**Figure 18A -** crT7m, **Figure 18B -** crT4, and **Figure 18C -** crT7) comprising the transcriptional activator LeuO along with a CRISPR array as compared to their wild-type variants. Target *E. coli* were incubated for 2 or 5 hours for crT7m, crT4 and crT7, respectively, in growth media at the indicated multiplicity-of-infection (ratio of phage to bacteria) for each phage. Significant differences were observed in CFU reduction across all three crPhages.

[0035] Figure 19A- Figure 19E exemplify dose-response *in vitro* kill curves for LeuO enhanced crPhages crT4, crT7, and crT7m against *E. coli* strain MG1655 for each crPhage or crPhage cocktail and resultant changes in population were measured by optical density. *E. coli* was grown to mid-log phase and treated with multiplicity-of-infection (MOI; ratio of phage to bacteria) as follows: **Figure 19A**, crT7 was incubated at MOIs of 0.0001, 0.01, and 1.0; **Figure 19B**, crT7m was incubated at MOIs of 0.0009, 0.09, and 9.0; and **Figure 19C**, crT4 was incubated at MOIs of 0.0006, 0.06, and 6.0. Each phage was mixed in equal amounts to create a crPhage cocktail ('Cocktail') and was incubated at MOIs (for each crPhage) of 0.0006, 0.06, and 6.0, as seen in **Figure 19D**. **Figure 19E** is a zoomed in graph from **Figure 19D**.

[0036] Figure 20 exemplifies the dose-dependent relationship observed between concentrations of LeuO enhanced crPhages and the resultant time-to-lysis in *E. coli* MG1655. *E. coli* was grown to mid-log phase and treated with multiplicity-of-infection (MOI; ratio of phage to bacteria) as indicated for each crPhage. For all three crPhages tested, MOI in excess of 1.0 resulted in the fastest time-to-lysis, presumably being limited by the lytic period of each phage. The observed time-to-lysis for each phage was approximately 15-20 minutes for crT7m and crT7 and approximately 45-50 minutes for crT4.

[0037] Figure 21A-Figure 21G illustrate a schematic timeline representation of the dosing parameters for an *in vivo* tolerability of the three LeuO enhanced crPhages crT4, crT7, and crT7m. No overt toxicity was observed during veterinary observation and no measurable changes in body temperature or body weight were noted after dosing with each crPhage preparation as shown in

Figure 21B-Figure 21G. **Figure 21B and Figure 21E** illustrates crT7 body temperature and body weight after dosing, respectively. **Figure 21C and Figure 21F** illustrates crT7M body temperature and body weight after dosing, respectively. **Figure 21D and Figure 21G** illustrates crT4 body temperature and body weight after dosing, respectively.

[0038] Figure 22A-Figure 22D illustrate a schematic timeline representation for the treatment parameters for a murine *in-vivo* peritonitis model with *E. coli* with the LeuO enhanced crPhages and the results. Female CD-1 mice were injected intraperitoneally with a lethal dose of *E. coli* (~$5x10^7$ CFU/ mouse of ATCC 8739) followed within 30 minutes by intraperitoneal injections of saline or crPhages. Single-dose administration of crPhage ($2.0x10^{11}$ PFU/dose of crT7 (**Figure 22B**), $3.7x10^9$ PFU/dose of crT7m (**Figure 22C**) or $6.0x10^8$ PFU/dose of crT4 (**Figure 22D**)) resulted in significant protection.

[0039] Figure 23A-Figure 23E illustrate a schematic timeline representation for the treatment parameters for a murine *in-vivo* thigh infection model with *E. coli* treated with the LeuO enhanced crPhages for monitoring the effect upon bacterial bioburden reduction and the results. Mice were inoculated with 10^5 CFU of *E. coli* MG1655 by intramuscular injection into the thigh 30 minutes prior to intramuscular injection with the indicated crPhage or crPhage cocktail at corresponding doses of $4.0x10^{11}$ PFU/dose of crT7, $2.0x10^{11}$ PFU/dose of crT7M, $2.0x10^{10}$ PFU/dose of crT4 or the cocktail containing $1.0x10^{10}$ PFU/dose of each phage. After injection with each crPhage, whole thigh muscles were excised at the indicated time points, homogenized and immediately diluted and plated to count surviving bacterial colonies per gram of tissue. CFU reductions measured approximately 2-log for crT4 (**Figure 23C**), 3-log for crT7M (**Figure 23D**), and >5-log for both crT7 (**Figure 23B**) and the combined crPhage cocktail (**Figure 23E**).

[0040] Figure 24 illustrates *in-vivo* persistence and distribution of LeuO enhanced crPhages**.** Female CD-1 mice were treated with approximately $1.0x10^9$ PFU/dose/phage of a crT7/crT7m cocktail by intraurethral instillation directly into the bladder. At time points of 0, 0.5, 1, 6, 12, 24 and 72 hours post-inoculation, 3 mice per time point were sacrificed and collected bladder, kidney, blood, liver and spleen whole tissue homogenates were diluted and subjected to phage titration analysis to quantify the total combined amount of crT7 and crT7m. Presence of active crPhage was detected up to 72 hours after dosing. crPhage levels decreased over time in bladder and were undetectable in kidney, liver, blood and spleen by 72 hours. Significant phage titers were observed in the kidneys suggesting that intraurethral route of administration, in some instances, results in exposure in the lower and upper urinary tract. Also, phage titers were detected in blood, liver and spleen tissues, showing that crPhages appear to enter circulation by crossing the urothelium.

[0041] Figure 25A- Figure 25C exemplify a small scale *in-vivo* persistence and distribution study of LeuO enhanced crPhages similar to that seen in **Figure 24** using quantitative PCR detection instead of PFU assay. A single dose of $2.7x10^9$ PFU total of each crT7, crT7m and crT4 was administered by oral gavage. Treated mice were sacrificed and total DNA from whole tissue homogenates was extracted and subjected to qPCR analysis to quantify the amount of crT7 (**Figure 25A**) , crT4 (**Figure 25B**) or crT7m (**Figure 25C**) present.

[0042] Figure 26A- Figure 26B exemplify reduction in lysogeny formation rate and reduction in viable CD19 cells treated with a cI-knockout bacteriophage. **Figure 26A** exemplifies a reduction in viable CD19 cells when treated with ΔcI CD24-2 as compared to CD19 cells treated with WT CD24-2. **Figure 26B** exemplifies a reduction in the percent lysogens in the surviving CD19 cells that were treated with ΔcI CD24-2 as compared to in CD19 cells treated with WT CD24-2.

[0043] Figure 27A- Figure 27B exemplify comparative CFU reduction in bladder via i.v. delivery (**Figure 27A**) or local delivery (**Figure 27B**) of wild type phage (wtPhage), crPhage and ciprofloxacin. Results exemplify improved CFU reduction with crPhage compared to wtPhage.

[0044] Figure 28A- Figure 28B exemplify dose response of crPhage treatment in bladder (**Figure 28A**) and in kidney (**Figure 28B**). crPhage were delivered intraurethrally.

[0045] Figure 29 illustrates a schematic of an exemplary UTI efficacy study with research-grade material compared WT versus engineered cocktail via intravenous (IV), intra-urethral (IU), or concurrent IV and IU delivery.

[0046] Figure 30A – Figure 30D exemplify a UTI efficacy study demonstrating reduction in E.coli in bladder (**Figure 30A** and **Figure 30B**) and kidney (**Figure 30C** and **Figure 30D**) following intraurethral (IU) or intravenous (IV) administration. Measurements were taken 54 hour post infection in **Figure 30A** and **Figure 30C**. Measurements were taken 102 hour post infection in **Figure 30B** and **Figure 30D**. The results exemplify that crPhage cocktail has 1.5 to 3.5-log improved kill over wtPhage cocktail at 120h in the bladder. The results also exemplify that regardless of delivery route, at 120h crPhage cocktail performs comparable with ciprofloxacin in the bladder.

[0047] Figure 31A – Figure 31D illustrate route-dependent penetration of phage into different tissues, such as in urine 78 hour post infection (**Figure 31A**), into kidney 102 hour post infection (**Figure 31B**), into bladder102 hour post infection (**Figure 31C**), and into spleen 102 hour post infection (**Figure 31D**).

[0048] Figure 32 illustrates a schematic of an exemplary UTI efficacy study with research-grade material compared WT versus engineered cocktail via intravenous (IV), intra-urethral (IU), or concurrent IV and IU delivery.

[0049] **Figure 33A – Figure 33D** illustrate IV dosing of crPhage cocktail requires high doses for efficacy, while IU delivery is effective even at low doses and high dose crPhage outperforms ciprofloxacin. CFUs in **Figure 33A** (bladder) and **Figure 33C** (kidney) are analyzed 54 hour post infection. CFUs in **Figure 33B** (bladder) and **Figure 33D** (kidney) are analyzed 102 hour post infection.

[0050] **Figure 34A** is a schematic of an exemplary human study conducted in adults with reoccurring and asymptomatic E.coli colonization of the urinary tract.

[0051] **Figure 34B** is an exemplary study participant inclusion and exclusion criteria for the UTI Phase 1b study.

[0052] **Figure 35A – Figure 35F** illustrate engineered phage (p33s and p33s-6) show increased killing against both Type IE and Type IF E.coli strains.

[0053] **Figure 36A – Figure 36F** illustrate engineered phage (CRISPR phage crp0046) show increased killing against both Type IE and Type IF E.coli strains.

[0054] **Figure 37A – Figure 37C** illustrate switching phage cocktails overcomes target bacterial resistance in E.coli.

[0055] **Figure 38A – Figure 38B** illustrate a comparison of wildtype phage PB1 and CRISPR-enhanced PB1 (cr-PB1) against P.aeruginosa strains.

[0056] **Figure 39A – Figure 39B** illustrate plasmid based killing in E.coli and P.aeruginosa by Type I CRISPR-Cas systems.

DETAILED DESCRIPTION

Definitions

[0057] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this disclosure belongs. The terminology used in the description of the disclosure herein is for the purpose of describing particular embodiments only and is not intended to be limiting of the disclosure.

[0058] Unless the context indicates otherwise, it is specifically intended that the various features of the disclosure described herein are able of being used in any combination. Moreover, the present disclosure also contemplates that in some embodiments, any feature or combination of features set forth herein are excluded or omitted. To illustrate, if the specification states that a composition comprises components A, B and C, it is specifically intended that any of A, B or C, or a combination thereof, are omitted and disclaimed singularly or in any combination.

[0059] One of skill in the art will understand the interchangeability of terms designating the various CRISPR-Cas systems and their components due to a lack of consistency in the literature and an

ongoing effort in the art to unify such terminology. Likewise, one of skill in the art will also understand the interchangeability of terms designating the various anti-CRISPR proteins due to a lack of consistency in the literature and an ongoing effort in the art to unify such terminology.

[0060] As used in the description and the appended claims, the singular forms "a," "an" and "the" are intended to include the plural forms as well, unless the context clearly indicates otherwise. Also as used herein, "and/or" refers to and encompasses any and all possible combinations of one or more of the associated listed items, as well as the lack of combinations when interpreted in the alternative ("or").

[0061] The term "about" as used herein when referring to a measurable value such as a dosage or time period and the like refers to variations of ± 20%, ± 10%, ± 5%, ± 1%, + 0.5%, or even ± 0.1% of the specified amount. As used herein, phrases such as "between X and Y" and "between about X and Y" should be interpreted to include X and Y. As used herein, phrases such as "between about X and Y" mean "between about X and about Y" and phrases such as "from about X to Y" mean "from about X to about Y."

[0062] The term "comprise", "comprises", and "comprising", "includes", "including", "have" and "having", as used herein, specify the presence of the stated features, steps, operations, elements, and/or components, but do not preclude the presence or addition of one or more other features, steps, operations, elements, components, and/or groups thereof.

[0063] As used herein, the transitional phrase "consisting essentially of" means that the scope of a claim is to be interpreted to encompass the specified materials or steps recited in the claim and those that do not materially affect the basic and novel characteristic(s) of the claimed disclosure. Thus, the term "consisting essentially of" when used in a claim of this disclosure is not intended to be interpreted to be equivalent to "comprising."

[0064] The term "consists of" and "consisting of", as used herein, excludes any features, steps, operations, elements, and/or components not otherwise directly stated. The use of "consisting of" limits only the features, steps, operations, elements, and/or components set forth in that clause and does exclude other features, steps, operations, elements, and/or components from the claim as a whole.

[0065] As used herein, "chimeric" refers to a nucleic acid molecule or a polypeptide in which at least two components are derived from different sources (e.g., different organisms, different coding regions).

[0066] "Complement" as used herein mean 100% complementarity or identity with the comparator nucleotide sequence or it mean less than 100% complementarity (e.g., about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and

the like, complementarity). Complement or complementable may also be used in terms of a "complement" to or "complementing" a mutation.

[0067] The terms "complementary" or "complementarity", as used herein, refer to the natural binding of polynucleotides under permissive salt and temperature conditions by base-pairing. For example, the sequence "A-G-T" binds to the complementary sequence "T-C-A." Complementarity between two single-stranded molecules is "partial," in which only some of the nucleotides bind, or it is complete when total complementarity exists between the single stranded molecules. The degree of complementarity between nucleic acid strands has significant effects on the efficiency and strength of hybridization between nucleic acid strands.

[0068] As used herein, the term "gene" refers to a nucleic acid molecule capable of being used to produce mRNA, tRNA, rRNA, miRNA, anti-microRNA, regulatory RNA, and the like. Genes may or may not be capable of being used to produce a functional protein or gene product. Genes include both coding and non-coding regions (e.g., introns, regulatory elements, promoters, enhancers, termination sequences and/or 5' and 3' untranslated regions). A gene is "isolated" by which is meant a nucleic acid that is substantially or essentially free from components normally found in association with the nucleic acid in its natural state. Such components include other cellular material, culture medium from recombinant production, and/or various chemicals used in chemically synthesizing the nucleic acid.

[0069] As used herein, a "target nucleotide sequence" refers to the portion of a target gene that is complementary to the spacer sequence of the recombinant CRISPR array.

[0070] As used herein, a "target DNA," "target nucleotide sequence," "target region," or a "target region in the genome" refers to a region of an organism's genome that is fully complementary or substantially complementary (e.g., at least 70% complementary (e.g., 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more)) to a spacer sequence in a CRISPR array. In some embodiments, a target region is about 10 to about 40 consecutive nucleotides in length located immediately adjacent to a PAM sequence (PAM sequence located immediately 3' of the target region) in the genome of the organism. In some embodiments, a target nucleotide sequence is located adjacent to or flanked by a PAM (protospacer adjacent motif). While PAMs are often specific to the particular CRISPR- Cas system, a PAM sequence is determined by a suitable method. Thus, for example, experimental approaches include targeting a sequence flanked by all possible nucleotides sequences and identifying sequence members that do not undergo targeting, such as through in vitro cleavage of target DNA or the transformation of target plasmid DNA. In some embodiments, a computational approach includes performing BLAST searches of natural

spacers to identify the original target DNA sequences in bacteriophages or plasmids and aligning these sequences to determine conserved sequences adjacent to the target sequence.

[0071] As used herein, the term "protospacer adjacent motif" or "PAM" refers to a DNA sequence present on the target DNA molecule adjacent to the sequence matching the guide RNA spacer. This motif is found in the target gene next to the region to which a spacer sequence binds as a result of being complementary to that region and identifies the point at which base pairing with the spacer nucleotide sequence begins. For type I systems, the PAM is located immediately 5' to the sequence that matches the spacer, and thus is 3' to the sequence that base pairs with the spacer nucleotide sequence. Non-limiting examples of PAMs include CCA, CCT, CCG, CCT, CCA, TTC, AAG, AGG, ATG, GAG, and/or CC. For type I systems, PAM is directly recognized by Cascade. The exact PAM sequence that is required varies between each different CRISPR-Cas system and is identified through established bioinformatics and experimental procedures. Once a protospacer is recognized, Cascade generally recruits the endonuclease Cas3, which cleaves and degrades the target DNA.

[0072] For type II systems, the PAM is required for a Cas9/sgRNA to form an R-loop to interrogate a specific DNA sequence through Watson-Crick pairing of its guide RNA with the genome. The PAM specificity is a function of the DNA-binding specificity of the Cas9 protein (e.g., a —protospacer adjacent motif recognition domain at the C-terminus of Cas9).

[0073] As used herein, type I Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated complex for antiviral defense (Cascade) refers to a complex of polypeptides involved in processing of pre-crRNAs and subsequent binding to the target DNA in type I CRISPR-Cas systems. These polypeptides include, but are not limited to, the Cascade polypeptides of type I subtypes l-A, l-B, l-C, l-D, l-E and l-F. Non-limiting examples of type I -A polypeptides include Cas7 (Csa2), Cas8a1 (Csx13), Cas8a2 (Csx9), Cas5, Csa5, Cas6a, Cas3' and/or a Cas3". Non-limiting examples of type l-B polypeptides include Cas6b, Cas8b (Csh1), Cas7 (Csh2) and/or Cas5. Non-limiting examples of type-IC polypeptides include Cas5d, Cas8c (Csd1), and/or Cas7 (Csd2). Non-limiting examples of type-ID polypeptides include Cas10d (Csc3), Csc2, Csc1, and/or Cas6d. Non-limiting examples of type l-E polypeptides include Cse1 (CasA), Cse2 (CasB), Cas7 (CasC), Cas5 (CasD) and/or Cas6e (CasE). Non- limiting examples of type I-F polypeptides include Cys1, Cys2, Cas7 (Cys3) and/or Cas6f (Csy4). In some embodiments, a recombinant nucleic acid described herein comprises, consists essentially of, or consists of, a nucleotide sequence encoding a subset of type-l Cascade polypeptides that function to process a CRISPR array and subsequently bind to a target DNA using the spacer of the processed CRISPR RNA as a guide.

[0074] A "CRISPR array" as used herein means a nucleic acid molecule that comprises at least two repeat sequences, or a portion of each of said repeat sequences, and at least one spacer sequence. One of the two repeat sequences, or a portion thereof, is linked to the 5' end of the spacer sequence and the other of the two repeat sequences, or portion thereof, is linked to the 3' end of the spacer sequence. In a recombinant CRISPR array, the combination of repeat sequences and spacer sequences is synthetic, made by man and not found in nature. In some embodiments, a "CRISPR array" refers to a nucleic acid construct that comprises from 5' to 3' at least one repeat-spacer sequences (e.g., about 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25 or more repeat-spacer sequences, and any range or value therein), wherein the 3' end of the 3' most repeat-spacer sequence of the array are linked to a repeat sequence, thereby all spacers in said array are flanked on both the 5' end and the 3' end by a repeat sequence.

[0075] As used herein, "spacer sequence" or "spacer refers to a nucleotide sequence that is complementary to a target DNA (i.e., target region in the genome or the "protospacer sequence," which is adjacent to a protospacer adjacent motif (PAM) sequence). The spacer sequence is fully complementary or substantially complementary (e.g., at least about 70% complementary (e.g., about 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more)) to a target DNA.

[0076] A "repeat sequence" as used herein, refers to, for example, any repeat sequence of a wild-type CRISPR locus or a repeat sequence of a synthetic CRISPR array that are separated by "spacer sequences" (e.g., a repeat-spacer-repeat sequence). A repeat sequence useful with this disclosure is any known or later identified repeat sequence of a CRISPR locus or it is a synthetic repeat designed to function in a CRISPR system, for example CRISPR Type I system.

[0077] As used herein, the term "CRISPR phage", "CRISPR enhanced phage", and "crPhage" refers to a bacteriophage particle comprising bacteriophage DNA comprising at least one heterologous polynucleotide. In some embodiments, the polynucleotide encodes at least one component of a CRISPR-Cas system (e.g., CRISPR array, crRNA; e.g., PI bacteriophage comprising an insertion of crRNA targeting). In some embodiments, the polynucleotide encodes at least one transcriptional activator of a CRISPR-Cas system. In some embodiments, the polynucleotide encodes at least one component of an anti-CRISPR polypeptide of a CRISPR-Cas system.

[0078] As used herein, the phrase "substantially identical," or "substantial identity" in the context of two nucleic acid molecules, nucleotide sequences or protein sequences, refers to two or more sequences or subsequences that have at least about 50%, 51 %, 52%, 53%, 54%, 55%, 56%, 57%,

58%, 59%, 60%, 61 %, 62%, 63%, 64%, 65%, 66%, 67%, 68%, 69%, 70%, 71 %, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81 %, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and/or 100% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. In some embodiments, substantial identity refer to two or more sequences or subsequences that have at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95, 96, 96, 97, 98, or 99% identity. 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 entered 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.

[0079] Optimal alignment of sequences for aligning a comparison window are conducted by tools such as the local homology algorithm of Smith and Waterman, the homology alignment algorithm of Needleman and Wunsch, the search for similarity method of Pearson and Lipman, and optionally by computerized implementations of these algorithms such as GAP, BESTFIT, FASTA, and TFASTA available as part of the GCG® Wisconsin Package® (Accelrys Inc., San Diego, CA). An "identity fraction" for aligned segments of a test sequence and a reference sequence is the number of identical components which are shared by the two aligned sequences divided by the total number of components in the reference sequence segment, i.e., the entire reference sequence or a smaller defined part of the reference sequence. Percent sequence identity is represented as the identity fraction multiplied by 100. The comparison of one or more polynucleotide sequences is to a full-length polynucleotide sequence or to a portion thereof, or to a longer polynucleotide sequence. In some instances, "Percent identity" is determined using BLASTX version 2.0 for translated nucleotide sequences and BLASTN version 2.0 for polynucleotide sequences.

[0080] In some embodiments, the recombinant nucleic acids molecules, nucleotide sequences and polypeptides disclosed herein are "isolated." An "isolated" nucleic acid molecule, an "isolated" nucleotide sequence or an "isolated" polypeptide is a nucleic acid molecule, nucleotide sequence or polypeptide that exists apart from its native environment. In some instances, an isolated nucleic acid molecule, nucleotide sequence or polypeptide exists in a purified form that is at least partially separated from at least some of the other components of the naturally occurring organism or virus, for example, the cell or viral structural components or other polypeptides or nucleic acids commonly found associated with the polynucleotide. In representative embodiments, the isolated

nucleic acid molecule, the isolated nucleotide sequence and/or the isolated polypeptide is at least about 1%, 5%, 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, or more pure.

[0081] As used herein, the terms “anti-CRISPR” or “Acr” refers to any protein or gene product with functional anti-CRISPR activity. Due to a lack of consistency in the literature, one of skill in the art will understand the interchangeability of terms designating the various anti-CRISPR proteins. For example, as used herein the designation of Acr1-Bo is interchangeable with AcrIIC1Boe and the designation of Acr2-Nm is interchangeable with AcrIIC2Nme. Also, as used herein, the designation of Acr88a-32 is interchangeable with AcrE2. An anti-CRISPR protein is any bacteriophage protein with activity that prevents the function of a bacterial CRISPR-Cas system. Activity of an anti-CRISPR protein prevents a host bacterium from mounting a CRISPR-Cas system based defense against the invading bacteriophage.

[0082] By the terms "treat," "treating," or "treatment," it is intended that the severity of the subject's condition is reduced or at least partially improved or modified and that some alleviation, mitigation or decrease in at least one clinical symptom is achieved, and/or there is a delay in the progression of the disease or condition, and/or delay of the onset of a disease or illness. With respect to an infection, a disease or a condition, the term refers to a decrease in the symptoms or other manifestations of the infection, disease or condition. In some embodiments, treatment provides a reduction in symptoms or other manifestations of the infection, disease or condition by at least about 5%, e.g., about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50% or more.

[0083] The terms with respect to an “infection”, “a disease”, or “a condition”, used herein, refer to any adverse, negative, or harmful physiological condition in a subject. In some embodiments, the source of an “infection”, “a disease”, or “a condition”, is the presence of a target bacterial population in and/or a subject. In some embodiments, the bacterial population comprises one or more target bacterial species. In some embodiments, the one or more bacteria in the bacterial population comprise one or more strains of one or more bacteria. In some embodiments, the target bacterial population causing an “infection”, “a disease”, or “a condition” is acute or chronic. In some embodiments, the target bacterial population causing an “infection”, “a disease”, or “a condition” is localized or systemic. In some embodiments, the target bacterial population causing an “infection”, “a disease”, or “a condition” is idiopathic. In some embodiments, the target bacterial population causing an “infection”, “a disease”, or “a condition” is acquired through means, including but not limited to, respiratory inhalation, ingestion, skin and wound infections, blood stream infections, middle-ear infections, gastrointestinal tract infections, peritoneal membrane infections, urinary tract infections, urogenital tract infections, oral soft tissue infections, intra-abdominal infections, epidermal or mucosal absorption, eye infections (including contact lens

contamination), endocarditis, infections in cystic fibrosis, infections of indwelling medical devices such as joint prostheses, dental implants, catheters and cardiac implants, sexual contact, and/or hospital-acquired and ventilator-associated bacterial pneumonias.

[0084] As used herein the term "biofilm" means an accumulation of microorganisms embedded in a matrix of polysaccharide. Biofilms form on solid biological or non-biological surfaces and are medically important, accounting for over 80 percent of microbial infections in the body.

[0085] The terms "prevent," "preventing," and "prevention" (and grammatical variations thereof) refer to prevention and/or delay of the onset of an infection, disease, condition and/or a clinical symptom(s) in a subject and/or a reduction in the severity of the onset of the infection, disease, condition and/or clinical symptom(s) relative to what would occur in the absence of carrying out the methods disclosed herein prior to the onset of the disease, disorder and/or clinical symptom(s). Thus, in some embodiments, to prevent infection, food, surfaces, medical tools and devices are treated with compositions and by methods disclosed herein.

[0086] A "subject" disclosed herein includes any animal that has or is susceptible to an infection, disease or condition involving bacteria. Thus, in some embodiments, subjects are mammals, avians, reptiles, amphibians, or fish. Mammalian subjects include but are not limited to humans, non-human primates (e.g., gorilla, monkey, baboon, and chimpanzee, etc.), dogs, cats, goats, horses, pigs, cattle, sheep, and the like, and laboratory animals (e.g., rats, guinea pigs, mice, gerbils, hamsters, and the like). Avian subjects include but are not limited to chickens, ducks, turkeys, geese, quail, pheasants, and birds kept as pets (e.g., parakeets, parrots, macaws, cockatoos, canaries, and the like). In some embodiments, suitable subjects include both males and females and subjects of any age, including embryonic (e.g., *in-utero* or *in-ovo*), infant, juvenile, adolescent, adult and geriatric subjects. In some embodiments, a subject is a human.

[0087] By "pharmaceutically acceptable" it is meant a material that is not biologically or otherwise undesirable, i.e., the material are administered to a subject without causing any undesirable biological effects such as toxicity.

[0088] Provided in various embodiments are bacteriophages, characterized and/or comprising any nucleic acid described herein. In some embodiments, provided herein is a temperate bacteriophage (e.g., a bacteriophage having lytic activity, such as described herein). In specific embodiments, the temperate bacteriophage comprises a removal, replacement, or inactivation of at least one lysogeny gene. In certain embodiments, provided herein are bacteriophages having lytic activity and comprising (a) a nucleic acid encoding a spacer sequence and/or (b) a crRNA transcribed therefrom. In some specific embodiments, provided in certain embodiments herein are bacteriophages comprising (i) a first nucleic acid encoding a spacer sequence and/or a crRNA

transcribed therefrom, and (ii) a gene that is capable of inducing lysis of a bacterium (e.g., a target bacterium). In more specific embodiments, the spacer sequence is complementary to a nucleic acid sequence of a target gene of or in a bacterium (e.g., target bacterium). Provided in some embodiments herein are bacteriophages comprising a nucleic acid encoding a transcriptional activator for the CRISPR-Cas system in a bacterium (e.g., a target bacterium). In certain embodiments, provided herein are bacteriophages having lytic activity and a first nucleic acid sequence encoding an anti-CRISPR polypeptide (and/or comprising an anti-CRISPR polypeptide). In various embodiments, a bacteriophage provided herein has any one or more of the above references characteristics and/or activities. Moreover, in various embodiments herein, such bacteriophages comprise any one or more characteristic or activity described in the summary or detailed description herein.

[0089] In some embodiments, such bacteriophages are utilized and various compositions (e.g., pharmaceutical compositions) and methods, such as described herein. In certain embodiments, such bacteriophages are useful in any number of applications and methods (e.g., of tuning the microbiome of an individual or subject, such as one in need thereof), such as those described herein. In some embodiments, such bacteriophages are utilized in methods of or that involve killing (e.g., selectively killing) a bacterium (e.g., a target bacterium). In specific embodiments, the target bacterium is in a mixed population of bacteria, such as in an individual, environment, or other suitable location, such as described herein.

[0090] In certain embodiments, a bacteriophage provided herein selectively kills a target bacteria or bacterium, e.g., such that the bacteria that is not the target bacterium or bacteria is killed at a lesser rate than the target bacteria, such as at less than 50% the rate, less than 25% the rate, less than 10% the rate, or about 0% the rate (i.e., not at all) relative to the target bacterium or bacteria. In some instances, such as in certain methods provided herein, less than 50% of the non-target bacterium is killed, less than 25%, less than 20%, less than 10%, less than 5% killed, or the like is killed.

CRISPR Array

[0091] Disclosed herein are CRISPR arrays. In some embodiments, a nucleic acid encoding a CRISPR array comprises at least one repeat sequence and at least one spacer sequences complementary to a target nucleotide sequence from a target gene in the target bacterium. In some embodiments, a CRISPR array is of any length and comprises any number of spacer nucleotide sequences alternating with repeat nucleotide sequences necessary to achieve the desired level of killing of the target bacterium by use of one or more target genes. In some embodiments, the CRISPR array comprise, consist essentially of, or consist of 1 to about 100 spacer nucleotide

sequences, each linked on its 5' end and its 3' end to a repeat nucleotide sequence. In some embodiments, a recombinant CRISPR array of disclosed herein, consist essentially of, or consist of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1 , 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 , 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 , 32, 33, 34, 35, 36, 37, 38, 39, 40, 41 , 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 , 52, 53, 54, 55, 56, 57, 58, 59, 60, 61 , 62, 63, 64, 65, 66, 67, 68, 69, 70, 71 , 72, 73, 74, 75, 76, 77, 78, 79, 80, 81 , 82, 83, 84, 85, 86, 87, 88, 89, 90, 91 , 92, 93, 94, 95, 96, 97, 98, 99, 100, or more, spacer nucleotide sequences.

Spacer

[0092] In some embodiments, the spacer sequence described herein comprises one, two, three, four, or five mismatches as compared to the target DNA. In some embodiments, mismatches are contiguous. In some embodiments, mismatches are noncontiguous. In some embodiments, the spacer sequence has 70% complementarity to a target DNA. In some embodiments, the spacer nucleotide sequence has 80% complementarity to a target DNA. In some embodiments, the spacer nucleotide sequence is 85%, 90%, 95%, 96%, 97%, 98%, 99% or 100% complementarity to a target nucleotide sequence of a target gene. In some embodiments, the spacer sequence has 100% complementarity to the target DNA. In some embodiments, a spacer sequence has complete complementarity or substantial complementarity over a region of a target nucleotide sequence that are at least about 8 nucleotides to about 150 nucleotides in length. In some embodiments, a spacer sequence have complete complementarity or substantial complementarity over a region of a target nucleotide sequence that is at least about 20 nucleotides to about 100 nucleotides in length. In some embodiments, the 5 ' region of a spacer sequence is 100% complementary to a target DNA while the 3' region of the spacer is substantially complementary to the target DNA and therefore the overall complementarity of the spacer sequence to the target DNA is less than 100%. For example, in some embodiments, the first 7, 8, 9, 10, 11, 12, 13, 14, 15, 16 nucleotides in the 3' region of a 20 nucleotide spacer sequence (seed region) is 100% complementary to the target DNA, while the remaining nucleotides in the 5' region of the spacer sequence are substantially complementary (e.g., at least about 70% complementary) to the target DNA. In some embodiments, the first 7 to 12 nucleotides of the 3' end of the spacer sequence is 100% complementary to the target DNA, while the remaining nucleotides in the 5' region of the spacer sequence are substantially complementary (e.g., at least about 50% complementary (e.g., 50%, 55%, 60%, 65%, 70%, 71%, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more)) to the target DNA. In some embodiments, the first 7 to 10 nucleotides in the 3' end of the spacer sequence is 75%-99% complementary to the target DNA, while the remaining nucleotides in the 5' region of the spacer sequence are at least

about 50% to about 99% complementary to the target DNA. In some embodiments, the first 7 to 10 nucleotides in the 3' end of the spacer sequence is 100% complementary to the target DNA, while the remaining nucleotides in the 5' region of the spacer sequence are substantially complementary (e.g., at least about 70% complementary) to the target DNA. In some embodiments, the first 10 nucleotides (within the seed region) of the spacer sequence is 100% complementary to the target DNA, while the remaining nucleotides in the 5' region of the spacer sequence are substantially complementary (e.g., at least about 70% complementary) to the target DNA. In some embodiment, the 5' region of a spacer sequence (e.g., the first 8 nucleotides at the 5' end, the first 10 nucleotides at the 5' end, the first 15 nucleotides at the 5' end, the first 20 nucleotides at the 5' end) have about 75% complementarity or more (75% to about 100% complementarity) to a target DNA, while the remainder of the spacer sequence have about 50% or more complementarity to the target DNA. In some embodiments, the first 8 nucleotides at the 5' end of a spacer sequence have 100% complementarity to the target nucleotide sequence or have one or two mutations and therefore is about 88% complementary or about 75% complementary to a target DNA, respectively, while the remainder of the spacer nucleotide sequence is at least about 50% or more complementary to the target DNA.

[0093] In some embodiments, a spacer sequence described herein is about 15 nucleotides to about 150 nucleotides in length. In some embodiments, a spacer nucleotide sequence is about 15 nucleotides to about 100 nucleotides in length (e.g., about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100 nucleotides or more). In some embodiments, a spacer nucleotide sequence is a length of about 8 to about 150 nucleotides, about 8 to about 100 nucleotides, about 8 to about 50 nucleotides, about 8 to about 40 nucleotides, about 8 to about 30 nucleotides, about 8 to about 25 nucleotides, about 8 to about 20 nucleotides, about 10 to about 150 nucleotides, about 10 to about 100 nucleotides, about 10 to about 80 nucleotides, about 10 to about 50 nucleotides, about 10 to about 40, about 10 to about 30, about 10 to about 25, about 10 to about 20, about 15 to about 150, about 15 to about 100, about 15 to about 50, about 15 to about 40, about 15 to about 30, about 20 to about 150 nucleotides, about 20 to about 100 nucleotides, about 20 to about 80 nucleotides, about 20 to about 50 nucleotides, about 20 to about 40, about 20 to about 30, about 20 to about 25, at least about 8, at least about 10, at least about 15, at least about 20, at least about 25, at least about 30, at least about 32, at least about 35, at least about 40, at least about 44, at least about 50, at least about 60, at least about 70, at least about 80, at least about 90, at least about 100, at least about 110, at least about 120, at least about

130, at least about 140, at least about 150 nucleotides in length, or more, and any value or range therein.

[0094] In some embodiments, the identity of two or more spacer nucleotide sequences of a CRISPR array disclosed herein is the same. In some embodiments, the identity of two or more spacer nucleotide sequences of a CRISPR array disclosed herein is different. In some embodiments, the identity of two or more spacer nucleotide sequences of a CRISPR array is different but are complementary to one or more target nucleotide sequences. In some embodiments, the identity of two or more spacer nucleotide sequences of a CRISPR array is different and are complementary to one or more target nucleotide sequences that are overlapping sequences. In some embodiments, the identity of two or more spacer nucleotide sequences of a CRISPR array is different and are complementary to one or more target nucleotide sequences that are not overlapping sequences.

Codon Optimization

[0095] In some embodiments, a polynucleotide, nucleotide sequence and/or recombinant nucleic acid molecule described herein (e.g., polynucleotides comprising a CRISPR array, Cascade polypeptides, Cas9 polypeptides, Cas3 polypeptides, Cas3' polypeptides, Cas3" polypeptides, recombinant Type I or Type II, Type III, Type IV, Type V, Type VI CRISPR-Cas systems of the disclosure, polynucleotides encoding transcriptional activators, and the like) is codon optimized for expression in any species of interest. Codon optimization involves modification of a nucleotide sequence for codon usage bias using species-specific codon usage tables. The codon usage tables are generated based on a sequence analysis of the most highly expressed genes for the species of interest. When the nucleotide sequences are to be expressed in the nucleus, the codon usage tables are generated based on a sequence analysis of highly expressed nuclear genes for the species of interest. The modifications of the nucleotide sequences are determined by comparing the species specific codon usage table with the codons present in the native polynucleotide sequences. Codon optimization of a nucleotide sequence results in a nucleotide sequence having less than 100% identity (e.g., 50%, 60%, 70%, 71 %, 72%, 73%, 74%, 75%, 76%, 77%, 78%, 79%, 80%, 81 %, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and the like) to the native nucleotide sequence but which still encodes a polypeptide having the same function as that encoded by the original nucleotide sequence. In some embodiments, the nucleotide sequence and/or recombinant nucleic acid molecule of this disclosure are codon optimized for expression in the organism/species of interest.

Repeat Nucleotide sequences

[0096] In some embodiments, a repeat nucleotide sequence of a CRISPR array comprises a nucleotide sequence of any known repeat nucleotide sequence of a CRISPR-Cas system. In some

embodiment, the CRISPR-Cas system is a type-I CRISPR-Cas system. In some embodiment, a repeat nucleotide sequence is of a synthetic sequence comprising the secondary structure of a native repeat from a type-I CRISPR-Cas system (e.g., an internal hairpin).

[0097] In some embodiments, a spacer nucleotide sequence of a CRISPR array described herein is linked at its 5' end to the 3' end of a repeat sequence. In some embodiments, the spacer nucleotide sequence is linked at its 5' end to about 1 to about 8, about 1 to about 10, or about 1 to about 15 nucleotides of the 3' end of a repeat nucleotide sequence. In some embodiments, the about 1 to about 8, about 1 to about 10, about 1 to about 15 nucleotides of the repeat nucleotide sequence are a portion of the 3' end of a repeat nucleotide sequence. In some embodiments, spacer nucleotide sequence is linked at its 3' end to the 5' end of a repeat nucleotide sequence. In some embodiments, the spacer is linked at its 3' end to about 1 to about 8, about 1 to about 10, or about 1 to about 15 nucleotides of the 5' end of a repeat nucleotide sequence. In some embodiments, the about 1 to about 8, about 1 to about 10, about 1 to about 15 nucleotides of the repeat nucleotide sequence are a portion of the 5' end of a repeat nucleotide sequence.

[0098] In some embodiments, a spacer nucleotide sequence described herein is linked at its 5' end to a first repeat nucleotide sequence and linked at its 3' end to a second repeat nucleotide sequence to form a repeat-spacer-repeat sequence. In some embodiments, a spacer described herein is linked at its 5' end to about 1 to about 8, about 1 to about 10, or about 1 to about 15 nucleotides of the 3' end of a first repeat sequence and is linked at its 3' end to about 1 to about 8, about 1 to about 10, or about 1 to about 15 nucleotides of the 5' end of a second repeat sequence. In some embodiments, the about 1 to about 8, about 1 to about 10, about 1 to about 15 nucleotides of the first repeat sequence are a portion of the 3' end of the first repeat nucleotide sequence. In some embodiments, the about 1 to about 8, about 1 to about 10, about 1 to about 15 nucleotides of the first second sequence are a portion of the 3' end of the second repeat nucleotide sequence. In some embodiments, a spacer nucleotide sequence disclosed herein is linked at its 5' end to the 3' end of a first repeat nucleotide sequence and is linked at its 3' end to the 5' of a second repeat nucleotide sequence where the spacer nucleotide sequence and the second repeat nucleotide sequence are repeated to form a repeat-(spacer-repeat)n sequence such that n is any integer from 1 to 100. Thus, in some embodiments, a repeat-(spacer-repeat)n sequence disclosed herein comprise, consist essentially of, or consist of 1 , 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1 , 12, 13, 14, 15, 16, 17, 18, 19, 20, 21 , 22, 23, 24, 25, 26, 27, 28, 29, 30, 31 , 32, 33, 34, 35, 36, 37, 38, 39, 40, 41 , 42, 43, 44, 45, 46, 47, 48, 49, 50, 51 , 52, 53, 54, 55, 56, 57, 58, 59, 60, 61 , 62, 63, 64, 65, 66, 67, 68, 69, 70, 71 , 72, 73, 74, 75, 76, 77, 78, 79, 80, 81 , 82, 83, 84, 85, 86, 87, 88, 89, 90, 91 , 92, 93, 94, 95, 96, 97, 98, 99, 100, or more, spacer nucleotide sequences.

[0099] Thus, in some embodiments, a repeat sequence is identical to or substantially identical to a repeat sequence from a wild-type CRISPR Type I, II, or III loci. In some embodiments, a repeat sequence comprises a portion of a wild type repeat sequence (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 1 1, 12, 13, 14, 15 or more contiguous nucleotides of a wild type repeat sequence). In some embodiments, a repeat sequence comprises, consists essentially of, or consists of at least one nucleotide (e.g., 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, or more nucleotides, or any range therein).

Regulatory Elements

[0100] In some embodiments, recombinant CRISPR arrays, nucleotide sequences, and/or nucleic acid molecules disclosed herein are operatively associated with a variety of promoters, terminators and other regulatory elements for expression in various organisms or cells. In some embodiments, at least one promoter and/or terminator is operably linked to a recombinant nucleic acid molecule and/or a recombinant CRISPR array disclosed herein. Any promoter useful with this disclosure is used and includes, for example, promoters functional with the organism of interest as well as constitutive, inducible, developmental regulated, tissue-specific/preferred- promoters, and the like, as described herein. A regulatory element as used herein is endogenous or heterologous. In some embodiments, an endogenous regulatory element derived from the subject organism is inserted into a genetic context in which it does not naturally occur (e.g. a different position in the genome than as found in nature), thereby producing a recombinant or non-native nucleic acid.

[0101] In some embodiments, expression of a construct disclosed herein is constitutive, inducible, temporally regulated, developmentally regulated, or chemically regulated. In some embodiments, a construct is made constitutive, inducible, temporally regulated, developmentally regulated, or chemically regulated by operatively linking the construct to a promoter functional in an organism of interest. In some embodiments, repression is made reversible by operatively linking a recombinant nucleic acid construct disclosed herein to an inducible promoter that is functional in an organism of interest. The choice of promoter described herein will vary depending on the quantitative, temporal and spatial requirements for expression, and also depending on the host cell to be transformed.

[0102] Exemplary promoters for use with the methods, bacteriophage and composition disclosed herein include promoters that are functional in bacteria. For example, L-arabinose inducible (*araBAD, P_{BAD}*) promoter, any *lac* promoter, L-rhamnose inducible (rhaPBAD) promoter, T7 RNA polymerase promoter, *trc* promoter, *tac* promoter, lambda phage promoter (p_Lp_L-9G-50), anhydrotetracycline-inducible (*tetA*) promoter, *trp*, *Ipp, phoA*, *recA*, *proU*, *cst*-1, *cadA*, *nar*, *Ipp*-

lac, *cspA*, *11-lac* operator, T3-*lac* operator, T4 gene *32*, T5-*lac* operator, *nprM*- lac operator, Vhb, Protein A, corynebacterial-*E. coli* like promoters, *thr*, *horn*, diphtheria toxin promoter, *sig* A, *sig* B, *nusG*, *SoxS*, *katb*, *a-amylase* (*Pamy*), *Ptms*, *P43* (comprised of two overlapping RNA polymerase σ factor recognition sites, σA, σB), *Ptms*, *P43*, *rplK-rplA*, ferredoxin promoter, and/or xylose promoter.

[0103] In some embodiments, inducible promoters are used. In some embodiment, chemical-regulated promoters are used to modulate the expression of a gene in an organism through the application of an exogenous chemical regulator. The use of chemically regulated promoters enables RNAs and/or the polypeptides disclosed herein to be synthesized only when, for example, an organism is treated with the inducing chemicals. In some embodiments where a chemical-inducible promoter is used, the application of a chemical induces gene expression. In some embodiments wherein a chemical-repressible promoter is used, the application of the chemical represses gene expression. In some embodiments, the promoter is a light-inducible promoter, where application of specific wavelengths of light induces gene expression. In some embodiments, a promoter is a light-repressible promoter, where application of specific wavelengths of light represses gene expression.

Transformation

[0104] In some embodiments, the nucleotide sequences, constructs, and expression cassettes disclosed herein are expressed transiently and/or stably incorporated into the genome of a host organism. In some embodiments, a polynucleotide disclosed herein is introduced into a cell by any method known to those of skill in the art. Exemplary methods of transformation include transformation via electroporation of competent cells, passive uptake by competent cells, chemical transformation of competent cells, as well as any other electrical, chemical, physical (mechanical) and/or biological mechanism that results in the introduction of nucleic acid into a cell, including any combination thereof. In some embodiments, transformation of a cell comprises nuclear transformation. In some embodiments, transformation of a cell comprises plasmid transformation and conjugation.

[0105] In some embodiments, when more than one nucleotide sequence is introduced, the nucleotide sequences are assembled as part of a single nucleic acid construct, or as separate nucleic acid constructs, and are located on the same or different nucleic acid constructs. In some embodiments, nucleotide sequences are introduced into the cell of interest in a single transformation event, or in separate transformation events.

Expression Cassette

[0106] In some embodiments, a nucleic acid construct is an "expression cassette" or in an expression cassette. As used herein, "expression cassette" means a recombinant nucleic acid

molecule comprising a nucleotide sequence of interest (e.g., the recombinant nucleic acid molecules and CRISPR arrays disclosed herein), wherein the nucleotide sequence is operably associated with at least a control sequence (e.g., a promoter). In some embodiments, the expression cassettes are designed to express the recombinant nucleic acid molecules and/or the recombinant CRISPR arrays disclosed herein.

[0107] In some embodiments, an expression cassette comprising a nucleotide sequence of interest is chimeric, meaning that at least one of its components is heterologous with respect to at least one of its other components. In some embodiments, an expression cassette is naturally occurring but has been obtained in a recombinant form useful for heterologous expression.

[0108] In some embodiments, an expression cassette includes a transcriptional and/or translational termination region (i.e. termination region) that is functional in the selected host cell. In some embodiments, termination regions are responsible for the termination of transcription beyond the heterologous nucleotide sequence of interest and for correct mRNA polyadenylation. In some embodiments, the termination region is native to the transcriptional initiation region, is native to the operably linked nucleotide sequence of interest, is native to the host cell, or is derived from another source (i.e., foreign or heterologous to the promoter, to the nucleotide sequence of interest, to the host, or any combination thereof). In some embodiments, terminators are operably linked to the recombinant nucleic acid molecule and CRISPR array disclosed herein.

[0109] In some embodiments, an expression cassette includes a nucleotide sequence for a selectable marker. As used herein, "selectable marker" means a nucleotide sequence that when expressed imparts a distinct phenotype to the host cell expressing the marker and thus allows such transformed cells to be distinguished from those that do not have the marker. In some embodiments, a nucleotide sequence encode either a selectable or screenable marker, depending on whether the marker confers a trait that is selected for by chemical means, such as by using a selective agent (e.g. an antibiotic), or on whether the marker is simply a trait that one identifies through observation or testing, such as by screening (e.g., fluorescence).

Vectors

[0110] In addition to expression cassettes, the nucleic acid molecules and nucleotide sequences described herein (e.g. polynucleotides comprising a CRISPR array, polynucleotides encoding a transcriptional activator, or anti-CRISPR polypeptides) are used in connection with vectors. The term "vector" refers to a composition for transferring, delivering or introducing a nucleic acid (or nucleic acids) into a cell. A vector comprises a nucleic acid molecule comprising the nucleotide sequence(s) to be transferred, delivered or introduced. Non-limiting examples of general classes of vectors include but are not limited to a viral vector, a plasmid vector, a phage vector, a phagemid

vector, a cosmid vector, a fosmid vector, a bacteriophage, an artificial chromosome, or an agrobacterium binary vector in double or single stranded linear or circular form which may or may not be self-transmissible or mobilizable. A vector as defined herein transforms prokaryotic or eukaryotic host either by integration into the cellular genome or exist extrachromosomally (e.g. autonomous replicating plasmid with an origin of replication). Additionally included are shuttle vectors by which is meant a DNA vehicle capable, naturally or by design, of replication in two different host organisms. In some embodiments, a shuttle vector replicates in actinomycetes and bacteria and/or eukaryotes. In some embodiments, the nucleic acid in the vector are under the control of, and operably linked to, an appropriate promoter or other regulatory elements for transcription in a host cell. In some embodiments, the vector is a bi-functional expression vector which functions in multiple hosts.

CRISPR/CAS Systems

[0111] CRISPR-Cas systems are naturally adaptive immune systems found in bacteria and archaea. The CRISPR system is a nuclease system involved in defense against invading phages and plasmids that provides a form of acquired immunity. There is a diversity of CRISPR-Cas systems based on the set of *cas* genes and their phylogenetic relationship. There are at least six different types (I through VI) where Type I represents over 50% of all identified systems in both bacteria and archaea. In some embodiments, a Type I, Type II, Type II, Type IV, Type V, or Type VI CRISPR-Cas system is used herein.

[0112] In some embodiments, processing of a CRISPR-array disclosed herein includes, but is not limited to, the following processes: 1) transcription of the nucleic acid encoding a CRISPR array into a pre-crRNA and optional tracrRNA; 2) pre-crRNA processing by either Cas6 or Cas9/Rnase III into mature crRNAs; 3) mature crRNA complexation Cas9 or Cascade; 4) target recognition by the complexed mature crRNA/Cas9 or crRNA/Cascade complexes; and 5) nuclease activity at the target leading to double or single stranded DNA breakage.

[0113] With regard to Type I systems, Type I systems are divided into seven subtypes including: type I-A, type I-B, type I-C, type I-D, type I-E, type I-F, and type I-U. Type I CRISPR-Cas systems include a multi-subunit complex called Cascade (for complex associated with antiviral defense), Cas3 (a protein with nuclease, helicase, and exonuclease activity that is responsible for degradation of the target DNA), and crRNA (stabilizes Cascade complex and directs Cascade and Cas3 to DNA target). Cascade forms a complex with the crRNA, and the protein-RNA pair recognizes its genomic target by complementary base pairing between the 5' end of the crRNA sequence and a predefined protospacer. This complex is directed to homologous loci of pathogen DNA via regions

encoded within the crRNA and protospacer-adjacent motifs (PAMs) within the pathogen genome. Base pairing occurs between the crRNA and the target DNA sequence leading to a conformational change. In the Type I-E system, the PAM is recognized by the CasA protein within Cascade, which then unwinds the flanking DNA to evaluate the extent of base pairing between the target and the spacer portion of the crRNA. Sufficient recognition leads Cascade to recruit and activate Cas3. Cas3 then nicks the non-target strand and begins degrading the strand in a 3'-to-5' direction.

[0114] In some embodiments, a CRISPR array disclosed herein comprises a nucleic acid that encodes a processed, mature crRNA. In some embodiments, a mature crRNA is introduced into a phage or a target bacterium described herein. In some embodiments, a phage comprises a nucleic acid that encodes a processed, mature crRNA. In some embodiments, an endogenous or exogenous Cas6 processes a CRISPR array into mature crRNA. In some embodiments, an exogenous Cas6 is introduced into a phage. In some embodiments, a phage comprises an exogenous Cas6. In some embodiments, an exogenous Cas6 is introduced into a target bacterium.

[0115] In some embodiments, the CRISPR-Cas system is endogenous to the target bacterium. In some embodiments, the CRISPR-Cas system is exogenous to the target bacterium. In some embodiments, the CRISPR-Cas system is a type II CRISPR-Cas system. In some embodiments, the CRISPR-Cas system is a type III CRISPR-Cas system.

[0116] In some embodiments, a Type I CRISPR-Cas system is used herein. As described above, Type-I Cascade polypeptides process CRISPR arrays to produce a processed RNA that is then used to bind the complex to a DNA that is complementary to a spacer in the processed RNA. In some embodiments, a first nucleic acid that is introduced into the bacteriophage encodes the Cascade polypeptides that are involved in processing of the first nucleic acid disclosed herein.

[0117] In some embodiments, a type-I Cascade polypeptide disclosed herein have an amino acid sequence having substantial identity to a wild-type type-I Cascade polypeptide. In some embodiments, a Cascade polypeptide described herein is a functional fragment of any full length type-l Cascade polypeptides. In some embodiments, the type I Cascade complex is a type I-A Cascade polypeptides, a type I-B Cascade polypeptides, a type I-C Cascade polypeptides, a type I-D Cascade polypeptides, a type I-E Cascade polypeptides, a type I-F Cascade polypeptides, or a type I-U Cascade polypeptides.

[0118] In some embodiments, the type I Cascade complex comprises: (a) a nucleotide sequence encoding a Cas6b polypeptide, a nucleotide sequence encoding a Cas8b (Csh1) polypeptide, a nucleotide sequence encoding a Cas7 (Csh2) polypeptide, and a nucleotide sequence encoding a Cas5 polypeptide (Type l-B); (b) a nucleotide sequence encoding a Cas5d polypeptide, a nucleotide sequence encoding a Cas8c (Csd1) polypeptide, and a nucleotide sequence encoding a Cas7 (Csd2)

polypeptide (Type I- C); (c) a nucleotide sequence encoding a Cse1 (CasA) polypeptide, a nucleotide sequence encoding a Cse2 (CasB) polypeptide, a nucleotide sequence encoding a Cas7 (CasC) polypeptide, a nucleotide sequence encoding a Cas5 (CasD) polypeptide, and a nucleotide sequence encoding a Cas6e (CasE) polypeptide (Type l-E); (d) a nucleotide sequence encoding a Cys1 polypeptide, a nucleotide sequence encoding a Cys2 polypeptide, a nucleotide sequence encoding a Cas7 (Cys3) polypeptide, and a nucleotide sequence encoding a Cas6f polypeptide (Type l-F); (e) a nucleotide sequence encoding a Cas7 (Csa2) polypeptide, a nucleotide sequence encoding a Cas8a1 (Csx13) polypeptide or a Cas8a2 (Csx9) polypeptide, a nucleotide sequence encoding a Cas5 polypeptide, a nucleotide sequence encoding a Csa5 polypeptide, a nucleotide sequence encoding a Cas6a polypeptide, a nucleotide sequence encoding a Cas3' polypeptide, and a nucleotide sequence encoding a Cas3" polypeptide having no nuclease activity (Type I -A); and/or (f) a nucleotide sequence encoding a Casl Od (Csc3) polypeptide, a nucleotide sequence encoding a Csc2 polypeptide, a nucleotide sequence encoding a Csc1 polypeptide, and a nucleotide sequence encoding a Cas6d polypeptide (Type l-D).

Bacteriophages

[0119] Bacteriophages or “phages” represent a group of bacterial viruses and are engineered or sourced from environmental sources. Individual bacteriophage host ranges are usually narrow, meaning, phages are highly specific to one strain or few strains of a bacterial species and this specificity makes them unique in their antibacterial action. Bacteriophages are bacterial viruses that rely on the host's cellular machinery to replicate. Generally, phages generally fall into three categories: lytic, lysogenic, and temperate. Lytic bacteriophages infect a host cell, undergo numerous rounds of replication, and trigger cell lysis to release newly made bacteriophage particles. In some embodiments, the lytic bacteriophages disclosed herein retain their replicative ability. In some embodiments, the lytic bacteriophages disclosed herein retain their ability to trigger cell lysis. In some embodiments, the lytic bacteriophages disclosed herein retain both they replicative ability and the ability to trigger cell lysis. In some embodiments, the bacteriophages disclosed herein comprise a CRISPR array. In some embodiments, the CRISPR array does not affect the bacteriophages ability to replicate and/or trigger cell lysis. Lysogenic bacteriophages permanently reside within the host cell, either within the bacterial genome or as an extrachromosomal plasmid. Temperate bacteriophages are capable of being lytic or lysogenic, and choose one versus the other depending on growth conditions and the physiological state of the cell. Anytime a lysogenic bacterium is exposed to adverse conditions, the lysogenic state is terminated. This process is called induction. Adverse conditions which favor the termination of the lysogenic

state include desiccation, exposure to UV or ionizing radiation, and exposure to mutagenic chemicals. This leads to the expression of the phage genes, reversal of the integration process, and lytic multiplication.

[0120] Bacteriophages package and deliver synthetic DNA using three general approaches. Under the first approach, the synthetic DNA is randomly recombined into the bacteriophage genome, which usually involves a selectable marker. Under the second approach, restriction sites within the phage are used to introduce synthetic DNA *in-vitro*. Under the third approach, a plasmid generally encoding the phage packaging sites and lytic origin of replication is packaged as part of the assembly of the bacteriophage particle. The resulting plasmids have been coined "phagemids."

[0121] Phages are limited to a given bacterial strain for evolutionary reasons. In some cases, injecting their genetic material into an incompatible strain is counterproductive. Phages have therefore evolved to specifically infect a limited cross-section of strains. However, some phages have been discovered that inject their genetic material into a wide range of bacteria. The classic example is the PI phage, which has been shown to inject DNA in a range of gram-negative bacteria.

[0122] In some embodiments, the bacteriophage or phagemid DNA is from a lysogenic or temperate bacteriophage. In some embodiments, the bacteriophage or phagemid DNA is from an obligate lytic bacteriophage. In some embodiments, the bacteriophages or phagemids include but are not limited to PI phage, a Ml 3 phage, a λ phage, a T4 phage, a ϕC2 phage, a ϕCD27 phage, a ϕNMl phage, Bc431 v3 phage, ϕ10 phage, ϕ25 phage, ϕ151 phage, A511-like phages, B054, 0176 - like phages, or Campylobacter phages (such as NCTC 12676 and NCTC 12677). In some embodiments, the bacteriophage is ϕCD146 *C. difficile* bacteriophage. In some embodiments, the bacteriophage is ϕCD24-2 *C. difficile* bacteriophage. In some embodiments, the bacteriophage is T4 *E. coli* bacteriophage. In some embodiments, the bacteriophage is T7 *E. coli* bacteriophage. In some embodiments, the bacteriophage is T7m *E. coli* bacteriophage.

[0123] In some embodiments, a plurality of bacteriophages are used together. In some embodiments, the plurality of bacteriophages used together targets the same or different bacteria within a sample or subject. In some embodiments, the bacteriophages used together comprises T4 phage, T7 phage, T7m phage, or any combination of bacteriophages described herein.

[0124] In some embodiments, bacteriophages of interest are obtained from environmental sources. or commercial research vendors. In some embodiments, obtained bacteriophages are screened for lytic activity against a library of bacteria and their associated strains. In some embodiments, the bacteriophages are screened against a library of bacteria and their associated strains for their ability to generate primary resistance in the screened bacteria.

[0125] In some embodiments, disclosed herein are method for killing a target bacterium comprising introducing into a target bacterium a bacteriophage comprising: a nucleic acid encoding a spacer sequence or a crRNA transcribed therefrom, wherein the spacer sequence is complementary to a target nucleotide sequence from a target gene in the target bacterium; and a gene that is capable of inducing lysis of the target bacterium, wherein the target bacterium is killed by lytic activity of the bacteriophage or activity of a CRISPR-Cas system using the spacer sequence or the crRNA transcribed therefrom. In some embodiments, disclosed herein are bacteriophages comprising: a nucleic acid encoding a spacer sequence or a crRNA transcribed therefrom, wherein the spacer sequence is complementary to a target nucleotide sequence from a target gene in a target bacterium; and a gene that is capable of inducing lysis of the target bacterium, wherein the target bacterium is killed by the lytic activity of the bacteriophage or activity of a CRISPR-Cas system using the spacer sequence or the crRNA transcribed therefrom.

Insertion Sites

[0126] In some embodiments, the introduction of a nucleic acid encoding a CRISPR array into a bacteriophage does not disrupt the lytic activity of the bacteriophage. In some embodiments, the introduction of a nucleic acid encoding a CRISPR array into a bacteriophage preserves the lytic activity of the bacteriophage. In some embodiments, the nucleic acid is inserted into the bacteriophage genome. In some embodiments, the nucleic acid is inserted into the bacteriophage genome at a transcription terminator site at the end of an operon of interest. In some embodiments, the nucleic acid is inserted into the bacteriophage genome as a replacement for one or more removed non-essential genes. In some embodiments, the nucleic acid is inserted into the bacteriophage genome as a replacement for one or more removed lysogenic genes. In some embodiments, the replacement of non-essential and/or lysogenic genes with the nucleic acid does not affect the lytic activity of the bacteriophage. In some embodiments, the replacement of non-essential and/or lysogenic genes with the nucleic acid preserves the lytic activity of the bacteriophage. In some embodiments, the replacement of non-essential and/or lysogenic genes with the nucleic acid enhances the lytic activity of the bacteriophage. In some embodiments, the replacement of non-essential and/or lysogenic genes with the nucleic acid renders a lysogenic bacteriophage lytic.

[0127] In some embodiments, the nucleic acid is introduced into the bacteriophage genome at a first location while one or more non-essential and/or lysogenic genes are separately removed and/or inactivated from the bacteriophage genome at a separate location. In some embodiments, the removal and/or inactivation of one or more non-essential and/or lysogenic genes does not affect the lytic activity of the bacteriophage. In some embodiments, the removal and/or inactivation of one or

more non-essential and/or lysogenic genes preserves the lytic activity of the bacteriophage. In some embodiments, the removal of one or more non-essential and/or lysogenic genes renders a lysogenic bacteriophage into a lytic bacteriophage. Similarly, in some embodiments, one or more lytic genes are introduced into the bacteriophage so as to render a non-lytic, lysogenic bacteriophage into a lytic bacteriophage.

[0128] In some embodiments, the bacteriophage is a temperate bacteriophage which has been rendered lytic by any of the aforementioned means. In some embodiments, a temperate bacteriophage is rendered lytic by the removal, replacement, or inactivation of one or more lysogenic genes. In some embodiments, the lytic activity of the bacteriophage is due to the removal, replacement, or inactivation of at least one lysogeny gene. In some embodiments, a temperate bacteriophage is rendered lytic by the removal, replacement, or inactivation of one or more lysogenic gene and comprises a CRISPR array comprising at least one spacer that is complementary to a target nucleotide sequence in a target gene in a target bacterium. In some embodiments, a temperate bacteriophage is rendered lytic by the removal, replacement, or inactivation of one or more lysogenic gene via a CRISPR array comprising a spacer directed to the one or more lysogenic gene and comprises a CRISPR array comprising at least one spacer that is complementary to a target nucleotide sequence in a target gene in a target bacterium. In some embodiments, the lysogenic gene plays a role in the maintenance of lysogenic cycle in the bacteriophage. In some embodiments, the lysogenic gene plays a role in establishing the lysogenic cycle in the bacteriophage. In some embodiments, the lysogenic gene plays a role in both establishing the lysogenic cycle and in the maintenance of the lysogenic cycle in the bacteriophage. In some embodiments, the lysogenic gene is a repressor gene. In some embodiments, the lysogenic gene is *c*I repressor gene. In some embodiments, the lysogenic gene is an activator gene. In some embodiments, the lysogenic gene is *c*II gene. In some embodiments, the lysogenic gene is *lexA* gene. In some embodiments, the lysogenic gene is *int* (integrase) gene. In some embodiments, two or more lysogeny genes are removed, replaced, or inactivated to cause arrest of a bacteriophage lysogeny cycle and/or induction of a lytic cycle. In some embodiments, a temperate bacteriophage is rendered lytic by the insertion of one or more lytic genes. In some embodiments, a temperate bacteriophage is rendered lytic by the insertion of one or more genes that contribute to the induction of a lytic cycle. In some embodiments, a temperate bacteriophage is rendered lytic by altering the expression of one or more genes that contribute to the induction of a lytic cycle. In some embodiments, a temperate bacteriophage phenotypically changes from a lysogenic bacteriophage to a lytic bacteriophage. In some embodiments, a temperate bacteriophage is rendered lytic by environmental alterations. In some embodiments, environmental alterations

include, but are not limited to, alterations in temperature, pH, or nutrients, exposure to antibiotics, hydrogen peroxide, foreign DNA, or DNA damaging agents, presence of organic carbon, and presence of heavy metal (e.g. in the form of chromium (VI). In some embodiments, a temperate bacteriophage that is rendered lytic is prevented from reverting to lysogenic state. In some embodiments, a temperate bacteriophage that is rendered lytic is prevented from reverting back to lysogenic state by way of introducing an additions CRIPSR array. In some embodiments, the bacteriophage does not confer any new properties onto the target bacterium beyond cellular death cause by lytic activity of the bacteriophage and/or the activity of the CRISPR array.

[0129] In some embodiments, the replacement, removal, inactivation, or any combination thereof, of one or more non-essential and/or lysogenic genes is achieved by chemical, biochemical, and/or any suitable method. In some embodiments, the insertion of one or more lytic genes is achieved by any suitable chemical, biochemical, and/or physical method by homologous recombination.

[0130] In some embodiments, the bacteriophage is an obligate lytic bacteriophage. In some embodiments, the bacteriophage is ϕCD146 *C. difficile* bacteriophage. In some embodiments, the bacteriophage is ϕCD24-2 *C. difficile* bacteriophage. In some embodiments, the bacteriophage is T4 *E. coli* bacteriophage. In some embodiments, the bacteriophage is T7 *E. coli* bacteriophage. In some embodiments, the bacteriophage is T7m *E. coli* bacteriophage.

Non-Essential Gene

[0131] In some embodiments, the non-essential gene to be removed and/or replaced from the bacteriophage is a gene that is non-essential for the survival of the bacteriophage. In some embodiments, the non-essential gene to be removed and/or replaced from the bacteriophage is a gene that is non-essential for the induction and/or maintenance of lytic cycle. In some embodiments, the non-essential gene to be removed and/or replaced from the bacteriophage is *gp49* from ϕCD146 *C. difficile* bacteriophage. In some embodiments, the non-essential gene to be removed and/or replaced from the bacteriophage is *gp75* from ϕCD24-2*C. difficile* bacteriophage. In some embodiments, the non-essential gene to be removed and/or replaced from the bacteriophage is the *hoc* gene from a T4 *E. coli* bacteriophage. In some embodiments, the non-essential gene to be removed and/or replaced include *gp0.7*, *gp4.3*, *gp4.5*, *gp4.7*, or any combination thereof from a T7 *E. coli* bacteriophage. In some embodiments, the non-essential gene to be removed and/or replaced is *gp0.6*, *gp0.65*, *gp0.7*, *gp4.3*, *gp4.5*, or any combination thereof from a T7m *E. coli* bacteriophage.

Transcriptional Activators

[0132] In some embodiments, a bacteriophage disclosed herein further comprises a transcriptional activator. In some embodiments, the transcriptional activator encoded regulates the expression of

genes of interest within the target bacterium. In some embodiments, the transcriptional activator activates the expression of genes of interest within the target bacterium whether exogenous or endogenous. In some embodiments, the transcriptional activator activates the expression genes of interest within the target bacterium by disrupting the activity of one or more inhibitory elements within the target bacterium. In some embodiments, the inhibitory element comprises a transcriptional repressor. In some embodiments, the inhibitory element comprises a global transcriptional repressor. In some embodiments the inhibitory element is a histone-like nucleoid-structuring (H-NS) protein or homologue or functional fragment thereof. In some embodiments, the inhibitory element is a leucine responsive regulatory protein (LRP). In some embodiments, the inhibitory element is a CodY protein.

[0133] In some bacteria, the CRISPR-Cas system is poorly expressed and considered silent under most environmental conditions. In these bacteria, the regulation of the CRISPR-Cas system is the result of the activity of transcriptional regulators, for example histone-like nucleoid-structuring (H-NS) protein which is widely involved in transcriptional regulation of the host genome. H-NS exerts control over host transcriptional regulation by multimerization along AT-rich sites resulting in DNA bending. In some bacteria, such as *E. coli*, the regulation of the type-I CRISPR-Cas3 operon is regulated by H-NS.

[0134] Similarly, in some bacteria, the repression of the CRISPR-Cas system is controlled by an inhibitory element, for example the leucine responsive regulatory protein (LRP). LRP has been implicated in binding to upstream and downstream regions of the transcriptional start sites. Notably, the activity of LRP in regulating expression of the CRISPR-Cas system varies from bacteria to bacteria. Unlike, H-NS which has broad inter-species repression activity, LRP has been shown to differentially regulate the expression of the host CRISPR-Cas system. As such, in some instances, LRP reflects a host-specific means of regulating CRISPR-Cas system expression in different bacteria.

[0135] In some instances, the repression of CRISPR-Cas system is also controlled by inhibitory element CodY. CodY is a GTP-sensing transcriptional repressor that acts through DNA binding. The intracellular concentration of GTP acts as an indicator for the environmental nutritional status. Under normal culture conditions, GTP is abundant and binds with CodY to repress transcriptional activity. However, as GTP concentrations decreases, CodY becomes less active in binding DNA, thereby allowing transcription of the formerly repressed genes to occur. As such, CodY acts as a stringent global transcriptional repressor.

[0136] In some embodiments, the transcriptional activator is a LeuO polypeptide, any homolog or functional fragment thereof, a nucleic acid sequence encoding the same, or an agent that

upregulates LeuO. In some embodiments, the transcriptional activator comprises any ortholog or functional equivalent of LeuO. In some bacteria, LeuO acts in opposition to H-NS by acting as a global transcriptional regulator that responds to environmental nutritional status of a bacterium. Under normal conditions, LeuO is poorly expressed. However, under amino acid starvation and/or reaching of the stationary phase in the bacterial life cycle, LeuO is upregulated. Increased expression of LeuO leads to it antagonizing H-NS at overlapping promoter regions to effect gene expression. Overexpression of LeuO upregulates the expression of the CRISPR-Cas system. In *E. coli* and *S. tphyimurium*, LeuO drives increased expression of the casABCDE operon which has predicted LeuO and H-NS binding sequences upstream of CasA.

[0137] In some embodiments, the expression of LeuO leads to disruption of an inhibitory element. In some embodiments, the disruption of an inhibitory element due to expression of LeuO removes the transcriptional repression of a CRISPR-Cas system. In some embodiments, the expression of LeuO removes transcriptional repression of a CRISPR-Cas system due to activity of H-NS. In some embodiments, the expression of LeuO removes transcriptional repression of a CRISPR-Cas system due to activity of LRP. In some embodiments, the disruption of an inhibitory element due to the expression of LeuO causes an increase in the expression of a CRISPR-Cas system. In some embodiments, the increase in the expression of a CRISPR-Cas system due to the disruption of an inhibitory element caused by the expression of LeuO causes an increase in the CRISPR-Cas processing of a nucleic acid encoding a CRISPR array. In some embodiments, the increase in the expression of a CRISPR-Cas system due to the disruption of an inhibitory element by the expression of LeuO causes an increase in the CRISPR-Cas processing of a nucleic acid encoding a CRISPR array so as to increase the level of lethality of the CRISPR array against a bacterium. In some embodiments, transcriptional activator described herein, causes increase activity of a bacteriophage and/or CRISPR-Cas system described herein.

[0138] In some embodiments, the sequence for LeuO or any homolog or functional fragments thereof from *E. coli* strain K12 includes but is not limited to GenBank accession number: AP0090408.1. The protein sequence for LeuO is listed below in FASTA format as SEQ ID NO. 1:

MPEVQTDHPETAELSKPQLRMVDLNLLTVFDAVMQEQNITRAAHVLGMSQPAVSNAVAR
LKVMFNDELFVRYGRGIQPTARAFQLFGSVRQALQLVQNELPGSGFEPASSERVFHLCVCS
PLDSILTSQIYNHIEQIAPNIHVMFKSSLNQNTEHQLRYQETEFVISYEDFHRPEFTSVPLFKD
EMVLVASKNHPTIKGPLLKHDVYNEQHAAVSLDRFASFSQPWYDTVDKQASIAYQGMAM
MSVLSVVSQTHLVAIAPRWLAEEFAESLELQVLPLPLKQNSRTCYLSWHEAAGRDKGHQ
WMEEQLVSICKR

[0139] In some embodiments, the transcriptional activator is a CD2983 polypeptide or any homolog or functional fragment thereof, or a nucleic acid encoding the same. In some embodiments, the transcriptional activator is any ortholog or functional equivalent of CD29883. In some bacteria, CD2983 act as a specific transcriptional regulator that responds to environmental nutritional status of a bacterium. In some bacteria, the CRISPR-Cas system is regulated by the environmental nutritional status of glucose in a ccpA dependent manner. However, under normal conditions CD2983 is suppressed by CodY. Under nutritional stress, CodY becomes less active to allow expression of CD2983. Upregulation of CD2983 is associated with CRISPR-Cas system upregulation.

[0140] In some embodiments, the expression of CD2983 leads to disruption of an inhibitory element. In some embodiments, the disruption of an inhibitory element due to expression of CD2983 removes the transcriptional repression of a CRISPR-Cas system. In some embodiments, the expression of CD2983 removes transcriptional repression of a CRISPR-Cas system due to activity of CodY. In some embodiments, the disruption of an inhibitory element due to the expression of CD2983 causes an increase in the expression of a CRISPR-Cas system. In some embodiments, the increase in the expression of a CRISPR-Cas system due to the disruption of an inhibitory element caused by the expression of CD2983 causes an increase in the CRISPR-Cas processing of the first nucleic acid encoding a CRISPR array. In some embodiments, the increase in the expression of a CRISPR-Cas system due to the disruption of an inhibitory element by the expression of CD2983 causes an increase in the CRISPR-Cas processing of the first nucleic acid encoding a CRISPR array so as to increase the level of lethality of the CRISPR array against a bacterium.

[0141] In some embodiments, the sequence for CD2983 or any homolog or functional fragments thereof from *C. difficile* strain 630 includes but is not limited to GenBank accession number: CAJ69877.1. The protein sequence for CD2983 is listed below in FASTA format as SEQ ID NO. 2:

MMILIQSRGKMKCKELSEELEVSERQIKSYKMYLEQAGIFINSTPGIYGGYEIDKCNSISLIK
LLDSEVSILDMINSQLEYNNDIYKNEFNNIVEKIKAVLNTGEKSDTYMDYFTVQAQRNCDY
ESEKNKCNEIIRAYTTKHKFWIEYYSLNSGNSERIVHPYGLFNYKSDTYMVAFCEKRFKFID
FKLCRIKDYKVLEEKYNVDKSFSWDEYSKNSIGIYKGEEINVVIKISHPFSTIIKEKVWVNN
QQIIEYDDKSIMFKAKMRGYEEIKSWILSMGAYVEVVEPDRLRNDILSEIEKMKKIY

[0142] In some embodiments, killing of the target bacterium is achieved by the lytic activity of the bacteriophage. In some embodiments, killing of the target bacterium is achieved by the activity of

the first nucleic acid encoding a CRISPR array comprising at least one spacer that is complementary to a target nucleotide sequence in a target gene in the target bacterium. In some embodiments, killing of the bacterium is achieved by the processing of the CRISPR array by a type I, type II, or a type III CRISPR-Cas system to produce a processed crRNA capable of directing CRISPR-Cas based endonuclease activity and/or cleavage at the target nucleotide sequence in the target gene of the bacterium. In some embodiments, the killing of the bacterium is achieved by the enhanced processing of the CRISPR array due to the expression of a second nucleic acid encoding a CRISPR-Cas system transcriptional activator.

[0143] In some embodiments, killing of the bacterium is achieved by the lytic activity of the bacteriophage and by the activity of a nucleic acid encoding a CRISPR array comprising at least one spacer that is complementary to a target nucleotide sequence in a target gene in the target bacterium in combination. In some embodiments, killing of the bacterium is achieved by the lytic activity of the bacteriophage and by the enhanced activity of the nucleic acid encoding a CRISPR array due to the activity of the expressed transcriptional activator. In some embodiments, the killing of the bacterium by a combination of the lytic activity of the bacteriophage and by the activity of the nucleic acid encoding a CRISPR array is synergistic. In some embodiments, the killing of the bacterium by a combination of the lytic activity of the bacteriophage and by the activity of the nucleic acid encoding a CRISPR array is synergistic due to the expression of the CRISPR-Cas transcriptional activator encoded by the second nucleic acid.

Enhanced Lytic Bacteriophages

[0144] Disclosed herein, are methods of producing a bacteriophage that comprises a nucleic acid encoding a transcriptional activator for a CRISPR-Cas system. Also, disclosed herein, are bacteriophages that comprise a nucleic acid encoding a transcriptional activator for a CRISPR-Cas system.

[0145] In some embodiments, the introduction of a nucleic acid encoding a transcriptional activator for a CRISPR-Cas into a bacteriophage is used to modulate the activity of a CRISPR-Cas system in the target bacterium. In some embodiments, the transcriptional activator introduced by the bacteriophage increases the expression of a CRISPR-Cas system in the target bacterium. In some embodiments, the increased expression of a CRISPR-Cas system in the target bacterium due to the introduction of a transcriptional activator by a first bacteriophage, enhances the lethality of a second different bacteriophage comprising a CRISPR array as described by previous embodiments. In some embodiments, the increased expression of a CRISPR-Cas system in the target bacterium due to the introduction of a transcriptional activator by a first bacteriophage, enhances the lethality

of a second different bacteriophage comprising a pre-processed immature or a processed mature crRNA as described by previous embodiments.

[0146] In addition to regulation by transcriptional activators, the CRISPR-Cas system is tightly controlled by other means and mechanisms of regulation. Quorum sensing (QS) is the chemical communication between bacteria within a bacterial population which permits the coordination of gene expression with respect to the population density. QS relies upon chemical signals that are produced and accumulate during bacterial growth. Upon hitting a threshold level, QS signals bind to transcriptional regulators to influence bacterial gene expression. In some bacteria, QS signaling enhances the CRISPR-Cas system for bacterial defense by de-repressing its expression. In addition to QS signaling, the regulation of CRISPR-Cas system expression is believed to be sensitive to perturbations in the host bacterium's membrane integrity. BaeSR is a two component response regulator system that links host membrane envelope stress to the activation of Cas genes. Likewise, heat shock protein G (HtpG) has been shown to stabilize Cas upon induction by phage infection. Additionally, metabolic sensing proteins such as the cAMP metabolite sensing cAMP receptor protein (CRP) are able to activate CRISPR-Cas expression. Other metabolic sensing proteins which regulate Cas expression includes sigma factor RpoN (σ^{54}) which responds to nitrogen starvation.

[0147] In some embodiments, the transcriptional activator comprises a QS signal. In some embodiments, the transcriptional activator comprises a protein involved in sensing stress to the membrane of the host bacterium. In some embodiments, this protein comprises BaeSR. In some embodiments, the transcriptional activator comprises a protein which stabilizes Cas. In some embodiments, this protein comprises HtpG. In some embodiments, the transcriptional activator is a metabolic sensing protein. In some embodiments, the metabolic sensing protein comprises CRP or RpoN (σ^{54}). In some embodiments, a nucleic acid encoding a transcriptional activator or a functional fragment thereof is introduced into the target bacteria. In some embodiments, a nucleic acid encoding a transcriptional activator or a functional fragment thereof is introduced into the target bacteria via a CRISPR array described herein. In some embodiments, the methods disclosed herein comprises: introducing a bacteriophage comprising a nucleic acid encoding a transcriptional activator for the CRISPR-Cas system in the target bacterium. In some embodiments, disclosed herein are bacteriophages comprising a nucleic acid encoding a transcriptional activator for a CRISPR-Cas system in a target bacterium.

Anti-CRISPR Array

[0148] In some embodiments, a bacteriophage disclosed herein further comprises an Anti-CRISPR.

[0149] In some embodiments, a method disclosed herein comprises introducing into a target bacterium a bacteriophage comprising: lytic activity, and a first nucleic acid sequence encoding an anti-CRISPR polypeptide, wherein the anti-CRISPR polypeptide enhances the lytic activity of the bacteriophage. In some embodiments, disclosed herein are bacteriophages comprising: lytic activity, and a first nucleic acid sequence encoding an anti-CRISPR polypeptide, wherein the anti-CRISPR polypeptide enhances the lytic activity of the bacteriophage.

[0150] In some embodiments, the nucleic acid encoding an anti-CRISPR polypeptide directly enhances the lytic activity of the bacteriophage or another bacteriophage. In some embodiments, enhancement of the lytic activity of the bacteriophage is due to the anti-CRISPR polypeptide inhibiting, inactivating, and/or repressing the activity of a CRISPR-Cas system in the host target bacterium. An anti-CRISPR polypeptide is any bacteriophage protein with activity that prevents the function of a bacterial CRISPR-Cas system. Activity of an anti-CRISPR protein prevents a host bacterium from mounting a CRISPR-Cas system based defense against the invading bacteriophage. In some embodiments, the anti-CRISPR polypeptide inactivates the host bacterium's CRISPR-Cas system using a process comprising gene regulation interference. In some embodiments, the anti-CRISPR polypeptide inactivates the host bacterium's CRISPR-Cas system using a process comprising nuclease recruitment interference. In some embodiments, the anti-CRISPR polypeptide inhibits, inactivates, and/or represses the activity of a type I CRISPR-Cas system, type II CRISPR-Cas system, or a type III CRISPR-Cas system, Type IV CRISPR-Cas system, Type V CRISPR-Cas system, or Type VI CRISPR-Cas system. In some embodiments, the protein product of a nucleic acid encoding an anti-CRISPR polypeptide or the introduced anti-CRISPR polypeptide binds directly or indirectly to a Cascade or a Cascade-like complex.

[0151] In some embodiments, the anti-CRISPR polypeptide is a truncated, mutated, or fused to another protein of interest. In some embodiments, the anti-CRISPR polypeptide is a dimer protein. In some embodiments, the anti-CRISPR polypeptide is a homodimer or heterodimer protein. In one embodiment, the anti-CRISPR polypeptide comprises AcrIIC1Boe, AcrIIC1Nme, AcrIIC2Nme, AcrIIC3Nme, AcrIIC4Hpa, AcrIIC5Smu, or any functional fragments thereof. In one embodiment, the anti-CRISPR polypeptide binds with specific affinity to a specific binding site upon the CRISPR-Cas system.

[0152] In some embodiments, the anti-CRISPR polypeptide inhibits, inactivates, or represses the activity of a CRISPR-Cas system in the target bacterium, wherein said CRISPR-Cas system targets the bacteriophage comprising the nucleic acid encoding the anti-CRISPR polypeptide. In some embodiments, the anti-CRISPR polypeptide inhibits, inactivates, or represses the activity of a CRISPR-Cas system in the target bacterium, wherein said CRISPR-Cas system targets a second

orthogonal bacteriophage different than a first bacteriophage. In some embodiments, the second orthogonal bacteriophage is different than the first bacteriophage. In some embodiments, the inhibition, inactivation, or repression of the CRISPR-Cas system activity in the target bacterium by the anti-CRISPR polypeptide from a first bacteriophage enhances the activity of the first bacteriophage or a second orthogonal bacteriophage. In some embodiments, the second orthogonal bacteriophage has lytic activity. In some embodiments, the second orthogonal bacteriophage comprises a bacteriophage of any of the embodiments disclosed herein.

Methods of Killing a Target Bacterium

[0153] Disclosed herein, in certain embodiments, are methods of killing bacteria. In some embodiments, killing of the target bacterium is achieved by the lytic activity of the bacteriophage. In some embodiments, the lytic activity of the bacteriophage is due to the removal, replacement, or inactivation of at least one lysogeny gene. In some embodiments, the lysogenic gene plays a role in the maintenance of lysogenic cycle in the bacteriophage. In some embodiments, the lysogenic gene plays a role in establishing the lysogenic cycle in the bacteriophage. In some embodiments, the lysogenic gene plays a role in both establishing the lysogenic cycle and in the maintenance of the lysogenic cycle in the bacteriophage. In some embodiments, the lysogenic gene is a repressor gene. In some embodiments, the lysogenic gene is *c*I repressor gene. In some embodiments, the lysogenic gene is an activator gene. In some embodiments, the lysogenic gene is *c*II gene. In some embodiments, the lysogenic gene is *lexA* gene. In some embodiments, the lysogenic gene is *int* (integrase) gene. In some embodiments, a temperate bacteriophage is rendered lytic by the insertion of one or more genes that contribute to the induction of a lytic cycle. In some embodiments, a temperate bacteriophage is rendered lytic by altering the expression of one or more genes that contribute to the induction of a lytic cycle. In some embodiments, a temperate bacteriophage phenotypically changes from a lysogenic bacteriophage to a lytic bacteriophage. In some embodiments, a temperate bacteriophage is rendered lytic by environmental alterations. In some embodiments, environmental alterations include, but are not limited to, alterations in temperature, pH, or nutrients, exposure to antibiotics, hydrogen peroxide, foreign DNA, or DNA damaging agents, presence of organic carbon, and presence of heavy metal (e.g. in the form of chromium (VI). In some embodiments, a temperate bacteriophage that is rendered lytic is prevented from reverting to lysogenic state. In some embodiments, a temperate bacteriophage that is rendered lytic is prevented from reverting back to lysogenic state by way of introducing an additions CRIPSR array. In some embodiments, killing of a target bacterium is achieved by the activity of a CRISPR array comprising at least one spacer that is complementary to a target nucleotide sequence

in a target gene in the target bacterium. In some embodiments, killing of the target bacterium is achieved by the activity of a mature crRNA. In some embodiments, killing of the bacterium is achieved by the processing of the CRISPR array by a Type I, Type II, Type III, Type IV, Type V, or a Type VI CRISPR-Cas system to produce a processed crRNA capable of directing CRISPR-Cas based endonuclease activity and/or cleavage at the target nucleotide sequence in the target gene of the bacterium. In some embodiments, killing of a target bacterium is achieved by the activity of the CRISPR array independent to the lytic and/or non-lytic activity of the bacteriophage. In some embodiments, the killing of a target bacterium is by any method or combination of methods disclosed herein.

[0154] In some embodiments, killing of the bacterium are achieved solely by the lytic activity of the bacteriophage. In some embodiments, killing of the bacterium is achieved solely by the activity of the nucleic acid encoding a CRISPR array comprising at least one spacer. In some embodiments, killing of the bacterium is achieved solely by the activity of the nucleic acid encoding a mature crRNA. In some embodiments, killing of the bacterium is achieved by a combination of the lytic activity of the bacteriophage and the activity of the CRISPR array or mature crRNA. In some embodiments, killing of the bacterium by a combination of the lytic activity of the bacteriophage and by the activity of the first nucleic acid encoding a CRISPR array is synergistic. In some embodiments, the killing activity of the CRISPR array or mature crRNA supplements or enhances the lytic activity of the bacteriophage. In some embodiments, killing of a target bacterium is a synergistic effect of two or more systems.

[0155] In some embodiments, the synergistic killing of the bacterium is modulated by the concentration of the bacteriophage and/or the design of the CRISPR array. In some embodiments, the synergistic killing of the bacterium is modulated to favor killing by the lytic activity of the bacteriophage over the activity of the CRISPR array by increasing the concentration of bacteriophage administered to the bacterium. In some embodiments, the synergistic killing of the bacterium is modulated to disfavor killing by the lytic activity of the bacteriophage over the activity of the CRISPR array by decreasing the concentration of bacteriophage administered to the bacterium. In some embodiments, at low concentrations, lytic replication allows for amplification and killing of the target bacteria. In some embodiments, at high concentrations, amplification of a phage is not required.

[0156] In some embodiments, the synergistic killing of the bacterium is modulated to favor killing by the activity of the CRISPR array over the lytic activity of the bacteriophage by altering the number, the length, the composition, the identity, or any combination thereof, of the spacers so as to increase the lethality of the CRISPR array. In some embodiments, the synergistic killing of the

bacterium is modulated to disfavor killing by the activity of the CRISPR array over the lytic activity of the bacteriophage by altering the number, the length, the composition, the identity, or any combination thereof, of the spacers so as to decrease the lethality of the CRISPR array.

[0157] In some embodiments, the target nucleotide sequence in the bacterium to be killed is any essential target nucleotide sequence of interest. In some embodiments, the target nucleotide sequence is a non-essential sequence. In some embodiments, a target nucleotide sequence comprises, consists essentially of or consist of all or a part of a nucleotide sequence encoding a promoter, or a complement thereof, of a target gene. In some embodiments, the spacer nucleotide sequence is complementary to a promoter, or a part thereof, of a target gene.

[0158] In some embodiments, the target nucleotide sequence comprises all or a part of a nucleotide sequence located on a coding or a non-coding strand of DNA. In some embodiments, the target nucleotide sequence comprises all or a part of a nucleotide sequence located on a coding of a transcribed region of a target gene.

[0159] An essential gene is any gene of an organism that is critical for its survival. However, being essential is highly dependent on the circumstances in which an organism lives. For instance, a gene required to digest starch is only essential if starch is the only source of energy. In some embodiments, the essential gene includes but is not limited to: *acpP, csrA, eno, fusA, gapA, glyQ, infA, nusG, secY, trmD, Tsf, and ftsA*. In some embodiments, a non-essential gene is any gene of an organism that is not critical for survival. However, being non-essential is highly dependent on the circumstances in which an organism lives.

[0160] In some embodiments, non-limiting examples of a target gene of interest includes a gene encoding a transcriptional regulator, a translational regulator, a polymerase gene, a metabolic enzyme, a transporter, an RNase, a protease, a DNA replication enzyme, a DNA modifying or degrading enzyme, a regulatory RNA, a transfer RNA, or a ribosomal RNA. In some embodiments, a target gene is a gene involved in cell-division, cell structure, metabolism, motility, pathogenicity or virulence. In some embodiments, a target gene includes a hypothetical gene whose function is not yet characterized. Thus, for example, the target genes are any gene from any bacterium.

Antimicrobial Agents and Peptides

[0161] In some embodiments, a bacteriophage disclosed herein is further genetically modified to express an antibacterial peptide, a functional fragment of an antibacterial peptide or a lytic gene. In some embodiments, a bacteriophage disclosed herein express at least one antimicrobial agent or peptide disclosed herein. In some embodiments, a bacteriophage disclosed herein comprises a nucleic acid sequence that encodes an enzybiotic where the protein product of the nucleic acid sequence targets phage resistant bacteria. In some embodiments, the bacteriophage comprises

nucleic acids which encode enzymes which assist in breaking down or degrading biofilm matrix. In some embodiments, a bacteriophage disclosed herein comprises nucleic acids encoding Dispersin D aminopeptidase, amylase, carbohydrase, carboxypeptidase, catalase, cellulase, chitinase, cutinase, cyclodextrin glycosyltransferase, deoxyribonuclease, esterase, alpha-galactosidase, beta-galactosidase, glucoamylase, alpha-glucosidase, beta-glucosidase, haloperoxidase, invertase, laccase, lipase, mannosidase, oxidase, pectinolytic enzyme, peptidoglutaminase, peroxidase, phytase, polyphenoloxidase, proteolytic enzyme, ribonuclease, transglutaminase, xylanase or lyase. In some embodiments, the enzyme is selected from the group consisting of cellulases, such as glycosyl hydroxylase family of cellulases, such as glycosyl hydroxylase 5 family of enzymes also called cellulase A; polyglucosamine (PGA) depolymerases; and colonic acid depolymerases, such as 1,4-L-fucodise hydrolase Characterisation of a 1,4-beta- fucoside hydrolase degrading colanic acid, depolymerazing alginase, DNase I, or combinations thereof. In some embodiments, a bacteriophage disclosed herein secretes an enzyme disclosed herein.

[0162] In some embodiments, an antimicrobial agent or peptide is expressed and/or secreted by a bacteriophage disclosed herein. In some embodiments, a bacteriophage disclosed herein secretes and expresses an antibiotic such as ampicillin, penicillin, penicillin derivatives, cephalosporins, monobactams, carbapenems, ofloxacin, ciproflaxacin, levofloxacin, gatifloxacin, norfloxacin, lomefloxacin, trovafloxacin, moxifloxacin, sparfloxacin, gemifloxacin, pazufloxacin or any antibiotic disclosed herein. In some embodiments, a bacteriophage disclosed herein comprises a nucleic acid sequence encoding an antibacterial peptide, expresses an antibacterial peptide, or secretes a peptide that aids or enhances killing of a target bacterium. In some embodiments, a bacteriophage disclosed herein comprises a nucleic acid sequence encoding a peptide, a nucleic acid sequence encoding an antibacterial peptide, expresses an antibacterial peptide, or secretes a peptide that aids or enhances the activity of a CRISPR-Cas system.

Uses

Bacterial Infections

[0163] Disclosed herein, are methods of treating bacterial infections. In some embodiments, the bacteriophages disclosed herein treat or prevent diseases or conditions mediated or caused by bacteria as disclosed herein in a human or animal subjects. Such bacteria are typically in contact with tissue of the subject including: gut, oral cavity, lung, armpit, ocular, vaginal, anal, ear, nose or throat tissue. In some embodiments, a bacterial infection is treated by modulating the activity of the bacteria and/or by directly killing of the bacteria.

[0164] In some embodiments, one or more target bacteria present in a bacterial population are pathogenic. In some embodiments, the pathogenic bacteria are uropathogenic. In some embodiments, the pathogenic bacterium is uropathogenic *E. Coli* (UPEC). In some embodiments, the pathogenic bacteria are diarreagenic. In some embodiments, the pathogenic bacteria are diarreagenic *E.coli* (DEC). In some embodiments, the pathogenic bacteria are Shiga-toxin producing. In some embodiments, the pathogenic bacterium is Shiga-toxin producing *E.coli* (STEC). In some embodiments, the pathogenic bacteria are Shiga-toxin producing. In some embodiments, the pathogenic bacterium is Shiga-toxin producing *E.coli* (STEC). In some embodiments, the pathogenic bacterium is Shiga-toxin producing *E.coli* (STEC). In some embodiments, the pathogenic bacteria are various O-antigen:H-antigen serotype *E. coli*. In some embodiments, the pathogenic bacteria are entereopathogenic. In some embodiments, the pathogenic bacterium is entereopathogenic *E.coli* (EPEC).

[0165] In some embodiments, the pathogenic bacteria are various strains of *C. difficile* including: CD043, CD05, CD073, CD093, CD180, CD106, CD128, CD199, CD111, CD108, CD25, CD148, CD154, FOBT195, CD03, CD038, CD112, CD196, CD105, UK1, UK6, BI-9, CD041, CD042, CD046, CD19, or R20291.

[0166] In some embodiments, the bacteriophages disclosed herein are used to treat an infection, a disease, or a condition, in the gastrointestinal tract of a subject. In some embodiments, the bacteriophages are used to modulate and/or kill target bacteria within the microbiome or gut flora of a subject. In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target bacteria from a plurality of bacteria within the microbiome or gut flora of a subject. In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target entereopathogenic bacteria from a plurality of bacteria within the microbiome or gut flora of a subject. In some embodiments, the target entereopathogenic bacterium is entereopathogenic *E. Coli* (EPEC). In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target diarreagenic bacteria from a plurality of bacteria within the microbiome or gut flora of a subject. In some embodiments, the target diarreagenic bacterium is diarreagenic *E.coli* (DEC). In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target Shiga-toxin producing bacteria from a plurality of bacteria within the microbiome or gut flora of a subject. In some embodiments, the target Shiga-toxin producing bacterium is Shiga-toxin producing *E.coli* (STEC).

[0167] In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target enteropathogenic *C. difficile* bacteria strains within the microbiome or gut flora of a subject including: CD043, CD05, CD073, CD093, CD180, CD106, CD128, CD199, CD111,

CD108, CD25, CD148, CD154, FOBT195, CD03, CD038, CD112, CD196, CD105, UK1, UK6, BI-9, CD041, CD042, CD046, CD19, or R20291.

[0168] In some embodiments, the bacteriophages disclosed herein are used to treat an infection, a disease, or a condition, in the urinary tract of a subject. In some embodiments, the bacteriophages are used to modulate and/or kill target bacteria within the urinary tract flora of a subject. The urinary tract flora includes, but is not limited, to *Staphylococcus epidermidis, Enterococcus faecalis,* and some alpha-hemolytic *Streptococci*. In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target uropathogenic bacteria from a plurality of bacteria within the urinary tract flora of a subject. In some embodiments, the target bacterium is uropathogenic *E. Coli* (UPEC).

[0169] In some embodiments, the bacteriophages disclosed herein are used to treat an infection, a disease, or a condition, on the skin of a subject. In some embodiments, the bacteriophages are used to modulate and/or kill target bacteria on the skin of a subject.

[0170] In some embodiments, the bacteriophages disclosed herein are used to treat an infection, a disease, or a condition, on a mucosal membrane of a subject. In some embodiments, the bacteriophages are used to modulate and/or kill target bacteria on the mucosal membrane of a subject.

[0171] In some embodiments, the pathogenic bacteria are antibiotic resistant. In one embodiment, the pathogenic bacterium is methicillin-resistant Staphylococcus aureus (MRSA).

[0172] In some embodiments, the one or more target bacteria present in the bacterial population form a biofilm. In some embodiments, the biofilm comprises pathogenic bacteria. In some embodiments, the bacteriophage disclosed herein is used to treat a biofilm.

[0173] In some embodiments, non-limiting examples of target bacteria includes *Escherichia spp., Salmonella spp., Bacillus spp. , Corynebacterium Clostridium spp., Clostridium spp., Psuedomonas spp., Clostridium spp., Lactococcus spp., Acinetoibacter spp., Mycobacterium spp., Myxococcus spp., Staphylococcus spp., Streptococcus spp., or cyanobacteria.* In some embodiments, non-limiting examples of bacteria include *Escherichia coli, Salmonella enterica, Bacillus subtilis, Clostridium acetobutylicum, Clostridium ljungdahlii, Clostridium difficile, Acinetobacter baumannii, Mycobacterium tuberculosis, Myxococcus xanthus, Staphylococcus aureus, Streptococcus pyogenes,* or *cyanobacteria*. In some embodiments, non-limiting examples of bacteria include *Staphylococcus aureus, methicillin resistant Staphylococcus aureus, Streptococcus pneumonia, carbapenem-resistant Enteroacteriaceae, Staphylococcus epidermidis, Staphylococcus salivarius, Corynebacterium minutissium, Corynebacterium pseudodiphtheriae, Corynebacterium stratium, Corynebacterium* group G1, *Corynebacterium* group G2, *Streptococcus pneumonia,*

Streptococcus mitis, Streptococcus sanguis, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Burkholderia cepacia, Serratia marcescens, Haemophilus influenzae, Moraxella sp., Neisseria meningitidis, Neisseria gonorrhoeae, Salmonella typhimurium, Actinomyces spp., Porphyromonas spp., Prevotella melaninogenicus, Helicobacter pylori, Helicobacter felis, or *Campylobacter jejuni*. Further non-limiting examples of bacteria include lactic acid bacteria including but not limited to *Lactobacillus spp.* and *Bifidobacterium spp.*; electrofuel bacterial strains including but not limited to *Geobacter spp.*, *Clostridium spp.*, or *Ralstonia eutropha*; or bacteria pathogenic on, for example, plants and mammals. In some embodiments, the bacterium is *Escherichia coli*. In some embodiments, the bacterium is *Clostridium difficile*.

[0174] In some embodiments, the bacteriophage treats acne and other related skin infections.

[0175] In some embodiments, a target bacterium is a multiple drug resistant (MDR) bacteria strain. An MDR strain is a bacteria strain that is resistant to at least one antibiotic. In some embodiments, a bacteria strain is resistant to an antibiotic class such as a cephalosporin, a fluoroquinolone, a carbapenem, a colistin, an aminoglycoside, vancomycin, streptomycin, and methicillin. In some embodiments, a bacteria strain is resistant to an antibiotic such as a Ceftobiprole, Ceftaroline, Clindamycin, Dalbavancin, Daptomycin, Linezolid, Mupirocin, Oritavancin, Tedizolid, Telavancin, Tigecycline, Vancomycin, an Aminoglycoside, a Carbapenem, Ceftazidime, Cefepime, Ceftobiprole, a Fluoroquinolone, Piperacillin, Ticarcillin, Linezolid, a Streptogramin, Tigecycline, Daptomycin, or any combination thereof. Examples of MDR strains include: Vancomycin-Resistant *Enterococci* (VRE), Methicillin-Resistant *Staphylococcus aureus* (MRSA), Extended-spectrum β-lactamase (ESBLs) producing Gram-negative bacteria, *Klebsiella* pneumoniae carbapenemase (KPC) producing Gram-negatives, and Multidrug-Resistant gram negative rods (MDR GNR) MDRGN bacteria such as *Enterobacter species E.coli, Klebsiella* pneumoniae, *Acinetobacter baumannii*, or *Pseudomonas aeruginosa*.

[0176] In some embodiments the target bacterium is *Klebsiella* pneumoniae. In some embodiments, the target bacterium is *Staphylococcus aureus*. In some embodiments, the target bacterium is *Enterococci*. In some embodiments, the target bacterium is *Acinetobacter*. In some embodiments, the target bacterium is *Pseudomonas*. In some embodiments, the target bacterium is *Enterobacter*. In some embodiments, the target bacterium is *Clostridium difficile*. In some embodiments, the target bacterium is *E.coli*. In some embodiments, the target bacterium is *Clostridium bolteae*. In some embodiments, the methods and compositions disclosed herein are for use in veterinary and medical applications as well as research applications

Microbiome

[0177] "Microbiome", "microbiota", and "microbial habitat" are used interchangeably hereinafter and refer to the ecological community of microorganisms that live on or in a subject's bodily surfaces, cavities, and fluids. Non-limiting examples of habitats of microbiome include: gut, colon, skin, skin surfaces, skin pores, vaginal cavity, umbilical regions, conjunctival regions, intestinal regions, stomach, nasal cavities and passages, gastrointestinal tract, urogenital tracts, saliva, mucus, and feces. In some embodiments, the microbiome comprises microbial material including, but not limited to, bacteria, archaea, protists, fungi, and viruses. In some embodiments, the microbial material comprises a gram-negative bacterium. In some embodiments, the microbial material comprises a gram-positive bacterium. In some embodiments, the microbial material comprises *Proteobacteria, Actinobacteria, Bacteriodetes,* or *Firmicutes.*

[0178] In some embodiments, the bacteriophages as disclosed herein are used to modulate or kill target bacteria within the microbiome of a subject. In some embodiments, the bacteriophages are used to modulate and/or kill target bacteria within the microbiome by the CRISPR-Cas system, lytic activity, or a combination thereof. In some embodiments, the bacteriophages are used to modulate and/or kill target bacteria within the microbiome of a subject. In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target bacteria from a plurality of bacteria within the microbiome of a subject. In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target entereopathogenic bacteria from a plurality of bacteria within the microbiome of a subject. In some embodiments, the target entereopathogenic bacterium is entereopathogenic E. Coli (EPEC). In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target diarreagenic bacteria from a plurality of bacteria within the microbiome of a subject. In some embodiments, the target diarreagenic bacterium is diarreagenic E.coli (DEC). In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target Shiga-toxin producing bacteria from a plurality of bacteria within the microbiome of a subject. In some embodiments, the target Shiga-toxin producing bacterium is Shiga-toxin producing E.coli (STEC).

[0179] In some embodiments, the bacteriophages are used to selectively modulate and/or kill one or more target enteropathogenic *C. difficile* bacteria strains within the microbiome of a subject including: CD043, CD05, CD073, CD093, CD180, CD106, CD128, CD199, CD111, CD108, CD25, CD148, CD154, FOBT195, CD03, CD038, CD112, CD196, CD105, UK1, UK6, BI-9, CD041, CD042, CD046, CD19, or R20291.

[0180] In some embodiments, the bacteriophages are used to modulate or kill target single or plurality of bacteria within the microbiome or gut flora of the gastrointestinal tract of a subject.

Modification (e.g., dysbiosis) of the microbiome or gut flora increases the risk for health conditions such as diabetes, mental disorders, ulcerative colitis, colorectal cancer, autoimmune disorders, obesity, diabetes, diseases of the central nervous system and inflammatory bowel disease. An exemplary list of the bacteria associated with diseases and conditions of gastrointestinal tract and are being modulated or killed by the bacteriophages include strains, sub-strains, and enterotypes of enterobacteriaceae, pasteurellaceae, fusobacteriaceae, neisseriaceae, veillonellaceae, gemellaceae, bacteriodales, clostridiales, erysipelotrichaceae, bifidobacteriaceae bacteroides, faecalibacterium, roseburia, blautia, ruminococcus, coprococcus, streptococcus, dorea, blautia, ruminococcus, lactobacillus, enterococcus, streptococcus, escherichia coli, fusobacterium nucleatum, haemophilus parainfluenzae (pasteurellaceae), veillonella parvula, eikenella corrodens (neisseriaceae), gemella moribillum, bacteroides vulgatus, bacteroides caccae, bifidobacterium bifidum, bifidobacterium longum, bifidobacterium adolescentis, bifidobacterium dentum, blautia hansenii, ruminococcus gnavus, clostridium nexile, faecalibacterium prausnitzii, ruminoccus torques, clostridium bolteae, eubacterium rectale, roseburia intestinalis, coprococcus comes, actinomyces, lactococcus, roseburia, streptococcus, blautia, dialister, desulfovibrio, escherichia, lactobacillus, coprococcus, clostridium, bifidobacterium, klebsiella, granulicatella, eubacterium, anaerostipes, parabacteroides, coprobacillus, gordonibacter, collinsella, bacteroides, , faecalibacterium, , anaerotruncus, alistipes, haemophilus, anaerococcus, veillonella, arevotella, akkermansia, bilophila, sutterella, eggerthella, holdemania, gemella, peptoniphilus, rothia, enterococcus, pediococcus, citrobacter, odoribacter, enterobacteria, fusobacterium, and proteus.

[0181] In some embodiments, a bacteriophage disclosed herein is administered to a subject to promote a healthy microbiome. In some embodiments, a bacteriophage disclosed herein is administered to a subject to restore a subject's microbiome to a microbiome composition that promotes health. In some embodiments, a composition comprising a bacteriophage disclosed herein comprises a prebiotic or a third agent. In some embodiment, microbiome related disease or disorder is treated by a bacteriophage disclosed herein.

Environmental Therapy

[0182] In some embodiments, bacteriophages disclosed herein are further used for food and agriculture sanitation (including meats, fruits and vegetable sanitation), hospital sanitation, home sanitation, vehicle and equipment sanitation, industrial sanitation, etc. In some embodiments, bacteriophages disclosed herein are used for the removal of antibiotic-resistant or other undesirable pathogens from medical, veterinary, animal husbandry, or any additional environments bacteria are passed to humans or animals.

[0183] Environmental applications of phage in health care institutions are for equipment such as endoscopes and environments such as ICUs which are potential sources of nosocomial infection due to pathogens that are difficult or impossible to disinfect. In some embodiments, a phage disclosed herein is used to treat equipment or environments inhabited by bacterial genera such as *Pseudomonas* which become resistant to commonly used disinfectants. In some embodiments, phage compositions disclosed herein are used to disinfect inanimate objects. In some embodiments, an environment disclosed herein is sprayed, painted, or poured onto with aqueous solutions with phage titers. In some embodiment a solution described herein comprises between 10^1-10^{20} plaque forming units (PFU)/ml. In some embodiments, a bacteriophage disclosed herein is applied by aerosolizing agents that include dry dispersants to facilitate distribution of the bacteriophage into the environment. In some embodiments, objects are immersed in a solution containing bacteriophage disclosed herein.

Sanitation

[0184] In some embodiments, bacteriophages disclosed herein are used as sanitation agents in a variety of fields. Although the terms "phage" or "bacteriophage" may be used, it should be noted that, where appropriate, this term should be broadly construed to include a single bacteriophage, multiple bacteriophages, such as a bacteriophage mixtures and mixtures of a bacteriophage with an agent, such as a disinfectant, a detergent, a surfactant, water, etc.

[0185] In some embodiments, bacteriophages are used to sanitize hospital facilities, including operating rooms, patient rooms, waiting rooms, lab rooms, or other miscellaneous hospital equipment. In some embodiments, this equipment includes electrocardiographs, respirators, cardiovascular assist devices, intraaortic balloon pumps, infusion devices, other patient care devices, televisions, monitors, remote controls, telephones, beds, etc. In some situations, the bacteriophage is applied through an aerosol canister. In some embodiments, bacteriophage is applied by wiping the phage on the object with a transfer vehicle.

[0186] In some embodiments, a bacteriophage described herein is used in conjunction with patient care devices. In some embodiment, bacteriophage is used in conjunction with a conventional ventilator or respiratory therapy device to clean the internal and external surfaces between patients. Examples of ventilators include devices to support ventilation during surgery, devices to support ventilation of incapacitated patients, and similar equipment. In some embodiments, the conventional therapy includes automatic or motorized devices, or manual bag-type devices such as are commonly found in emergency rooms and ambulances. In some embodiments, respiratory therapy includes inhalers to introduce medications such as bronchodilators as commonly used with

chronic obstructive pulmonary disease or asthma, or devices to maintain airway patency such as continuous positive airway pressure devices.

[0187] In some embodiment, a bacteriophage described herein is used to cleanse surfaces and treat colonized people in an area where highly-contagious bacterial diseases, such as meningitis or enteric infections are present.

[0188] In some embodiments, water supplies are treated with a composition disclosed herein. In some embodiments, bacteriophage disclosed herein is used to treat contaminated water, water found in cisterns, wells, reservoirs, holding tanks, aqueducts, conduits, and similar water distribution devices. In some embodiments, the bacteriophage is applied to industrial holding tanks where water, oil, cooling fluids, and other liquids accumulate in collection pools. In some embodiments, a bacteriophage disclosed herein is periodically introduced to the industrial holding tanks in order to reduce bacterial growth.

[0189] In some embodiments, bacteriophages disclosed herein are used to sanitize a living area, such as a house, apartment, condominium, dormitory, or any living area. In some embodiments, the bacteriophage is used to sanitize public areas, such as theaters, concert halls, museums, train stations, airports, pet areas, such as pet beds, or litter boxes. In this capacity, the bacteriophage is dispensed from conventional devices, including pump sprayers, aerosol containers, squirt bottles, pre-moistened towelettes, etc, applied directly to (e.g., sprayed onto) the area to be sanitized, or be transferred to the area via a transfer vehicle, such as a towel, sponge, etc. In some embodiments, a phage disclosed herein is applied to various rooms of a house, including the kitchen, bedrooms, bathrooms, garage, basement, etc. In some embodiments, a phage disclosed herein is in the same manner as conventional cleaners. In some embodiments, the phage is applied in conjunction with (before, after, or simultaneously with) conventional cleaners provided that the conventional cleaner is formulated so as to preserve adequate bacteriophage biologic activity.

[0190] In some embodiments, a bacteriophage disclosed herein is added to a component of paper products, either during processing or after completion of processing of the paper products. Paper products to which a bacteriophage disclosed herein is added include, but are not limited to, paper towels, toilet paper, moist paper wipes.

Food Safety

[0191] In some embodiments, a bacteriophage described herein is used in any food product or nutritional supplement, for preventing contamination. Examples for food or pharmaceuticals products are milk, yoghurt, curd, cheese, fermented milks, milk based fermented products, ice-creams, fermented cereal based products, milk based powders, infant formulae or tablets, liquid suspensions, dried oral supplement, wet oral supplement, or dry-tube-feeding.

[0192] The broad concept of bacteriophage sanitation is applicable to other agricultural applications and organisms. Produce, including fruits and vegetables, dairy products, and other agricultural products. For example, freshly-cut produce frequently arrive at the processing plant contaminated with pathogenic bacteria. This has led to outbreaks of food-borne illness traceable to produce. In some embodiments, the application of bacteriophage preparations to agricultural produce substantially reduce or eliminate the possibility of food-borne illness through application of a single phage or phage mixture with specificity toward species of bacteria associated with food-borne illness. In some embodiments, bacteriophages are applied at various stages of production and processing to reduce bacterial contamination at that point or to protect against contamination at subsequent points.

[0193] In some embodiments, specific bacteriophages are applied to produce in restaurants, grocery stores, produce distribution centers. In some embodiments, bacteriophages disclosed herein are periodically or continuously applied to the fruit and vegetable contents of a salad bar. In some embodiments, the application of bacteriophages to a salad bar or to sanitize the exterior of a food item is a misting or spraying process or a washing process.

[0194] In some embodiments, a bacteriophage described herein is used in matrices or support media containing with packaging containing meat, produce, cut fruits and vegetables, and other foodstuffs. In some embodiments, polymers that are suitable for packaging are impregnated with a bacteriophage preparation.

[0195] In some embodiments, a bacteriophage described herein is used in farm houses and livestock feed. In some embodiments, on a farm raising livestock, the livestock is provided with bacteriophage in their drinking water, food, or both. In some embodiments, a bacteriophage described herein is sprayed onto the carcasses and used to disinfect the slaughter area.

[0196] The use of specific bacteriophages as biocontrol agents on produce provides many advantages. For example, bacteriophages are natural, non-toxic products that will not disturb the ecological balance of the natural microflora in the way the common chemical sanitizers do, but will specifically lyse the targeted food-borne pathogens. Because bacteriophages, unlike chemical sanitizers, are natural products that evolve along with their host bacteria, new phages that are active against recently emerged, resistant bacteria are rapidly identified when required, whereas identification of a new effective sanitizer is a much longer process, several years.

Pharmaceutical Compositions

[0197] In some embodiments, the disclosure provides pharmaceutical compositions and methods of administering the same to treat bacterial, archaeal infections or to disinfect an area. In some

embodiments, the pharmaceutical composition comprises any of the reagents discussed above in a pharmaceutically acceptable carrier. In some embodiments, a pharmaceutical composition or method disclosed herein treats Lung infections (CFP, NCFB, HAP/VAP) systemic infections (bacteremia, SSSI) GI microbiome dysbiosis (CDI) and/ or urinary tract infections (cUTI).

[0198] In some embodiments, compositions disclosed herein comprise medicinal agents, pharmaceutical agents, carriers, adjuvants, dispersing agents, diluents, and the like.

[0199] In some embodiments, the bacteriophages disclosed herein are formulated for administration in a pharmaceutical carrier in accordance with suitable methods. In some embodiments, the manufacture of a pharmaceutical composition according to the disclosure, the bacteriophage is admixed with, inter alia, an acceptable carrier. In some embodiments, the carrier is a solid (including a powder) or a liquid, or both, and is preferably formulated as a unit-dose composition. In some embodiments, one or more bacteriophages are incorporated in the compositions disclosed herein, which are prepared by any suitable method of a pharmacy.

[0200] In some embodiment, a method of treating subject's *in-vivo*, comprising administering to a subject a pharmaceutical composition comprising a bacteriophage disclosed herein in a pharmaceutically acceptable carrier, wherein the pharmaceutical composition is administered in a therapeutically effective amount. In some embodiments, the administration of the bacteriophage to a human subject or an animal in need thereof are by any means known in the art.

[0201] In some embodiments, bacteriophages disclosed herein are for oral administration. In some embodiments, the bacteriophages are administered in solid dosage forms, such as capsules, tablets, and powders, or in liquid dosage forms, such as elixirs, syrups, and suspensions. In some embodiments, compositions and methods suitable for buccal (sub-lingual) administration include lozenges comprising the bacteriophages in a flavored base, usually sucrose and acacia or tragacanth; and pastilles comprising the bacteriophages in an inert base such as gelatin and glycerin or sucrose and acacia.

[0202] In some embodiments, methods and compositions of the present disclosure are suitable for parenteral administration comprising sterile aqueous and non-aqueous injection solutions of the bacteriophage. In some embodiments, these preparations are isotonic with the blood of the intended recipient. In some embodiments, these preparations comprise antioxidants, buffers, bacteriostals and solutes which render the composition isotonic with the blood of the intended recipient. In some embodiments, aqueous and non-aqueous sterile suspensions include suspending agents and thickening agents. In some embodiments, compositions disclosed herein are presented in unit\dose or multi-dose containers, for example sealed ampoules and vials, and are stored in a freeze-

dried(lyophilized) condition requiring only the addition of the sterile liquid carrier, for example, saline or water for injection on immediately prior to use.

[0203] In some embodiment, methods and compositions suitable for rectal administration are presented as unit dose suppositories. In some embodiments, these are prepared by admixing the bacteriophage with one or more conventional solid carriers, for example, cocoa butter, and then shaping the resulting mixture. In some embodiments, methods and compositions suitable for topical application to the skin are in the form of an ointment, cream, lotion, paste, gel, spray, aerosol, or oil. In some embodiments, carriers which are used include petroleum jelly, lanoline, polyethylene glycols, alcohols, transdermal enhancers, and combinations of two or more thereof.

[0204] In some embodiments, methods and compositions suitable for transdermal administration are presented as discrete patches adapted to remain in intimate contact with the epidermis of the recipient for a prolonged period of time.

[0205] In some embodiments, methods and compositions suitable for nasal administration or otherwise administered to the lungs of a subject include any suitable means, e.g., administered by an aerosol suspension of respirable particles comprising the bacteriophage compositions, which the subject inhales. In some embodiments, the respirable particles are liquid or solid. As used herein, "aerosol" includes any gas-borne suspended phase, which is capable of being inhaled into the bronchioles or nasal passages. In some embodiments, aerosols of liquid particles are produced by any suitable means, such as with a pressure-driven aerosol nebulizer or an ultrasonic nebulizer. In some embodiments, aerosols of solid particles comprising the composition is produced with any solid particulate medicament aerosol generator, by techniques known in the pharmaceutical art.

[0206] In some embodiment, methods and compositions suitable for administering bacteriophages disclosed herein to a surface of an object or subject includes aqueous solutions. In some embodiments, such aqueous solutions are sprayed onto the surface of an object or subject. In some embodiment, the aqueous solutions are used to irrigate and clean a physical wound of a subject form foreign debris including bacteria.

[0207] In some embodiments, the bacteriophages disclosed herein are administered to the subject in a therapeutically effective amount. In some embodiments, at least one bacteriophage composition disclosed herein is formulated as a pharmaceutical formulation. In some embodiments, a pharmaceutical formulation comprises 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more bacteriophage disclosed herein. In some instances, a pharmaceutical formulation comprises a bacteriophage described herein and at least one of: an excipient, a diluent, or a carrier.

[0208] In some embodiments, a pharmaceutical formulation comprises an excipient. Excipients are described in the Handbook of Pharmaceutical Excipients, American Pharmaceutical Association

(1986) and includes but are not limited to solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, solid binders, and lubricants.

[0209] Non-limiting examples of suitable excipients include but is not limited to a buffering agent, a preservative, a stabilizer, a binder, a compaction agent, a lubricant, a chelator, a dispersion enhancer, a disintegration agent, a flavoring agent, a sweetener, a coloring agent.

[0210] In some embodiments, an excipient is a buffering agent. Non-limiting examples of suitable buffering agents include but is not limited to sodium citrate, magnesium carbonate, magnesium bicarbonate, calcium carbonate, and calcium bicarbonate. In some embodiments, a pharmaceutical formulation comprises any one or more buffering agent listed: sodium bicarbonate, potassium bicarbonate, magnesium hydroxide, magnesium lactate, magnesium glucomate, aluminum hydroxide, sodium citrate, sodium tartrate, sodium acetate, sodium carbonate, sodium polyphosphate, potassium polyphosphate, sodium pyrophosphate, potassium pyrophosphate, disodium hydrogen phosphate, dipotassium hydrogen phosphate, trisodium phosphate, tripotassium phosphate, potassium metaphosphate, magnesium oxide, magnesium hydroxide, magnesium carbonate, magnesium silicate, calcium acetate, calcium glycerophosphate, calcium chloride, calcium hydroxide and other calcium salts.

[0211] In some embodiments an excipient is a preservative. Non-limiting examples of suitable preservatives include but is not limited to antioxidants, such as alpha-tocopherol and ascorbate, and antimicrobials, such as parabens, chlorobutanol, and phenol. In some embodiments, antioxidants include but not limited to EDTA, citric acid, ascorbic acid, butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), sodium sulfite, p-amino benzoic acid, glutathione, propyl gallate, cysteine, methionine, ethanol and N- acetyl cysteine. In some embodiments, preservatives include validamycin A, TL-3, sodium ortho vanadate, sodium fluoride, N-a-tosyl-Phe- chloromethylketone, N-a-tosyl-Lys-chloromethylketone, aprotinin, phenylmethylsulfonyl fluoride, diisopropylfluorophosphate, protease inhibitor, reducing agent, alkylating agent, antimicrobial agent, oxidase inhibitor, or other inhibitor.

[0212] In some embodiments, a pharmaceutical formulation comprises a binder as an excipient. Non-limiting examples of suitable binders include starches, pregelatinized starches, gelatin, polyvinylpyrolidone, cellulose, methylcellulose, sodium carboxymethylcellulose, ethylcellulose, polyacrylamides, polyvinyloxoazolidone, polyvinylalcohols, C_{12}-C_{18} fatty acid alcohol, polyethylene glycol, polyols, saccharides, oligosaccharides, and combinations thereof.

[0213] In some embodiments, the binders that are used in a pharmaceutical formulation are selected from starches such as potato starch, corn starch, wheat starch; sugars such as sucrose,

glucose, dextrose, lactose, maltodextrin; natural and synthetic gums; gelatine; cellulose derivatives such as microcrystalline cellulose, hydroxypropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, carboxymethyl cellulose, methyl cellulose, ethyl cellulose; polyvinylpyrrolidone (povidone); polyethylene glycol (PEG); waxes; calcium carbonate; calcium phosphate; alcohols such as sorbitol, xylitol, mannitol and water or a combination thereof.

[0214] In some embodiments, a pharmaceutical formulation comprises a lubricant as an excipient. Non-limiting examples of suitable lubricants include magnesium stearate, calcium stearate, zinc stearate, hydrogenated vegetable oils, sterotex, polyoxyethylene monostearate, talc, polyethylene glycol, sodium benzoate, sodium lauryl sulfate, magnesium lauryl sulfate, and light mineral oil. In some embodiments, lubricants that are in a pharmaceutical formulation are selected from metallic stearates (such as magnesium stearate, calcium stearate, aluminum stearate), fatty acid esters (such as sodium stearyl fumarate), fatty acids (such as stearic acid), fatty alcohols, glyceryl behenate, mineral oil, paraffins, hydrogenated vegetable oils, leucine, polyethylene glycols (PEG), metallic lauryl sulphates (such as sodium lauryl sulphate, magnesium lauryl sulphate), sodium chloride, sodium benzoate, sodium acetate and talc or a combination thereof.

[0215] In some embodiments, an excipient comprises a flavoring agent. In some embodiments, flavoring agents includes natural oils; extracts from plants, leaves, flowers, and fruits; and combinations thereof.

[0216] In some embodiments, an excipient comprises a sweetener. Non-limiting examples of suitable sweeteners include glucose (corn syrup), dextrose, invert sugar, fructose, and mixtures thereof (when not used as a carrier); saccharin and its various salts such as a sodium salt; dipeptide sweeteners such as aspartame; dihydrochalcone compounds, glycyrrhizin; Stevia Rebaudiana (Stevioside); chloro derivatives of sucrose such as sucralose; and sugar alcohols such as sorbitol, mannitol, sylitol, and the like.

[0217] In some instances, a pharmaceutical formulation comprises a coloring agent. Non-limiting examples of suitable color agents include food, drug and cosmetic colors (FD&C), drug and cosmetic colors (D&C), and external drug and cosmetic colors (Ext. D&C).

[0218] In some embodiments, the pharmaceutical formulation disclosed herein comprises a chelator. In some embodiments, a chelator includes ethylenediamine-N,N,N',N'-tetraacetic acid (EDTA); a disodium, trisodium, tetrasodium, dipotassium, tripotassium, dilithium and diammonium salt of EDTA; a barium, calcium, cobalt, copper, dysprosium, europium, iron, indium, lanthanum, magnesium, manganese, nickel, samarium, strontium, or zinc chelate of EDTA.

[0219] In some instances, a pharmaceutical formulation comprises a diluent. Non-limiting examples of diluents include water, glycerol, methanol, ethanol, and other similar biocompatible

diluents. In some embodiments, a diluent is an aqueous acid such as acetic acid, citric acid, maleic acid, hydrochloric acid, phosphoric acid, nitric acid, sulfuric acid, or similar.

[0220] In some embodiments, a pharmaceutical formulation comprises a surfactant. In some embodiments, surfactants are be selected from, but not limited to, polyoxyethylene sorbitan fatty acid esters (polysorbates), sodium lauryl sulphate, sodium stearyl fumarate, polyoxyethylene alkyl ethers, sorbitan fatty acid esters, polyethylene glycols (PEG), polyoxyethylene castor oil derivatives, docusate sodium, quaternary ammonium compounds, aminoacids such as L- leucine, sugar esters of fatty acids, glycerides of fatty acids or a combination thereof.

[0221] In some instances, a pharmaceutical formulation comprises an additional pharmaceutical agent. In some embodiments, an additional pharmaceutical agent is an antibiotic agent. In some embodiments, an antibiotic agent is of the group consisting of aminoglycosides, ansamycins, carbacephem, carbapenems, cephalosporins (including first, second, third, fourth and fifth generation cephalosporins), lincosamides, macrolides, monobactams, nitrofurans, quinolones, penicillin, sulfonamides, polypeptides or tetracycline.

[0222] In some embodiments, an antibiotic agent described herein is an aminoglycoside such as Amikacin, Gentamicin, Kanamycin, Neomycin, Netilmicin, Tobramycin or Paromomycin. In some embodiments, an antibiotic agent described herein is an Ansamycin such as Geldanamycin or Herbimycin

[0223] In some embodiments, an antibiotic agent described herein is a carbacephem such as Loracarbef. In some embodiments, an antibiotic agent described herein is a carbapenem such as Ertapenem, Doripenem, Imipenem/Cilastatin or Meropenem.

[0224] In some embodiments, an antibiotic agent described herein is a cephalosporins (first generation) such as Cefadroxil, Cefazolin, Cefalexin, Cefalotin or Cefalothin, or alternatively a Cephalosporins (second generation) such as Cefaclor, Cefamandole, Cefoxitin, Cefprozil or Cefuroxime. In some embodiments, an antibiotic agent is a Cephalosporins (third generation) such as Cefixime, Cefdinir, Cefditoren, Cefoperazone, Cefotaxime, Cefpodoxime, Ceftibuten, Ceftizoxime and Ceftriaxone or a Cephalosporins (fourth generation) such as Cefepime or Ceftobiprole.

[0225] In some embodiments, an antibiotic agent described herein is a lincosamide such as Clindamycin and Azithromycin, or a macrolide such as Azithromycin, Clarithromycin, Dirithromycin, Erythromycin, Roxithromycin, Troleandomycin, Telithromycin and Spectinomycin.

[0226] In some embodiments, an antibiotic agent described herein is a monobactams such as Aztreonam, or a nitrofuran such as Furazolidone or Nitrofurantoin.

[0227] In some embodiments, an antibiotic agent described herein is a penicillin such as Amoxicillin, Ampicillin, Azlocillin, Carbenicillin, Cloxacillin, Dicloxacillin, Flucloxacillin, Mezlocillin, Nafcillin, Oxacillin, Penicillin G or V, Piperacillin, Temocillin and Ticarcillin.

[0228] In some embodiments, an antibiotic agent described herein is a sulfonamide such as Mafenide, Sulfonamidochrysoidine, Sulfacetamide, Sulfadiazine, Silver sulfadiazine, Sulfamethizole, Sulfamethoxazole, Sulfanilimide, Sulfasalazine, Sulfisoxazole, Trimethoprim, or Trimethoprim-Sulfamethoxazole (Co-trimoxazole) (TMP-SMX).

[0229] In some embodiments, an antibiotic agent described herein is a quinolone such as Ciprofloxacin, Enoxacin, Gatifloxacin, Levofloxacin, Lomefloxacin, Moxifloxacin, Nalidixic acid, Norfloxacin, Ofloxacin, Trovafloxacin, Grepafloxacin, Sparfloxacin and Temafloxacin.

[0230] In some embodiments, an antibiotic agent described herein is a polypeptide such as Bacitracin, Colistin or Polymyxin B.

[0231] In some embodiments, an antibiotic agent described herein is a tetracycline such as Demeclocycline, Doxycycline, Minocycline or Oxytetracycline.

Dose

[0232] Dose and duration of the administration of a composition disclosed herein will depend on a variety of factors, including the subject's age, subject's weight, and tolerance of the phage. In some embodiments, a bacteriophage disclosed herein is administered to patients by oral administration. In some embodiments, a dose of phage between 10^3 and 10^{20} PFU is given. For example, in some embodiments, the bacteriophage is present in a composition in an amount between 10^3 and 10^{11} PFU. In some embodiments, the bacteriophage is present in a composition in an amount about 10^3, 10^4, 10^5, 10^6, 10^7, 10^8, 10^9, 10^{10}, 10^{11}, 10^{12}, 10^{13}, 10^{14}, 10^{15}, 10^{16}, 10^{17}, 10^{18}, 10^{19}, 10^{20}, 10^{21}, 10^{22}, 10^{23}, 10^{24} PFU or more. In some embodiments, the bacteriophage is present in a composition in an amount of less than10^1 PFU. In some embodiments, the bacteriophage is present in a composition in an amount between 10^1 and 10^8, 10^4 and 10^9, 10^5 and 10^{10}, or 10^7 and 10^{11} PFU. In some embodiments, a bacteriophage or a mixture is administered to a subject in need thereof 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, or 24 times a day. In some embodiments, a bacteriophage or a mixture is administered to a subject in need thereof at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 21 times a week. In some embodiments, a bacteriophage or a mixture is administered to a subject in need thereof at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, or 90 times a month.

[0233] In some embodiments, the compositions (bacteriophage) disclosed herein are administered before, during, or after the occurrence of a disease or condition. In some embodiment, the timing of administering the composition containing the bacteriophage varies. In some embodiments, the pharmaceutical compositions are used as a prophylactic and are administered continuously to subjects with a propensity to conditions or diseases in order to prevent the occurrence of the disease or condition. In some embodiments, pharmaceutical compositions are administered to a subject during or as soon as possible after the onset of the symptoms. In some embodiments, the administration of the compositions is initiated within the first 48 hours of the onset of the symptoms, within the first 24 hours of the onset of the symptoms, within the first 6 hours of the onset of the symptoms, or within 3 hours of the onset of the symptoms. In some embodiments, the initial administration of the composition is via any route practical, such as by any route described herein using any formulation described herein. In some embodiments, the compositions is administered as soon as is practicable after the onset of a disease or condition is detected or suspected, and for a length of time necessary for the treatment of the disease, such as, for example, from about 1 month to about 3 months. In some embodiments, the length of treatment will vary for each subject.

Kits

[0234] Disclosed herein are kits for use. In some embodiments, the kit comprises the nucleic acid constructs for the CRISPR arrays, transcriptional activators, and/or anti-CRISPR polypeptides, as well as the bacteriophages and/or any other vectors/expression cassettes disclosed herein in a form suitable for introduction into a cell and/or administration to a subject. In some embodiments, the kit comprises other therapeutic agents, carriers, buffers, containers, devices for administration, and the like. In some embodiments, the kit comprises labels and/or instructions for repression of expression a target gene and/or modulation of repression of expression of a target gene. In some embodiments, labeling and/or instructions includes, for example, information concerning the amount, frequency and method of introduction and/or administration of the nucleic acid constructs for the CRISPR arrays, transcriptional activators, and anti-CRISPR polypeptides, as well as the bacteriophages and/or any other vectors/expression cassettes.

[0235] In some embodiments, a kit for the killing of one target bacterium is provided, said kit comprising, consisting essentially of, consisting of nucleic acid constructs for the CRISPR arrays, transcriptional activators, and/or anti-CRISPR polypeptides, as well as the bacteriophages and/or any other vectors/expression cassettes necessary to achieve killing of the target bacteria by any embodiment disclosed herein.

[0236] In some embodiments, a kit is provided for modulating the activity of a CRISPR-Cas system in a target bacterium is provided, the kit comprising, consisting essentially of, consisting of nucleic acid constructs for the CRISPR arrays, transcriptional activators, and anti-CRISPR polypeptides, as well as the bacteriophages and/or any other vectors/expression cassettes necessary to achieve modulation of a CRISPR-Cas system in a target bacteria by any embodiment disclosed herein.

[0237] In some embodiments, the nucleic acid constructs for the CRISPR arrays, transcriptional activators, and/or anti-CRISPR polypeptides of said kits are comprised on a single vector or expression cassette or on separate vectors or expression cassettes or within a single bacteriophage or a plurality of bacteriophages. In some embodiments, a kit comprises one or more bacteriophage disclosed herein. In some embodiments, the kits comprise instructions for use. In some embodiments, the instructions for practicing the methods are recorded on a suitable recording medium. In some embodiments, the instructions are printed on a substrate, such as paper or plastic, etc. In some embodiments, the instructions are present in the kits as a package insert, in the labeling of the container of the kit or components thereof (i.e., associated with the packaging or subpackaging) etc. In some embodiments, the instructions are present as an electronic storage data file present on a suitable computer readable storage medium, e.g. CD-ROM, diskette, flash drive, etc. In some embodiments, the actual instructions are not present in the kit, but means for obtaining the instructions from a remote source (e.g. via the Internet), are provided. In some embodiments, the kit includes a web address where the instructions are viewed and/or from which the instructions are downloaded.

[0238] Certain embodiments disclosed herein, both in their methods and compositions, will now are described with reference to the following examples. It should be appreciated that these examples are not intended to limit the scope of the claims to the disclosure, but are rather intended to be exemplary of certain embodiments. Any variations in the exemplified methods that occur to the skilled artisan are intended to fall within the scope of the disclosure.

EXAMPLES

Example 1: Overview for generating CRISPR enhanced bacteriophages

[0239] CRISPR-enhanced bacteriophages are phages that have been engineered to express CRISPR RNA constructs from a bacteriophage genome that maintains the essential genes for lytic lifestyle. The steps involved are sourcing, isolating and identifying bacteriophages and cocktails of bacteriophages with broad host ranges against bacteria followed by engineering each phage to carry an expression construct (for example, crRNA) that targets the bacterium's genome and validating

optimized combinations of crPhages to be used as a clinical lead candidate. In some embodiments, the general processes are as schematically shown in steps 1-5 of **Figure 1**. Steps 1 – 5 are designed to identify a suitable number of wild-type bacteriophages such that they:

[0240] 1. Meet minimum quality standards (absence of lysogeny, virulence genes or antibiotic resistance genes) proposed in **Table 1** below:

Table 1: Summary of phage characterizations

Test/characteristic	**Method**
Genome size (kb)	Genome sequencing
Family of Caudovirales	Transmission electron microscopy
Host range activity	Host range analysis against uropathogenic *E. coli* clinical isolates and representative *E. coli* strains
Genome sequence	Genome sequencing
DNA restriction profile	Restriction enzyme digestion/electrophoresis
Typing	PCR specific to engineered insert
Lifestyle (lytic, temperate)	DNA analysis
Absence of generalized transduction	Microbiological transduction assay
Absence of virulence genes	Genome sequence analysis
Absence of antibiotic resistance genes	Genome sequence analysis

[0241] 2. Have collective activity against approximately 90% or greater of the clinical isolate panel.

[0242] 3. Result in infection of each strain by at least 2 phages within the cocktail (mixture of two or more phages), intended to ensure strain sensitivity to the cocktail in the event of resistance to any single bacteriophage. In some embodiments, a cocktail described herein in comprises at least 2, 3, 4, 5, 6, 7, 8, 9, 10, 20, 30, 50, 100 or more bacteriophages.

[0243] 4. Include genetic engineering of each candidate bacteriophage to express a crRNA construct from the wild-type genome. Each engineered crPhage is intended to retain lytic activity. crPhages will then be subjected to *in vitro* analyses to assess host range and *in vitro* efficacy. These studies are intended to confirm that crPhages retain broad host range, if not expanded host range by ability to transduce lethal crRNA constructs in the absence of productive lytic infection, and improved lethality for each crPhage to the cognate wild-type bacteriophage.

[0244] 5. Identifying crPhages for use in a cocktail that is optimized to improve host range, manufacturing limitations or nonclinical efficacy.

Example 2: Phage isolation

[0245] Bacteriophage vectors were obtained from environmental sources in North Carolina. Phages were directly isolated from these samples by co-incubating with uropathgenic *E. coli* s ECOR 14,

62, 64 and 71. Each phage was then subjected to clonal purification by single plaque isolation across a total of three rounds of double agar overlays prior to amplification, filtration and long-term storage at 4 degrees Celsius. Each phage was amplified, filtered, subjected to cesium chloride gradient purification and dialyzed into 1x tris-buffered saline. Phage genomic DNA was extracted from purified phage stocks using a column-based phage DNA preparation kit (Norgen) and submitted for genome sequencing by MiSeq or PacBio sequencing, as appropriate, and assembly at a third party vendor (Genewiz). A previously isolated phage, K1F was obtained that has been previously shown to infect uropathogenic *E. coli* isolates and have known genomes (K1F: NC_007456).

Example 3: Phage host range analysis

[0246] This library of wild-type bacteriophages was individually characterized against an *E. coli* panel for the ability to replicate and lyse each target. This process was expected to result in a wild-type bacteriophage panel that lyses approximately 90% of a panel of uropathogenic *E. coli* and displays minimal resistance during 24-hour challenge studies. From this master library, an abbreviated wild-type bacteriophage library was generated with retained predicted activity against approximately 90% of the uropathogenic *E. coli* panel.

[0247] Each phage was tested for lytic activity against an evolutionarily broad panel of *E. coli*. Briefly, phages were produced to high titer (10^9 – 10^{11} PFU/mL), filtered and left suspended in growth media. Each target host was grown to mid-log phase and incorporated in soft agar overlays to create bacterial lawns. Phages were serially diluted down to approximately 10^3 – 10^5 PFU/mL and 5 microliters of each dilution was spot plated on each bacterial lawn. Host sensitivity to each phage was defined as any observable zone of clearance within each spot. In some cases, this analysis includes some phages that adsorb to a target host and cause a phenomenon termed lysis-from-without rather than lysis-from-within caused by productive lytic infection. However, we intentionally chose not to exclude these data points under the assumption that lysis-from-without resulting from adsorption still permits DNA transduction even in the absence of productive lytic infection.

[0248] From this preliminary analysis, 10 phages were chosen that collectively infect 17 of 18 isolates tested (94%) in the panel, including 4 urinary pathogenic s isolated from human patients as shown in **Table 2** below. Phages were isolated or tested against ECOR 14, 62, 64 or 71 as highlighted and then tested against the broader panel shown. Importantly, phage host range was considered as all productive lysis events and include events that resulted from lysis-from-without.

Table 2: Host range analysis of isolated bacteriophages against diverse set of *E. coli* stains.

		Phage										# phages isolated per strain
		ΦECOR71-2	ΦECOR71-3	ΦECOR71-4	ΦECOR71-5	ΦECOR71-6	ΦECOR71-7	ΦECOR71-8	ΦECOR71-10	ΦECOR14-1	ΦK1F	
E. coli Strain	ECOR2	0	1	0	0	0	1	1	0	0	0	3/10
	ECOR5	0	1	0	1	1	1	0	0	0	0	4/10
	ECOR14 (UTI isolate)	0	0	0	0	0	0	0	0	0	0	0/10
	ECOR21	0	1	0	0	0	1	1	0	0	0	3/10
	ECOR27	0	1	0	0	0	1	1	0	0	0	3/10
	ECOR29	0	1	0	0	0	1	1	0	0	0	3/10
	ECOR35	1	0	0	0	0	0	0	0	1	1	3/10
	ECOR36	1	0	0	0	0	0	0	0	1	1	3/10
	ECOR41	1	0	0	0	0	0	0	1	1	1	4/10
	ECOR47	1	1	1	0	1	1	1	1	1	0	7/10
	ECOR51	0	0	0	0	0	1	1	1	0	0	3/10
	ECOR56	1	1	0	0	0	1	1	1	1	0	6/10
	ECOR58	0	1	0	0	0	1	1	0	0	0	3/10
	ECOR62 (UTI isolate)	1	1	0	0	0	1	1	1	0	1	6/10
	ECOR64 (UTI isolate)	0	1	1	1	0	1	1	0	0	1	6/10
	ECOR71 (UTI isolate)	1	1	1	0	1	1	1	1	1	1	9/10
	EV36	1	1	0	0	1	1	1	1	1	1	7/10
	CFT073 (UPEC Strain)	0	0	0	0	0	0	0	0	0	0	0/10
	# strains sensitive to phage	9/18	12/18	3/18	3/18	4/18	13/18	12/18	8/18	8/18	5/18	
	% of strains sensitive to phage	50%	67%	17%	17%	22%	72%	67%	44%	44%	28%	

Grey shaded rows demonstrated preliminary host range coverage against uropathogenic *E. coli* isolates.

Example 4: Primary resistance to isolate phages

[0249] Using selected *E. coli* that are susceptible to multiple wild-type bacteriophages in this abbreviated library, individual bacteriophages were then assessed for ability to generate resistant clones in treatment-naïve hosts after overnight incubation with a challenge phage. Apparent resistant colonies to each individual wild-type bacteriophage were clonally isolated and re-challenged with the original bacteriophage and the others within the library. These data were used to inform which bacteriophages are likely to generate rapid primary resistance, defined as stable resistance phenotypes after re-challenge with the same bacteriophage. Stable resistance phenotypes did not exhibit obvious lysis by inspection of growth curves during 24-hour cultures in media comprising a challenge bacteriophage. Bacteriophages that generate primary resistance in >50% of clones were excluded from further study. A further characterization step then measures the sensitivity of each clone to the other wild-type bacteriophages in the abbreviated library.

[0250] All phage stocks were produced as crude lysates of the wild-type, wild-type bacteriophage in growth media by fermentation, filtration and validation of $>10^{10}$ PFU/mL titers. To determine the empirical resistance profiles for each wild-type phage in the proposed 10-phage cocktail, putative resistant clones were generated for specific host: phage pairings by incubating ~108 colony forming units of an *E. coli* host with a high-titer lysate ($>10^8$ PFU, MOI > 1.0) in a double agar overlay. After overnight culture, putative resistant clones were isolated by triple colony purification. After outgrowth, each clone was subjected to challenge with each original phage from the abbreviated 10-phage cocktail. Notably, numerous clones with apparent resistance phenotypes, defined as survival after initial challenge, apparently regained sensitivity after outgrowth in the absence of phage challenge as seen **Table 3** below:

Table 3: Resistance profile data for selected crPhages against *E. coli* ECOR71.

Phage: host clone	Rep	PlateID	71-2	71-3	71-4	71-5	71-6	71-7	71-8	71-10	14-1	KIF
71-2	1	ECOR71:φECOR71-2-R1	**R**	S	R	R	R	S	S	S	R	S
71-2	2	ECOR71:φECOR71-2-R2	**R**	S	R	R	R	S	S	S	S	S
71-2	3	ECOR71:φECOR71-2-R3	**R**	S	R	R	R	S	S	S	S	T
71-2	4	ECOR71:φECOR71-2-R4	**R**	S	R	R	R	S	S	S	S	S
71-2	5	ECOR71:φECOR71-2-R5	**R**	S	R	R	R	S	S	S	S	S
71-2	6	ECOR71:φECOR71-2-R6	**R**	S	S	S	S	S	S	S	S	S
71-2	7	ECOR71:φECOR71-2-R7	**R**	S	R	R	R	S	S	S	S	S
71-2	8	ECOR71:φECOR71-2-R8	**R**	S	R	R	R	S	S	R	R	T
71-3	1	ECOR71:φECOR71-3-R1	R	**S**	S	R	R	S	S	S	S	T
71-3	2	ECOR71:φECOR71-3-R2	R	**S**	S	S	S	S	S	S	S	S
71-3	3	ECOR71:φECOR71-3-R3	R	**S**	S	R	R	S	S	S	S	S
71-3	4	ECOR71:φECOR71-3-R4	R	**S**	S	R	S	S	R	R	R	R
71-3	5	ECOR71:φECOR71-3-R5	R	**S**	S	R	R	S	R	S	S	S
71-3	6	ECOR71:φECOR71-3-R6	R	**S**	S	S	S	S	S	S	S	S
71-3	7	ECOR71:φECOR71-3-R7	R	**S**	S	R	R	S	S	R	S	S
71-3	8	ECOR71:φECOR71-3-R8	R	**S**	S	R	R	S	S	S	S	S
71-4	1	ECOR71:φECOR71-4-R1	S	S	**S**	S	S	S	S	S	S	S
71-4	2	ECOR71:φECOR71-4-R2	S	S	**S**	S	S	S	S	S	S	S
71-4	3	ECOR71:φECOR71-4-R3	S	S	**S**	S	S	S	S	S	S	S
71-4	4	ECOR71:φECOR71-4-R4	S	S	**S**	S	S	S	R	S	S	S
71-4	5	ECOR71:φECOR71-4-R5	S	S	**S**	S	S	S	S	S	S	S
71-4	6	ECOR71:φECOR71-4-R6	S	S	**S**	S	S	S	S	S	S	S
71-4	7	ECOR71:φECOR71-4-R7	S	S	**S**	S	S	S	S	S	S	S
71-4	8	ECOR71:φECOR71-4-R8	S	S	**S**	S	S	S	S	S	S	S
71-5	1	ECOR71:φECOR71-5-R1	S	S	S	**S**	S	S	S	S	S	S
71-5	2	ECOR71:φECOR71-5-R2	S	S	S	**S**	S	S	S	S	S	S
71-5	3	ECOR71:φECOR71-5-R3	S	S	S	**S**	S	S	S	S	S	S
71-5	4	ECOR71:φECOR71-5-R4	S	S	S	**S**	S	S	T	S	S	S
71-5	5	ECOR71:φECOR71-5-R5	S	S	S	**S**	S	S	S	S	S	S
71-5	6	ECOR71:φECOR71-5-R6	S	S	S	**S**	S	S	S	S	S	S
71-5	7	ECOR71:φECOR71-5-R7	S	S	S	**S**	S	S	R	S	S	S
71-5	8	ECOR71:φECOR71-5-R8	S	S	S	**S**	S	S	R	S	S	S
71-6	1	ECOR71:φECOR71-6-R1	S	S	S	S	**S**	S	S	S	S	S
71-6	2	ECOR71:φECOR71-6-R2	S	S	S	S	**S**	S	S	S	S	S
71-6	3	ECOR71:φECOR71-6-R3	S	S	S	S	**R**	S	S	S	S	S
71-6	4	ECOR71:φECOR71-6-R4	S	S	S	S	**S**	S	R	S	S	S
71-6	5	ECOR71:φECOR71-6-R5	S	S	S	S	**S**	S	S	S	R	S
71-6	6	ECOR71:φECOR71-6-R6	S	S	S	S	**S**	S	S	S	S	S
71-6	7	ECOR71:φECOR71-6-R7	S	S	S	S	**S**	S	S	S	S	S
71-6	8	ECOR71:φECOR71-6-R8	S	S	S	S	**S**	S	S	S	S	S
71-7	1	ECOR71:φECOR71-7-R1	R	X	X	X	X	**X**	X	S	X	X
71-7	2	ECOR71:φECOR71-7-R2	R	S	S	R	R	**S**	S	S	S	S

71-7	3	ECOR71:φECOR71-7-R3	R	S	S	R	R	**S**	S	S	S	S
71-7	4	ECOR71:φECOR71-7-R4	R	S	R	R	R	**S**	S	S	S	S
71-7	5	ECOR71:φECOR71-7-R5	R	S	S	R	R	**S**	R	R	S	S
71-7	6	ECOR71:φECOR71-7-R6	R	S	R	R	R	**S**	S	S	S	S
71-7	7	ECOR71:φECOR71-7-R7	R	S	R	R	R	**S**	S	S	S	S
71-7	8	ECOR71:φECOR71-7-R8	R	R	S	R	R	**S**	R	R	R	S
71-8	1	ECOR71:φECOR71-8-R1	R	S	R	X	R	X	**X**	X	X	X
71-8	2	ECOR71:φECOR71-8-R2	R	S	R	R	R	S	**T**	S	S	S
71-8	3	ECOR71:φECOR71-8-R3	R	S	X	R	X	R	**X**	X	X	X
71-8	4	ECOR71:φECOR71-8-R4	R	S	R	R	R	S	**S**	S	T	S
71-8	5	ECOR71:φECOR71-8-R5	R	R	S	R	R	S	**R**	R	S	S
71-8	6	ECOR71:φECOR71-8-R6	R	S	R	R	R	S	**S**	S	S	S
71-8	7	ECOR71:φECOR71-8-R7	R	S	S	R	R	S	**S**	S	S	S
71-8	8	ECOR71:φECOR71-8-R8	R	S	S	R	R	S	**R**	R	R	S
71-10	1	ECOR71:φECOR71-10-R1	R	S	S	S	S	S	S	**S**	X	S
71-10	2	ECOR71:φECOR71-10-R2	R	X	X	R	X	S	X	**S**	X	R
71-10	3	ECOR71:φECOR71-10-R3	R	S	S	S	S	S	S	**S**	S	S
71-10	4	ECOR71:φECOR71-10-R4	R	S	S	R	S	S	S	**S**	S	S
71-10	5	ECOR71:φECOR71-10-R5	R	S	R	R	R	S	S	**R**	S	S
71-10	6	ECOR71:φECOR71-10-R6	R	S	R	R	R	S	S	**S**	S	S
71-10	7	ECOR71:φECOR71-10-R7	R	S	S	S	S	S	S	**X**	S	S
71-10	8	ECOR71:φECOR71-10-R8	R	S	S	S	S	S	S	**S**	S	S
14-1	1	ECOR71:φECOR14-1-R1	R	S	S	R	R	R	S	S	**R**	X
14-1	2	ECOR71:φECOR14-1-R2	R	S	R	R	R	S	S	S	**S**	S
14-1	3	ECOR71:φECOR14-1-R3	S	S	S	S	S	S	S	S	**S**	S
14-1	4	ECOR71:φECOR14-1-R4	R	S	S	S	S	S	S	S	**S**	S
14-1	5	ECOR71:φECOR14-1-R5	R	S	S	S	S	S	S	S	**S**	S
14-1	6	ECOR71:φECOR14-1-R6	R	S	S	S	S	S	S	S	**S**	S
14-1	7	ECOR71:φECOR14-1-R7	R	S	S	R	R	S	S	S	**S**	S
14-1	8	ECOR71:φECOR14-1-R8	R	S	S	S	S	S	S	S	**S**	S
KIF	1	EV36:φK1F-R1	S	S	S	S	S	S	S	S	S	**S**
KIF	2	EV36:φK1F-R2	S	S	S	S	S	S	S	S	S	**S**
KIF	3	EV36:φK1F-R3	S	S	S	R	S	S	S	S	S	**S**
KIF	4	EV36:φK1F-R4	S	S	S	S	S	S	S	S	S	**S**
KIF	5	EV36:φK1F-R5	R	R	R	S	R	S	R	S	S	**S**
KIF	6	EV36:φK1F-R6	S	T	R	R	R	R	S	S	S	**S**
KIF	7	EV36:φK1F-R7	S	S	S	S	S	S	S	S	S	**S**
KIF	8	EV36:φK1F-R8	S	S	S	S	S	S	S	S	S	**X**

R: Resistant; S: Sensitive; T: Transient (defined initially as being sensitive followed by emergences of a resistant phenotype); X: Not determined; Grey shaded boxes indicate results from clones which were re-challenged with the indicated bacteriophage used to isolate the original putatively resistant clone.

[0251] In other cases, individual clones were readily identified with stable phenotypic resistance to the original challenge phage. However, each of these clones, while resistant to the original wild-type phage, retained sensitivity to at least one of the phages in the panel. Only one was tested per initial phage challenge. In some instances, resistance profiles across phages and for individual phages change dependent on the tested.

Example 5: Identification of lead bacteriophages for a cocktail

[0252] Potential wild-type bacteriophages and combinations therein were evaluated using data from individual wild-type phage host range and resistance results in **Table 2** and **Table 3**, respectively, against the following parameters: 1) phages have collective activity against approximately 90% of the clinical isolate panel or greater; 2) phages result in infection of each by at least 2 phages within the cocktail, intended to ensure sensitivity to the cocktail in the event of resistance to any single bacteriophage; 3) phages do not generate primary resistance in more than 50% of clones isolated, with primary resistance defined as emergence of escape clones after challenge with an individual phage that retain resistance to that particular phage after clonal outgrowth and re-challenge, and lastly 4) phages do not generate cross-resistance to all other phages in the cocktail, defined as emergence of escape clones after initial challenge and clonal outgrowth that display sustained resistance to the initial challenge phage and novel resistance to other phages in the proposed cocktail to which that clone was previously treatment-naïve.

[0253] Wild-type bacteriophage combinations shown in **Table 2** (cumulative host range of approximately 94%) were compared to resistance profiles shown in **Table 3**. From these data, and based on the optimization parameters described above, five candidate bacteriophages have been identified with an approximately 94% host range coverage when tested against a panel of 18 genetically diverse *E. coli* isolates, with each having susceptibility to a minimum of 2 of the 5 phages. Each wild-type phage is predicted to result in less than 50% emergent clones displaying primary resistance and no clones that display resistance to all phages in the proposed cocktail. These data are summarized in **Table 4**, **Table 5**, and **Table 6** and form the basis for preliminary development of a crPhage cocktail. A single crRNA expression construct is designed such that it directs the activity Type I-E and Type I-F CRISPR-Cas3 systems present in approximately 78% of *E. coli* genomes assessed (n=625) to target the host *E. coli* chromosome at multiple highly conserved loci collectively present in >99% of assessed genomes. Each individual crRNA was validated in vitro by transformation into *E. coli* isolates to demonstrate activity of each individual crRNA against targeted *E. coli* sequences. These crRNAs are then assembled into arrays that are

expressed from a bacteriophage genome and processed by endogenous CRISPR-Cas systems to target the host chromosome.

[0254] The proposed wild-type bacteriophages are currently being evaluated for the absence of lysogeny, virulence genes or antibiotic resistance genes as outlined in **Table 1.**

Table 4: Host range of 5 individual bacteriophages for proposed crPhage cocktail.

		ϕECOR71-3	ϕECOR71-7	ϕECOR71-10	ϕECOR14-1	ϕK1F****	# sensitive phages in cocktail
E. coli Strain	ECOR2	1	1	0	0	0	2/5
	ECOR5	1	1	0	0	0	2/5
	ECOR14 (UTI Isolate)	0	0	1	1	0	2/5
	ECOR21	1	1	0	0	0	2/5
	ECOR27	1	1	0	0	0	2/5
	ECOR29	1	1	0	0	0	2/5
	ECOR35	0	0	0	1	1	2/5
	ECOR36	0	0	0	1	1	2/5
	ECOR41	0	0	1	1	1	3/5
	ECOR47	1	1	1	1	0	4/5
	ECOR51	0	1	1	0	0	2/5
	ECOR56	1	1	1	1	0	4/5
	ECOR58	1	1	0	0	0	2/5
	ECOR62 (UTI Isolate)	1	1	1	0	1	4/5
	ECOR64 (UTI Isolate)	1	1	0	0	0	2/5
	ECOR73 (UTI Isolate)	1	1	1	1	1	5/5
	EV36	1	1	1	1	1	5/5
	CFT073 (UPEC Strain)	0	0	0	0	0	0/5
	# strains sensitive to phage	13/18	13/18	8/18	8/18	6/18	17/18
	% of strains sensitive to phage	67%	72%	44%	44%	33%	94.4%

Grey shaded rows demonstrated preliminary host range coverage against uropathogenic *E. coli* isolates.

Table 5: Resistance profiles for 5 individual bacteriophages for a proposed crPhage cocktail. (Revised from **Table 2** based on ECOR71 sensitivity to ΦK1F observed in **Table 3**)

Initial Challenge Phage/ Apparent Resistant Clone			Sensitivity after re-challenge with indicated phage				
Phage		Resistant Clone	ϕECOR71-3	ϕECOR71-7	ϕECOR71-10	ϕECOR14-1	ϕK1F
ϕECOR71-3	ECOR71	1	**Y**	Y	Y	Y	Y
ϕECOR71-3	ECOR71	2	**Y**	Y	Y	Y	Y
ϕECOR71-3	ECOR71	3	**Y**	Y	Y	Y	Y
ϕECOR71-3	ECOR71	4	**Y**	Y	N	N	N
ϕECOR71-3	ECOR71	5	**Y**	Y	Y	Y	Y
ϕECOR71-3	ECOR71	6	**Y**	Y	Y	Y	Y
ϕECOR71-3	ECOR71	7	**Y**	Y	N	Y	Y
ϕECOR71-3	ECOR71	8	**Y**	Y	Y	Y	Y
ϕECOR71-7	ECOR71	1	Y	**Y**	Y	Y	Y
ϕECOR71-7	ECOR71	2	Y	**Y**	Y	Y	Y
ϕECOR71-7	ECOR71	3	Y	**Y**	Y	Y	Y
ϕECOR71-7	ECOR71	4	Y	**Y**	N	Y	Y
ϕECOR71-7	ECOR71	5	Y	**Y**	Y	Y	Y
ϕECOR71-7	ECOR71	6	Y	**Y**	Y	Y	Y

ϕECOR71-7	ECOR71	7	N	**Y**	N	N	Y
ϕECOR71-10	ECOR71	1	Y	Y	**Y**	Y	Y
ϕECOR71-10	ECOR71	2	Y	Y	**Y**	Y	Y
ϕECOR71-10	ECOR71	3	Y	Y	**N**	Y	Y
ϕECOR71-10	ECOR71	4	Y	Y	**Y**	Y	Y
ϕECOR71-10	ECOR71	5	Y	Y	**Y**	Y	Y
ϕECOR14-1	ECOR71	1	Y	Y	Y	**Y**	Y
ϕECOR14-1	ECOR71	2	Y	Y	Y	**Y**	Y
ϕECOR14-1	ECOR71	3	Y	Y	Y	**Y**	Y
ϕECOR14-1	ECOR71	4	Y	Y	Y	**Y**	Y
ϕECOR14-1	ECOR71	5	Y	Y	Y	**Y**	Y
ϕECOR14-1	ECOR71	6	Y	Y	Y	**Y**	Y
ϕECOR14-1	ECOR71	7	Y	Y	Y	**Y**	Y
ϕK1F	EV36	1	Y	Y	Y	Y	**Y**
ϕK1F	EV36	2	Y	Y	Y	Y	**Y**
ϕK1F	EV36	3	Y	Y	Y	Y	**Y**
ϕK1F	EV36	4	Y	Y	Y	Y	**Y**
ϕK1F	EV36	5	N	Y	Y	Y	**Y**
ϕK1F	EV36	6	Y	N	Y	Y	**Y**
ϕK1F	EV36	7	Y	Y	Y	Y	**Y**

Grey shaded boxes indicate results from clones which were re-challenged with the indicated bacteriophage used to isolate the original putatively resistant clone.

Table 6: Summary data for 5 individual bacteriophages for proposed crPhage cocktail.

Component name	**Phage**	**Host Range**		**Resistance Data**			
		# s	**% s**	**# Primary Resistant Clones***	**# Total Clones***	**% Primary Resistance***	**Min. # phages per ****
LBx-UT01-ECΦ1	ϕECOR71-3	12/18	66.7%	0	8	0.0%	2
LBx-UT01-ECΦ2	ϕECOR71-7	13/18	72.2%	0	7	0.0%	2
LBx-UT01-ECΦ3	ϕECOR71-10	8/18	44.4%	1	7	14.3%	4
LBx-UT01-ECΦ4	ϕECOR14-1	8/18	44.4%	1	8	12.5%	5
LBx-UT01-ECΦ5	ϕK1F	6/18	33.3%	0	7	0.0%	4
LBx-UT01	**Cocktail**	**17/18**	**94.4%**				

*For resistance to primary challenge phage, excluded single data points where phage sensitivity data could not be determined (N=3).

**For cocktail susceptibility, excluded data for 6 resistant isolate as some individual phage library data points could not be determined (N=6).

[0255] The proposed crPhage cocktail is further summarized in **Table 7** below:

Table 7: Summary of proposed preliminary crPhage cocktail.

crPhage name	**Phage type**	**Replication host**	**Isolation source**	**Proposed concentration**
LBx-UT01-ECΦ1	Obligate lytic	*E. coli* strain ECOR71	Durham, NC Wastewater Treatment Plant	$10^9 – 10^{11}$ PFU/mL
LBx-UT01-ECΦ2	Obligate lytic	*E. coli* strain ECOR71	Durham Wastewater Treatment Plant	$10^9 – 10^{11}$ PFU/mL

crPhage name	Phage type	Replication host	Isolation source	Proposed concentration
LBx-UT01-ECΦ3	Obligate lytic	*E. coli* strain ECOR71	Durham Wastewater Treatment Plant	$10^9 - 10^{11}$ PFU/mL
LBx-UT01-ECΦ4	Obligate lytic	*E. coli* strain ECOR71	Durham Wastewater Treatment Plant	$10^9 - 10^{11}$ PFU/mL
LBx-UT01-ECΦ5	Obligate lytic	*E. coli* strain EV36	External source (K1F phage)	$10^9 - 10^{11}$ PFU/mL

[0256] The composition of the crRNA arrays for the proposed crPhage cocktail are summarized in **Table 8** below:

Table 8: Summary of individual crRNA information.

Array	crRNA name	Compatible Cas system	Target gene name	Target gene function	Target sequence	PAM
LBx-UT01-EC-IEa	LBx-UT01-EC-IE_spacer1	Type I-E	*acpP*	Lipid biosynthesis	SEQ ID NO. 3 ATTCCGGACGAAGAAGCTGAGAAAATC ACCAC	GAG
LBx-UT01-EC-IEa	LBx-UT01-EC-IE_spacer2	Type I-E	*gapA1*	Glycolysis	SEQ ID NO. 4 TATCAACGGTTTTGGCCGTATCGGTCGC ATTG	AGG
LBx-UT01-EC-IEa	LBx-UT01-EC-IE_spacer3	Type I-E	*secY1*	Secretory	SEQ ID NO. 5 TGCAAACTCTGATGATGTCCAGTCAGTA TGAG	AAG
LBx-UT01-EC-IEa	LBx-UT01-EC-IE_spacer4	Type I-E	*tsf*	Translation	SEQ ID NO. 6 AAAATGGTTGAAGGCCGCATGAAGAAA TTCAC	GAG
LBx-UT01-EC-IFa	LBx-UT01-EC-IF_spacer1	Type I-F	*csrA*	Glycolysis	SEQ ID NO. 7 AGGCTGAAAAATCCCAGCAGTCCAGTT ACTA	CC
LBx-UT01-EC-IFa	LBx-UT01-EC-IF_spacer2	Type I-F	*ftsA*	Cell division	SEQ ID NO. 8 GTATTATTCGACGGCGGTGGGATTGCTT CAC	CC
LBx-UT01-EC-IFa	LBx-UT01-EC-IF_spacer3	Type I-F	*fusA*	Translation	SEQ ID NO. 9 AAAGCTGACCAGGAAAAAATGGGTCTG GCTC	CC
LBx-UT01-EC-IFa	LBx-UT01-EC-IF_spacer4	Type I-F	*secY*	Secretory	SEQ ID NO. 10 GCTTTATGTGTTACTCTATGCGTCTGCA ATC	CC

Example 6: Optimizing the CRISPR array for lethality

[0257] To develop effective CRISPR-enhanced bacteriophages for use against *C. difficile*, various modifications were made to the CRISPR array.

[0258] **Figure 2A** shows a schematic diagram of the linear alignment of the identified CRISPR-Cas systems from within five strains of *C. difficile* with identification of the various CRISPR-Cas constituent components. The CRISPR-Cas system operon structures for strains 630 and R20291 are further diagrammed in **Figure 2B**.

[0259] Initial crRNA arrays within the *C. difficile* targeting crPhages comprised a native leader sequence for a CRISPR array from *C. difficile* 630 or R20291 that were combined with the consensus repeat sequences from the native CRISPR arrays found in *C. difficile*. The spacer sequence was defined by the consensus PAM sequence for the endogenous Type I-B systems in *C. difficile* and complementary to three selected *C. difficile* target host genes including: *dmsB*, *phi1*, and *phi2*. Two additional configurations were tested including: a crRNA array using an endogenous enolase promotor in lieu of the native leader sequence and a crRNA array wherein the second

repeat sequences was changed to facilitate IDT synthesis (R2 A>G). The configuration of the CRISPR-RNA for engineering the bacteriophage is thus leader-repeat-spacer-repeat. The engineered bacteriophage was created through homologous recombination in a native bacterial host as a lysogen using democratized plasmids for genetic manipulation of Clostridia.

[0260] *Clostridium difficile* strains 630 and R20291 were grown over night in brain heart infusion (BHI) medium at 37° C in an anaerobic environment and then sub-cultured into 5 mL of fresh BHI at a 1% (vol/vol) inoculum. *C. difficile* strains were then incubated until an OD of 0.2 before beginning a CFU reduction assay. All preparation and handling of bacteriophages was performed as described previously. To each culture, a total MOI of 10 wild-type or CRISPR phage lysate was added with a final concentration of 10 mM $MgCl_2$ and 1 mM $CaCl_2$. OD and CFU were then monitored over the course of 6 hours.

[0261] Similarly, alterations to the nucleotide composition of the repeat sequence within the CRISPR array had marginal impact on the overall lethality of the crRNA. *C. difficile* strain 630 consensus SEQ ID NO: 11 of 5'-GTTTTATATTAACTATATGGAATGTAAAT-3' was varied with single point nucleotide mutations for crRNAs comprising a native leader sequence for a CRISPR array from *C. difficile* 630 or R20291 or an endogenous enolase gene promoter and spacers complementary for *dmsB* or *int C. difficile* host genes. Changes in the repeat sequence are anticipated to alter the hairpin secondary structure which affects the overall crRNA activity. **Table 9** below summarizes the changes in the crRNA lethality for the various constructs.

Table 9: Alterations in the crRNA array repeat affect crRNA lethality.

Spacer	Promoter	Length	Repeat two (Alterations underlined)	Δlog
dmsB	Leader	36	SEQ ID NO. 12 GTTTTAGATTAACTATATGGAATGTAAAT	0.15
dmsB	Enolase	36	SEQ ID NO. 12 GTTTTAGATTAACTATATGGAATGTAAAT	0.01
dmsB	Leader	36	SEQ ID NO. 13 GTTTTAGATTAACTATATGGAATGTAA<u>G</u>T	0.00
int	Leader	36	SEQ ID NO. 14 GTTTTAGATTAACTATATGGAATGTAA<u>G</u>T	0.44
int	Leader	36	SEQ ID NO. 15 GTTTTAGATTAACTAT<u>G</u>TGGAATGTAAAT	
int	Leader	34	SEQ ID NO. 16 GTTTTAGATTAACTATATGGAATGTAA<u>G</u>T	
int	Leader	35	SEQ ID NO. 17 GTTTTAGATTAACTATATGGAATGTAA<u>G</u>T	
int	Leader	37	SEQ ID NO. 18 GTTTTAGATTAACTATATGGAATGTAA<u>G</u>T	
int	Leader	38	SEQ ID NO. 19 GTTTTAGATTAACTATATGGAATGTAA<u>G</u>T	

Example 7: crPhages have enhanced killing activity

[0262] CRISPR-enhanced bacteriophages against *E. coli* and *C. difficile* were developed from distinct obligate lytic bacteriophages that contain an identical DNA sequence encoding a functional self-targeting CRISPR RNA embedded in the wild-type phage genome. As seen in **Figure 3A**, treatment of an *E. coli* culture containing 10^{10} bacterial cells with the native, unmodified phage resulted in a 5-log reduction of bacterial cells by lytic activity of the phage. Treatment with the modified crPhage results in a further approximate 5-log improvement in killing activity of the *E. coli*. In some case, the improvement in anti-microbial activity is independent of the innate lytic activity of the phage and is a result of the anti-microbial activity of the CRISPR array itself. Treatment with the CRISPR array shows an approximate 7-log reduction in the bacterial cell population as seen in **Figure 3B**.

[0263] Similarly, treatment of a *C. difficile* culture containing approximately 10^8 bacterial cells with the native, unmodified phage resulted in almost an approximate 1.5-log reduction of bacterial cells by lytic activity of the phage. An additional 1-log reduction in killing activity of the bacterial cells was seen when treated with the modified crPhage as seen in **Figure 4A**. In some cases, the improvement in anti-microbial activity is independent of the innate lytic activity of the phage but is instead a result of the anti-microbial activity of the CRISPR array itself. Treatment with the CRISPR array only showed an approximate 3.5-log reduction in the *C. difficile* cell population as seen in **Figure 4B.**

Example 8: crPhages have expanded host range and associated killing activity

[0264] Identical concentrations of wild-type or crPhage were spotted onto an agar plate seeded with *C. difficile* strain 069 to identify crPhage sensitivity. Presence of phage plaques on the *C. difficile* bacterial lawn plate was used to identify *C. difficile* strains that were sensitive to killing by crPhage ϕCD146 but were insensitive to the wild-type phage ϕCD146 as seen in **Figure 5**. Cell death by the crPhage ϕCD146 but not the wild-type phage ϕCD146 is indicative of bacterial cell death being independent of phage based lytic activity. Instead, it is representative of bacterial cell death by the CRISPR array to *C. difficile* strains that are natively insensitive to lytic phage ϕCD146 infection. The crRNA array used targeted *R20291-3*.

Example 9: crPhages have enhanced killing activity over a wide range of *C. difficile* strains

[0265] The ability of a crPhage to be effective over a panel of different strains for *C. difficile* was determined. The CRISPR-RNA was designed by incorporating a native leader sequence for a CRISPR array from *Clostridium difficile* R20291 and then combined with the consensus repeat

sequences from the native CRISPR arrays found in *C. difficile*. The spacer sequence was defined by the consensus PAM sequence for the endogenous Type I-B systems in *C. difficile* and the length was determined by the most represented spacer length found in endogenous CRISPR arrays in *C. difficile*. The configuration of the CRISPR-RNA for engineering the bacteriophage is thus leader-repeat-spacer-repeat. The engineered bacteriophage was created through homologous recombination in a native bacterial host as a lysogen using democratized plasmids for genetic manipulation of *Clostridia*.

[0266] *Clostridium difficile* strains were grown over night in brain heart infusion (BHI) medium at 37° C in an anaerobic environment and then sub-cultured into 5 mL of fresh BHI at a 1% (vol/vol) inoculum. *C. difficile* strains were then incubated until an OD of 0.1 for the OD reduction assay and OD of 0.2 for the CFU reduction assay. All preparation and handling of bacteriophages was performed as described previously. To each culture, a total MOI of 10 wild-type or CRISPR phage lysate was added with a final concentration of 10 mM $MgCl_2$ and 1 mM $CaCl_2$. OD and CFU were then monitored over the course of 6 hours.

[0267] Comparison of wild-type phages against two crPhage variants was measure by optical density (OD600nm) against *C. difficile* strains 1-22 for crPhage ϕCD146 as shown in **Figure 6A- Figure 6V**. crPhage ϕCD24-2, an additional variant, was likewise tested against *C. difficile* strains 23-25 as shown in **Figure 7A- Figure 7C.**

[0268] A subset of selected strains were further examined using colony forming unit (CFU) enumeration to compare the bacterial killing activity of crPhage ϕCD146 and 2. CFU assays for *C. difficile* strains 1-4 against wild-type and crPhage ϕCD146 are shown in **Figure 8A- Figure 8D**. **Figure 9** shows a CFU assay for strain CD19 against wild-type and crPhage ϕCD24-2. A combinatorial comparison of crPhage ϕCD146 and crPhage ϕCD24-2 was tested in **Figure 10**. A CFU assay of crPhage ϕCD146 and crPhage ϕCD24-2 anti-bacterial activity was conducted for each crPhage individually as well as the when administered together. Co-administration showed improved killing efficacy as compared to treatment with a combination of both wild-type phages together. Although wild-type phage ϕCD24-2 demonstrated the stronger anti-bacterial killing activity as compared to wild-type phage ϕCD146, when administered together, the killing combined efficacy significantly diminished. This is suggestive of the wild-type ϕCD146 phage potentially interfering with the activity of wild-type phage ϕCD24-2. However, the combination of crPhage ϕCD146 and crPhage ϕCD24-2 together show equal to slightly improved bacterial killing ability as compared to that of wild-type phage ϕCD24-2 by itself.

[0269] To evaluate the effects of the MOI against bacterial growth, cultures of *C. difficile* strain R20291were grown and inoculated with wild-type ϕCD146 or crPhage ϕCD146 at various MOIs

from 0 to 16. The combined effects of lytic activity and CRISPR array activity against *R20291-3* upon the targeted host bacterium were evaluated. For both wildtype and crPhage, higher MOIs led to larger log reduction in the *C. difficile*. However, the crPhage ϕCD146 showed a consistent improvement in reducing the bacterial population compared to the wild-type phage ϕCD146 at all tested MOIs as showing in **Figure 11**.

Example 10: CRISPR enhanced crPhages in silico design of CRISPR array for overcoming resistance rates

[0270] In order to determine potential resistance rates to CRISPR-based targeting, a mathematical model was developed to determine the frequency of mutation at any given potential target site of approximately 32 base pairs in a targeted genome:

[0271] $$\#\ of\ cells\ with\ mutations\ conferring\ survival =$$

$$\left(1-\left(1-\left(\frac{\frac{mutation\ rate}{genome}}{generation}*\frac{genome}{\#\ basepairs}*\#\ generations\right)^{32}\right)^{n}\right)*(CFU\ load)$$

[0272] Calculations estimating escape mutants in populations assumed the general rate of mutation for any given gene is 1 in 1000 mutations per genome per generation, that a typical bacterial genome is approximately $5x10^6$ base pairs in length, and that the total number of generations is estimated as the total length of the infection divided by the doubling time of organism. Thus, this equation estimates the total number of surviving cells as those that acquire mutations over the course of the infection in all potential spacer targets assuming an independent mutational rate for a given genome multiplied by the total number of genomes (e.g. cells) in the population.

[0273] In silico prediction of number of resistant clones that emerge over time due to target site mutation as a function of the number of independent genes targeted by crRNAs. These models assume highly conservative assumptions that (1) the mutational rate is independent of gene target and (2) that all 32 bases of crRNA match for activity. Two sets of assumptions were tested to understand the impact of number of independently targeted genes on potential resistance to CRISPR. Two types of infection were modeled: acute infection rising to a total burden of 10^{10} CFU by doubling every 6 hours as seen in **Figure 12A** or an aggressive infection to a total burden of 1014 CFU by doubling every 20 minutes as seen in **Figure 12B**. Under these assumptions, both models show that 3 independent gene targets are sufficient to prevent mutational escape up to 28 days of infection length.

[0274] These estimates are considered to be conservative as: (1) crRNA targets are within highly conserved regions of essential genes and thus presumably be less likely to mutate than calculated here and (2) this model assumes that any single mutation in the 32 base pair crRNA target eliminates activity, contrary to data demonstrating that crRNAs tolerate 1 or more mismatches at its target site. CRISPR arrays were designed to express 4 independent crRNAs against 4 independent targets to ensure that resistance due to loss of CRISPR targets remains unlikely. To identify targets with the greatest prevalence amongst various *E. coli* strains, the strain coverage for each spacer target was analyzed as shown in **Figure 13**. crRNA array targets include the following genes in order of highest to lowest percentage of strain coverage: *Tsf* (100%), *cpP* (99%), *gapA* (99%), *infA* (99%), *secY* (99%), *secY'2* (99%), *csrA* (99%), *trmD* (99%), *ftsA* (99%), *musG* (99%), *fusA'2* (99%), *fusA* (98%), *glyQ* (98%), *eno* (95%), *gapA'2* (91%), *eno'2* (89%), and *musG'2* (73%).

[0275] A series of individual Type I-E crRNAs targeted to conserved regions of the *E. coli* genome were constructed as seen in **Figure 14**. crRNA targets include the following genes: *acpP, csra, eno, fusA, gapA, glyQ, infA, musG, secY, trmD,* and *Tsf.* These individual Type I-E crRNAs constructs were tested by transformation of crRNA expression constructs into *E. coli* cells constitutively expressing a Type I-E CRISPR-Cas3 system with transformation efficiency calculated as transformants per microgram of input DNA and each data point is a single replicate from three independent experiments. Each tested crRNA resulted in similar observed levels of lethality when transformed into recipient *E. coli*. From these spacers, 4 Type I-E crRNAs were selected from each subset to assemble into final arrays.

Example 11: crPhages enhanced with LeuO transcriptional activator

[0276] CRISPR-enhanced bacteriophages against *E. coli* were developed as a cocktail of up to three distinct obligate lytic bacteriophages that contain an identical DNA sequence encoding a functional self-targeting CRISPR RNA (crRNA cassette) embedded in the wild-type phage genome. Bacteriophages were engineered by one of two methods: (1) homologous recombination in *E. coli* cells with active phage infection or (2) by transformation of engineered phage DNA assembled outside of the cell to reconstitute active phages with engineered genomes. Each phage was engineered with a similar crRNA cassette that contained two elements: (1) a LeuO transcription factor gene derived from *E. coli* in front of a synthetic promoter, and (2) a repeat-spacer-repeat encoding a crRNA targeting the *ftsA* gene in front of a synthetic promoter. Three bacteriophage constructs were engineered: crT4, crT7, and crT7m. All three bacteriophage constructs are schematically illustrated in **Figure 15**. The crRNA expression cassette used in the engineered various crPhages contains a single crRNA as shown in **Table 10** below:

Table 10. crRNA expression cassette.

Array	crRNA name	Compatible Cas system	Target gene name	Target gene function	Target sequence	PAM
ftsA	ftsA	Type I-E	*ftsA*	Cell division	SEQ ID NO. 20 AGGGTCTCACCAACTCGACGAGTCAGAATCAG	AAG

[0277] Bacteriophage crT4 was engineered by deleting the *hoc* gene and replacing with a crRNA cassette. Bacteriophage crT7 was engineered by deleting *gp0.7*, *gp4.3*, *gp4.5* and *gp4.7* and replacing with a crRNA cassette. Bacteriophage crT7m was engineered by deleting *gp0.6*, *gp0.65*, *gp0.7*, *gp4.3*, and *gp4.5* and replacing with a crRNA cassette. Based on the observation that all phages were successfully engineered after these deletions, it was concluded that these early phage genes were non-essential for phage survival. Details of these engineered bacteriophages are summarized in **Table 11** below:

Table 11. Summary of engineered crPhages.

crPhage name	Phage type	Replication host	Isolation source
crT7m	Obligate lytic	*E. coli* B-strain	Commercial (ATCC T7m)
crT4	Obligate lytic	*E. coli* B-strain	Commercial (ATCC T4)
crT7	Obligate lytic	*E. coli* B-strain	Commercial (ATCC T7)

[0278] Upon DNA transduction during infection, LeuO is expressed from the phage genome and subsequently upregulate expression of the endogenous Type I-E CRISPR-Cas3 operon in *E. coli*. Concurrently, the synthetic *ftsA*-targeting crRNA is expressed from the phage genome that is recognized and processed by the endogenous Type I-E CRISPR-Cas3 protein complex. This crRNA is then loaded onto a CRISPR-Cas3 complex and thereby directs the targeting and degradation of target bacterial DNA.

Example 12: Prevalence and distribution of CRISPR-Cas systems in *E. coli*

[0279] There are a diverse range of CRISPR-Cas systems types and subtypes, with the majority (>60%) of discovered systems belonging to the Type I group that shares the unique feature of having the Cas3 signature nuclease. CRISPR-Cas3 systems are unique in that they generate single-strand nicks, followed by processive exonucleolytic degradation of targeted DNA. *E. coli* CRISPR-Cas systems belong to two distinct subtypes, Type I-E and Type I-F, that use this signature Cas3 nuclease for degradation.

[0280] To determine an approximate distribution of CRISPR-Cas systems in *E. coli*, 625 publicly available *E. coli* genomes were analyzed, spanning a diversity of strains including: uropathogenic *E. coli* (UPEC), Shiga toxin producing *E. coli*, (STEC), O157:H7 serotype *E. coli*, diarrheagenic *E.*

coli (DEC), non-157 O antigen type *E. coli*, and enteropathogenic *E. coli* (EPEC). Each genome was scanned for operons with similarity to canonical Type I-E or Type I-F *E. coli* CRISPR-Cas systems. **Figure 16A** shows the relative amounts of each of these *E. coli* genomes**. Figure 16B** shows that approximately 78% (487/625) of all strains already have the complete CRISPR-Cas3 system, either type I-E or type I-F. The proposed product exploits the presence of CRISPR-Cas3 proteins in the majority of *E. coli* by delivering only the guides and accessories required to activate endogenous CRISPR-Cas3 systems to target the genome.

Example 13: Presence of LeuO binding sites near Cas operon in *E. coli*

[0281] To determine the prevalence of LeuO binding sites near the Cas operon in *E. coli* genome, 628 E.coli genomes were downloaded directly from NCBI with accession numbers, spanning a diversity of s including: uropathogenic *E. coli* (UPEC), Shiga toxin producing *C.* (STEC), various O-antigen: H-antigen serotype *E. coli*, diarrheagenic *E. coli* (DEC), and enteropathogenic *E. coli* (EPEC). Genomes were then queried for genes annotated as "*CasB*". The *CasB* coding sequence and 5 kb flanking on either side were extracted for further annotation. Complete Cascade operons were determined by visual inspection for truncated genes in the *CasABCDE* or *Cas3* genes. There were 401 intact *Cascade-Cas3* operons detected (64% of all genomes queried). Next, 200-400 nucleotides upstream of *Cas3* and downstream of *Cas3* were extracted for analysis, respectively. Candidate LeuO binding sequences were retrieved and aligned to create a consensus sequence. Next, the individual and consensus sequences were queried against the *Cas3* upstream and downstream sequences with a threshold nucleotide identity of 60%. Collectively, candidate LeuO binding sites were observed in 88.5% of strains containing an intact *Cacade-Cas3* operon. **Table 12** summarizes the search analysis of the *E. coli* genomes below:

Table 12. Summary of LeuO prevalence in *E. coli* genomes.

***E. coli* Strain Designation**	**Accession No.**	**CRISPR**	**Cascade**	**Cas3-Cascade**	**LeuO site**
DEC1A	NZ_AIEV00000000				
DEC1B	NZ_AIEW00000000				
DEC1C	NZ_AIEX00000000				
DEC1D	NZ_AIEY00000000				
DEC1E	NZ_AIEZ00000000				
DEC2B	NZ_AFJB00000000				
DEC2C	NZ_AIFB00000000				
DEC2D	NZ_AIFC00000000				
DEC2E	NZ_AIFD00000000				
DEC3A	NZ_AIFE00000000	Yes		Yes	Yes
DEC3B	NZ_AIFF00000000	Yes		Yes	Yes
DEC3C	NZ_AIFG00000000	Yes		Yes	Yes

DEC3D	NZ_AIFH00000000	Yes		Yes	Yes
DEC3E	NZ_AIFI00000000	Yes		Yes	Yes
DEC3F	NZ_AIFJ00000000	Yes		Yes	Yes
DEC4A	NZ_AIFK00000000	Yes		Yes	Yes
DEC4B	NZ_AIFL00000000	Yes		Yes	Yes
DEC4C	NZ_AIFM00000000	Yes		Yes	Yes
DEC4D	NZ_AIFN00000000	Yes		Yes	Yes
DEC4E	NZ_AIFO00000000	Yes		Yes	Yes
DEC4F	NZ_AIFP00000000	Yes		Yes	Yes
DEC5A	NZ_AIFQ00000000	Yes		Yes	Yes
DEC5B	NZ_AIFR00000000	Yes		Yes	Yes
DEC5C	NZ_AIFS00000000	Yes		Yes	Yes
DEC5D	NZ_AIFT00000000	Yes		Yes	Yes
DEC5E	NZ_AIFU00000000	Yes		Yes	Yes
DEC6A	NZ_AIFV00000000				
DEC6B	NZ_AIFW00000000				
DEC6E	NZ_AIFZ00000000	Yes		Yes	
DEC7A	NZ_AIGA00000000	Yes		Yes	Yes
DEC7B	NZ_AIGB00000000				
DEC7C	NZ_AIGC00000000	Yes		Yes	Yes
DEC7D	NZ_AIGD00000000	Yes		Yes	Yes
DEC7E	NZ_AIGE00000000	Yes		Yes	Yes
DEC8A	NZ_AIGF00000000	Yes		Yes	Yes
DEC8B	NZ_AIGG00000000	Yes		Yes	Yes
DEC8C	NZ_AIGH00000000	Yes		Yes	Yes
DEC8D	NZ_AIGI00000000	Yes		Yes	Yes
DEC8E	NZ_AIGJ00000000	Yes		Yes	Yes
DEC9A	NZ_AIGK00000000	Yes		Yes	Yes
DEC9B	NZ_AIGL00000000	Yes		Yes	Yes
DEC9C	NZ_AIGM00000000	Yes		Yes	Yes
DEC9D	NZ_AIGN00000000	Yes		Yes	Yes
DEC9E	NZ_AIGO00000000	Yes		Yes	Yes
DEC10E	NZ_AIGT00000000	Yes		Yes	Yes
DEC10F	NZ_AIGU00000000	Yes		Yes	Yes
DEC11A	NZ_AIGV00000000	Yes		Yes	Yes
DEC11B	NZ_AIGW00000000	Yes		Yes	Yes
DEC11C	NZ_AIGX00000000	T			
DEC11D	NZ_AIGY00000000	Yes		Yes	Yes
DEC11E	NZ_AIGZ00000000	Yes		Yes	Yes
DEC12A	NZ_AIHA00000000	Yes		Yes	Yes
DEC12B	NZ_AIHB00000000	Yes		Yes	Yes
DEC12C	NZ_AIHC00000000	Yes		Yes	Yes
DEC12D	NZ_AIHD00000000	Yes		Yes	Yes
DEC12E	NZ_AIHE00000000	Yes		Yes	Yes
DEC13A	NZ_AIHF00000000	Yes		Yes	Yes

DEC13B	NZ_AIHG00000000	Yes		Yes	Yes
DEC13C	NZ_AIHH00000000	Yes		Yes	Yes
DEC13D	NZ_AIHI00000000	Yes		Yes	Yes
DEC13E	NZ_AIHJ00000000	Yes		Yes	Yes
DEC14A	NZ_AIHK00000000	Yes		Yes	Yes
DEC14B	NZ_AIHL00000000	Yes		Yes	
DEC14C	NZ_AIHM00000000	Yes		Yes	Yes
DEC14D	NZ_AIHN00000000	Yes		Yes	Yes
DEC15A	NZ_AIHO00000000	Yes		Yes	Yes
DEC15B	NZ_AIHP00000000	Yes		Yes	Yes
DEC15C	NZ_AIHQ00000000	Yes		Yes	Yes
DEC15D	NZ_AIHR00000000	Yes		Yes	Yes
DEC15E	NZ_AIHS00000000	Yes		Yes	Yes
EPECa12	NZ_AKNH00000000	Yes		Yes	Yes
EPECa14	NZ_ADUN00000000	Yes		Yes	Yes
EPEC C342-62	NZ_AKNI00000000	Yes		Yes	Yes
O5:K4(L):H4 str. ATCC 23502	NZ_CAPL00000000				
O6:H16:CFA/II str. B2C	NZ_AUZS00000000	Yes	Yes		Yes
O6:H16 str. 99-3165	NZ_JHJW00000000	Yes	Yes		Yes
O6:H16 str. F5656C1	NZ_JHJU00000000	Yes	Yes		Yes
O08	NZ_AOGM00000000	Yes		Yes	Yes
O10:K5(L):H4 str. ATCC 23506	NZ_CAPK00000000				
O15:H18 str. K1516	NZ_JHJE00000000	Yes		Yes	Yes
O25:NM str. E2539C1	NZ_JHJV00000000	Yes		Yes	
O26:H1 str. 2009C-4747	NZ_JHGM00000000	Yes		Yes	Yes
O26:H11 str. 2009C-3612	NZ_JHGZ00000000	Yes		Yes	Yes
O26:H11 str. 2011C-3655	NZ_JHLN00000000	Yes		Yes	Yes
O28ac:NM str. 02-3404	NZ_JHNY00000000				
O32:H37 str. P4	NZ_AJQW00000000	Yes		Yes	
O39:NM str. F8704-2	NZ_JHHJ00000000	Yes		Yes	Yes
O45:H2 str. 01-3147	NZ_JHOA00000000				
O45:H2 str. 03-EN-705	NZ_AGTK00000000				
O45:H2 str. 2009C-3686	NZ_JHGY00000000				
O45:H2 str. 2009C-4780	NZ_JHGJ00000000				
O45:H2 str. 2010C-3876	NZ_JHFI00000000				
O45:H2 str. 2010C-4211	NZ_JASS00000000				
O55:H7 str. 06-3555	NZ_JHNL00000000	Yes		Yes	Yes
O55:H7 str. 3256-97	NZ_AEUA00000000	Yes		Yes	Yes
O55:H7 str. USDA 5905	NZ_AEUB00000000	Yes		Yes	Yes
O69:H11 str. 06-3325	NZ_JHNP00000000	Yes		Yes	Yes
O69:H11 str. 07-3763	NZ_JASN00000000	Yes		Yes	Yes
O69:H11 str. 07-4281	NZ_JHLA00000000	Yes		Yes	Yes
O69:H11 str. 08-4661	NZ_JHHG00000000	Yes		Yes	Yes
O69:H11 str. 2009C-3601	NZ_JHHA00000000	Yes		Yes	Yes
O78:H12 str. 00-3279	NZ_JFBE00000000				

O79:H7 str. 06-3501	NZ_JHNM00000000	Yes		Yes	Yes
O81:NM str. 02-3012	NZ_JHNZ00000000				
O91:H21 str. B2F1	NZ_AGTI00000000	Yes		Yes	Yes
O104:H4 str. 11-02030	NZ_AMVR00000000	Yes		Yes	Yes
O104:H4 str. 11-02033-1	NZ_AMVS00000000	Yes		Yes	Yes
O104:H4 str. 11-02092	NZ_AMVT00000000	Yes		Yes	Yes
O104:H4 str. 11-02093	NZ_AMVU00000000	Yes		Yes	Yes
O104:H4 str. 11-02281	NZ_AMVV00000000	Yes		Yes	Yes
O104:H4 str. 11-02913	NZ_AMVX00000000	Yes		Yes	Yes
O104:H4 str. 11-03439	NZ_AMVY00000000	Yes		Yes	Yes
O104:H4 str. 11-03943	NZ_AMWA00000000	Yes		Yes	Yes
O104:H4 str. 11-04080	NZ_AMVZ00000000	Yes		Yes	Yes
O104:H4 str. E112/10	NZ_AHAV00000000	Yes		Yes	Yes
O104:H4 str. GOS1	NZ_AFWO00000000	Yes		Yes	Yes
O104:H4 str. GOS2	NZ_AFWP00000000	Yes		Yes	Yes
O104:H4 str. H112180280	NZ_AFPN00000000	Yes		Yes	Yes
O104:H4 str. H112180282	NZ_AFSO00000000				
O104:H4 str. ON2010	NZ_AHZE00000000				
O104:H4 str. ON2011	NZ_AHZF00000000				
O104:H4 str. TY-2482	NZ_AFVR00000000	Yes		Yes	Yes
O104:H4 str. TY-2482	NZ_AFOG00000000				
O104:H21 str. 94-3025	NZ_JHJZ00000000				
O111:H11 str. CVM9455	NZ_AKAX00000000	Yes		Yes	Yes
O111:H11 str. CVM9534	NZ_AJVS00000000	Yes		Yes	Yes
O111:H11 str. CVM9545	NZ_AJVT00000000	Yes		Yes	Yes
O111:H11 str. CVM9553	NZ_AKAY00000000	Yes		Yes	Yes
O111:H8 str. CVM9570	NZ_AJVU00000000	Yes		Yes	Yes
O111:H8 str. CVM9574	NZ_AJVV00000000	Yes		Yes	Yes
O111:H8 str. CVM9602	NZ_AKAV00000000	Yes		Yes	Yes
O111:H8 str. CVM9634	NZ_AKAW00000000	Yes		Yes	Yes
O111:H11 str. CFSAN001630	NZ_AMXP00000000	Yes		Yes	Yes
O111:NM str. 01-3076	NZ_JFGU00000000	Yes		Yes	Yes
O111:NM str. 03-3484	NZ_JHNU00000000	Yes		Yes	Yes
O111:NM str. 04-3211	NZ_JHNS00000000	Yes		Yes	Yes
O111:NM str. 08-4487	NZ_JHKU00000000	Yes		Yes	Yes
O111:NM str. 2009C-4006	NZ_JHGU00000000	Yes		Yes	Yes
O111:NM str. 2009C-4052	NZ_JHGS00000000	Yes		Yes	Yes
O111:NM str. 2010C-3053	NZ_JHFZ00000000	Yes		Yes	Yes
O111:NM str. 2010C-3977	NZ_JHFG00000000	Yes		Yes	Yes
O111:NM str. 2010C-4086	NZ_JHFF00000000	Yes		Yes	Yes
O111:NM str. 2010C-4221	NZ_JHFE00000000	Yes		Yes	Yes
O111:NM str. 2010C-4592	NZ_JHMY00000000	Yes		Yes	Yes
O111:NM str. 2010C-4622	NZ_JHMX00000000	Yes		Yes	Yes
O111:NM str. 2010C-4715	NZ_JHMW00000000	Yes		Yes	Yes
O111:NM str. 2010C-4735	NZ_JHMU00000000	Yes		Yes	Yes

O111:NM str. 2010C-4746	NZ_JHMT00000000	Yes		Yes	Yes
O111:NM str. 2011C-3170	NZ_JHMA00000000	Yes		Yes	Yes
O111:NM str. 2011C-3362	NZ_JHLW00000000	Yes		Yes	Yes
O111:NM str. 2011C-3573	NZ_JHLQ00000000	Yes		Yes	Yes
O111:NM str. 2011C-3632	NZ_JHLO00000000	Yes		Yes	Yes
O111:NM str. 2011C-3679	NZ_JHLM00000000	Yes		Yes	Yes
O111:NM str. K6904	NZ_JHHN00000000	Yes		Yes	Yes
O111:NM str. K6908	NZ_JHHM00000000	Yes		Yes	Yes
O111:NM str. K6915	NZ_JHHL00000000	Yes		Yes	Yes
O113:H21 str. CL-3	NZ_AGTH00000000	Yes		Yes	Yes
O118:H16 str. 07-4255	NZ_JASP00000000	Yes		Yes	Yes
O119:H6	NZ_BBUR00000000				
O119:H6	NZ_BBUT00000000				
O119:H6	NZ_BBUU00000000				
O119:H6	NZ_BBUS00000000				
O121:H7 str. 2009C-3299	NZ_JHHC00000000	Yes		Yes	Yes
O121:H19 str. 03-3227	NZ_JHNX00000000				
O121:H19 str. 06-3003	NZ_JHNR00000000				
O121:H19 str. 06-3822	NZ_JHNH00000000				
O121:H19 str. 2009C-4050	NZ_JHGT00000000				
O121:H19 str. 2009C-4659	NZ_JHGN00000000				
O121:H19 str. 2009C-4750	NZ_JHGL00000000				
O121:H19 str. 2009EL1302	NZ_JHGH00000000				
O121:H19 str. 2009EL1412	NZ_JHGG00000000				
O121:H19 str. 2010C-3609	NZ_JHFM00000000				
O121:H19 str. 2010C-3794	NZ_JHFL00000000				
O121:H19 str. 2010C-3840	NZ_JHFK00000000				
O121:H19 str. 2010C-4254	NZ_JHFC00000000				
O121:H19 str. 2010C-4824	NZ_JHMO00000000				
O121:H19 str. 2010C-4966	NZ_JHML00000000				
O121:H19 str. 2010C-4989	NZ_JHMJ00000000				
O121:H19 str. 2010EL1058	NZ_JHMG00000000				
O121:H19 str. 2011C-3537	NZ_JHLR00000000				
O121:H19 str. 2011C-3609	NZ_JASV00000000				
O121:H19 str. F6714	NZ_JHJR00000000				
O121:H19 str. K5198	NZ_JHIK00000000				
O121:H19 str. K5269	NZ_JHIJ00000000				
O121:H19 str. MT#2	NZ_AGTJ00000000				
O123:H11 str. 2009C-3307	NZ_JHHB00000000	Yes		Yes	Yes
O127:H6 str. E2348/69 substr. CVDNalr	NZ_ASZR00000000				
O127:H6 str. E2348/69 substr. UMD753	NZ_ASZS00000000				
O127:H27 str. C43/90	NZ_AHAW00000000				
O128:H2 str. 2011C-3317	NZ_JASU00000000	Yes		Yes	Yes
O145:H25 str. 07-3858	NZ_JASO00000000	Yes		Yes	Yes
O145:H28 str. 2009C-3292	NZ_JHHD00000000	Yes		Yes	Yes

O145:H28 str. 4865/96	NZ_JHEY00000000	Yes	Yes		Yes
O145:H28 str. 4865/96	NZ_AGTL00000000	Yes	Yes		Yes
O145:NM str. 06-3484	NZ_JHNN00000000				
O145:NM str. 08-4270	NZ_JHKV00000000	Yes	Yes		Yes
O145:NM str. 2010C-3507	NZ_JHFW00000000	Yes	Yes		Yes
O145:NM str. 2010C-3508	NZ_JHFV00000000	Yes	Yes		Yes
O145:NM str. 2010C-3509	NZ_JHFU00000000	Yes	Yes		Yes
O145:NM str. 2010C-3510	NZ_JHFT00000000	Yes	Yes		Yes
O145:NM str. 2010C-3511	NZ_JHFS00000000	Yes	Yes		Yes
O145:NM str. 2010C-3516	NZ_JHFR00000000	Yes	Yes		Yes
O145:NM str. 2010C-3517	NZ_JHFQ00000000	Yes	Yes		Yes
O145:NM str. 2010C-3518	NZ_JHFP00000000	Yes	Yes		Yes
O145:NM str. 2010C-3521	NZ_JHFO00000000	Yes	Yes		Yes
O145:NM str. 2010C-3526	NZ_JHFN00000000	Yes	Yes		Yes
O145:NM str. 2010C-4557C2	NZ_JHNA00000000	Yes	Yes		Yes
O146:H21 str. 2010C-3325	NZ_JASR00000000	Yes		Yes	Yes
O153:H2 str. 2010C-5034	NZ_JHMH00000000	Yes		Yes	Yes
O156:H25 str. 2011C-3602	NZ_JHLP00000000	Yes		Yes	Yes
O157:H7	NZ_LAYW00000000	Yes		Yes	Yes
O157:H7	NZ_LMXL00000000	Yes		Yes	Yes
O157:H7	NZ_LCWU00000000	Yes		Yes	Yes
O157:H7	NZ_LKAL00000000	Yes		Yes	Yes
O157:H7	NZ_LKAK00000000	Yes		Yes	Yes
O157:H7 str. 06-3745	NZ_JHNI00000000	Yes		Yes	Yes
O157:H7 str. 06-4039	NZ_JHNG00000000	Yes		Yes	Yes
O157:H7 str. 07-3091	NZ_JHNF00000000	Yes		Yes	Yes
O157:H7 str. 07-3391	NZ_JHNE00000000	Yes		Yes	Yes
O157:H7 str. 08-3037	NZ_JHKZ00000000	Yes		Yes	Yes
O157:H7 str. 08-3527	NZ_JHKY00000000	Yes		Yes	Yes
O157:H7 str. 08-4169	NZ_JHKW00000000	Yes		Yes	Yes
O157:H7 str. 08-4529	NZ_JHHI00000000	Yes		Yes	Yes
O157:H7 str. 08BKT061141	NZ_JJOL00000000				
O157:H7 str. 09BKT048303	NZ_JJOM00000000				
O157:H7 str. 1044	NZ_AERP00000000	Yes		Yes	Yes
O157:H7 str. 1125	NZ_AERR00000000	Yes		Yes	Yes
O157:H7 str. 2009C-4258	NZ_JHGQ00000000	Yes		Yes	Yes
O157:H7 str. 2009EL1449	NZ_JHGF00000000	Yes		Yes	Yes
O157:H7 str. 2009EL1705	NZ_JHGE00000000	Yes		Yes	Yes
O157:H7 str. 2009EL1913	NZ_JHGD00000000	Yes		Yes	Yes
O157:H7 str. 2009EL2109	NZ_JHGC00000000	Yes		Yes	Yes
O157:H7 str. 2010C-4979C1	NZ_JHMK00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-1107	NZ_JHLK00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2090	NZ_JHLI00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2091	NZ_JHLH00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2092	NZ_JHLG00000000	Yes		Yes	Yes

O157:H7 str. 2011EL-2093	NZ_JHLF00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2094	NZ_JHLE00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2096	NZ_JHLD00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2097	NZ_JHLC00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2098	NZ_JHLB00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2099	NZ_JHKT00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2099	NZ_JHKT00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2101	NZ_JHKS00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2101	NZ_JHKS00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2103	NZ_JHKR00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2104	NZ_JHKQ00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2105	NZ_JHKP00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2106	NZ_JHKO00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2107	NZ_JHKN00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2108	NZ_JHKM00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2109	NZ_JHKL00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2111	NZ_JHKK00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2112	NZ_JHKJ00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2113	NZ_JHKI00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2114	NZ_JHKH00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2286	NZ_JHKG00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2287	NZ_JHKF00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2288	NZ_JHKE00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2289	NZ_JHKD00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2290	NZ_JHKC00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2312	NZ_JHKB00000000	Yes		Yes	Yes
O157:H7 str. 2011EL-2313	NZ_JHKA00000000	Yes		Yes	Yes
O157:H7 str. EC508	NZ_ABHW00000000	Yes		Yes	Yes
O157:H7 str. EC536	NZ_ADVC00000000	Yes		Yes	Yes
O157:H7 str. EC869	NZ_ABHU00000000	Yes		Yes	Yes
O157:H7 str. EC1212	NZ_AERQ00000000	Yes		Yes	Yes
O157:H7 str. EC4009	NZ_ADMX00000000	Yes		Yes	Yes
O157:H7 str. EC4042	NZ_ABHM00000000	Yes		Yes	Yes
O157:H7 str. EC4045	NZ_ABHL00000000	Yes		Yes	Yes
O157:H7 str. EC4076	NZ_ABHQ00000000	Yes		Yes	Yes
O157:H7 str. EC4113	NZ_ABHP00000000	Yes		Yes	Yes
O157:H7 str. EC4127	NZ_ADUZ00000000	Yes		Yes	Yes
O157:H7 str. EC4191	NZ_ADVA00000000	Yes		Yes	Yes
O157:H7 str. EC4192	NZ_ADUX00000000	Yes		Yes	Yes
O157:H7 str. EC4196	NZ_ABHO00000000	Yes		Yes	Yes
O157:H7 str. EC4206	NZ_ABHK00000000	Yes		Yes	Yes
O157:H7 str. EC4401	NZ_ABHR00000000	Yes		Yes	Yes
O157:H7 str. EC4486	NZ_ABHS00000000	Yes		Yes	Yes
O157:H7 str. EC4501	NZ_ABHT00000000	Yes		Yes	Yes
O157:H7 str. F6142	NZ_JHJT00000000	Yes		Yes	Yes

O157:H7 str. F6749	NZ_JHJQ00000000	Yes		Yes	Yes
O157:H7 str. F6750	NZ_JHJP00000000	Yes		Yes	Yes
O157:H7 str. F6751	NZ_JHJO00000000	Yes		Yes	Yes
O157:H7 str. F7350	NZ_JHJN00000000	Yes		Yes	Yes
O157:H7 str. F7377	NZ_JHJM00000000	Yes		Yes	Yes
O157:H7 str. F7384	NZ_JHJL00000000	Yes		Yes	Yes
O157:H7 str. F7410	NZ_JHJK00000000	Yes		Yes	Yes
O157:H7 str. K1420	NZ_JHJF00000000	Yes		Yes	Yes
O157:H7 str. K1792	NZ_JHJD00000000	Yes		Yes	Yes
O157:H7 str. K1793	NZ_JHJC00000000	Yes		Yes	Yes
O157:H7 str. K1795	NZ_JHJB00000000	Yes		Yes	Yes
O157:H7 str. K1796	NZ_JHJA00000000	Yes		Yes	Yes
O157:H7 str. K1845	NZ_JHIZ00000000	Yes		Yes	Yes
O157:H7 str. K1921	NZ_JHIY00000000	Yes		Yes	Yes
O157:H7 str. K1927	NZ_JHIX00000000	Yes		Yes	Yes
O157:H7 str. K2188	NZ_JHIW00000000	Yes		Yes	Yes
O157:H7 str. K2191	NZ_JHIV00000000	Yes		Yes	Yes
O157:H7 str. K2192	NZ_JHIU00000000	Yes		Yes	Yes
O157:H7 str. K2324	NZ_JHIT00000000	Yes		Yes	Yes
O157:H7 str. K2581	NZ_JHIS00000000	Yes		Yes	Yes
O157:H7 str. K2622	NZ_JHIR00000000	Yes		Yes	Yes
O157:H7 str. K2845	NZ_JHIQ00000000	Yes		Yes	Yes
O157:H7 str. K2854	NZ_JHIP00000000	Yes		Yes	Yes
O157:H7 str. K4396	NZ_JHIO00000000	Yes		Yes	Yes
O157:H7 str. LSU-61	NZ_AEUC00000000	Yes		Yes	Yes
O157:H7 str. TW14313	NZ_AKMD00000000				
O157:H43 str. T22	NZ_AHZD00000000	Yes		Yes	Yes
O157:H- str. 493-89	NZ_AETY00000000	Yes		Yes	Yes
O157:H- str. 493-89	NZ_AGTG00000000	Yes		Yes	Yes
O157:H- str. H 2687	NZ_AETZ00000000	Yes		Yes	Yes
O157:NM str. 08-4540	NZ_JHHH00000000	Yes		Yes	Yes
O157: str. 2010EL-2044	NZ_JHME00000000	Yes		Yes	Yes
O157: str. 2010EL-2045	NZ_JHMD00000000	Yes		Yes	Yes
O157 str. NCCP15738	NZ_ASHB00000000				
O157 str. NCCP15739	NZ_ASHA00000000	Yes		Yes	Yes
STEC_7v	NZ_AEXD00000000				
STEC_B2F1	NZ_AFDQ00000000	Yes		Yes	Yes
STEC_C165-02	NZ_AFDR00000000	Yes		Yes	
STEC_EH250	NZ_AFDW00000000	Yes		Yes	
STEC_MHI813	NZ_AFDZ00000000	Yes		Yes	Yes
STEC_O31	NZ_AFEX00000000	Yes		Yes	Yes
STEC_S1191	NZ_AFEA00000000	Yes		Yes	
STEC O174:H8 str. 02-07607	NZ_AQGN00000000	Yes		Yes	
STEC 29	NZ_LNFU00000000	Yes		Yes	
STEC 66	NZ_LNFT00000000	Yes		Yes	

STEC 168	NZ_LNFV00000000	Yes		Yes	
STEC 169	NZ_LNZJ00000000				
STEC 196	NZ_LNZK00000000	Yes		Yes	
STEC 200	NZ_LNZL00000000	Yes		Yes	Yes
STEC 299	NZ_LOCR00000000	Yes		Yes	Yes
STEC 309	NZ_LOCS00000000	Yes		Yes	Yes
STEC 329	NZ_LOCT00000000	Yes		Yes	
STEC 343	NZ_LDOZ00000000	Yes		Yes	Yes
STEC 370	NZ_LOCU00000000	Yes		Yes	Yes
STEC 380	NZ_LOCV00000000	Yes		Yes	Yes
STEC 384	NZ_LOCW00000000	Yes		Yes	Yes
STEC 464	NZ_LOCX00000000	Yes		Yes	Yes
STEC 477	NZ_LOCY00000000	Yes		Yes	Yes
STEC 479	NZ_LOCZ00000000	Yes		Yes	Yes
STEC 487	NZ_LODA00000000	Yes		Yes	Yes
STEC 545	NZ_LODB00000000	Yes	Yes		Yes
STEC 559	NZ_LODC00000000	Yes		Yes	Yes
STEC 563	NZ_LODD00000000	Yes		Yes	Yes
STEC 565	NZ_LODE00000000				
STEC 605	NZ_LFUA00000000	Yes		Yes	Yes
STEC 623	NZ_LFUB00000000	Yes		Yes	Yes
STEC 627	NZ_LODF00000000	Yes		Yes	Yes
STEC 645	NZ_LODG00000000	Yes		Yes	
STEC 690	NZ_LOFJ00000000				
STEC 691	NZ_LOFK00000000	Yes		Yes	Yes
STEC 707	NZ_LOFL00000000	Yes		Yes	Yes
STEC 709	NZ_LOFM00000000	Yes		Yes	Yes
STEC 731	NZ_LOFN00000000	Yes	Yes		Yes
STEC 757	NZ_LOFO00000000	Yes		Yes	Yes
STEC 764	NZ_LOFP00000000	Yes		Yes	Yes
STEC 793	NZ_LOFQ00000000				
STEC 886	NZ_LOFR00000000	Yes		Yes	Yes
STEC 931	NZ_LOFS00000000	Yes		Yes	Yes
STEC 940	NZ_LOFT00000000				
STEC 1117	NZ_LOFU00000000	Yes		Yes	Yes
STEC 1161	NZ_LOFV00000000	Yes		Yes	Yes
STEC 1178	NZ_LOFW00000000				
STEC 1188	NZ_LOFX00000000	Yes		Yes	Yes
STEC 1198	NZ_LOFY00000000				
STEC 1201	NZ_LOFZ00000000	Yes		Yes	Yes
STEC 1225	NZ_LOGA00000000	Yes		Yes	Yes
STEC 1236	NZ_LOGB00000000	Yes		Yes	Yes
STEC 1255	NZ_LOGC00000000	Yes		Yes	Yes
STEC 1270	NZ_LOGD00000000				
STEC 1284	NZ_LOGE00000000	Yes		Yes	Yes

STEC 1293	NZ_LOGF00000000	Yes		Yes	Yes
STEC 1299	NZ_LOGG00000000	Yes		Yes	Yes
STEC 1303	NZ_LOGH00000000				
STEC 1363	NZ_LOGI00000000	Yes		Yes	Yes
STEC 1375	NZ_LOGJ00000000	Yes		Yes	Yes
STEC 1442	NZ_LOGK00000000	Yes	Yes		Yes
STEC 1465	NZ_LOGL00000000	Yes		Yes	Yes
STEC 1473	NZ_LOGM00000000				
STEC 1500	NZ_LOGN00000000	Yes		Yes	
STEC 1513	NZ_LOGO00000000	Yes		Yes	Yes
STEC 1528	NZ_LOGP00000000				
STEC 1532	NZ_LOGQ00000000	Yes		Yes	Yes
STEC 1585	NZ_LOGR00000000	Yes		Yes	
STEC 1634	NZ_LOGS00000000	Yes		Yes	Yes
STEC 1686	NZ_LOGT00000000	Yes		Yes	Yes
STEC 2064	NZ_LOJC00000000	Yes		Yes	
STEC 2074	NZ_LOJD00000000	Yes		Yes	Yes
STEC 2075	NZ_LGBD00000000	Yes		Yes	Yes
STEC 2110.1	NZ_LPWW00000000	Yes		Yes	
STEC 2110.3	NZ_LOJE00000000	Yes		Yes	Yes
STEC 2112	NZ_LGBE00000000	Yes		Yes	Yes
STEC 2144	NZ_LOGU00000000	Yes		Yes	Yes
STEC 2174	NZ_LOGV00000000	Yes		Yes	
STEC 2193	NZ_LOGW00000000	Yes		Yes	Yes
STEC 2211	NZ_LOGX00000000	Yes		Yes	Yes
STEC 2236	NZ_LOGY00000000				
STEC 2257	NZ_LGBF00000000	Yes		Yes	Yes
STEC 2270	NZ_LPWX00000000	Yes		Yes	Yes
STEC 2334	NZ_LOGZ00000000				
STEC 2346	NZ_LOHA00000000				
STEC 2359	NZ_LOHB00000000	Yes		Yes	Yes
STEC 2363	NZ_LPWY00000000	Yes		Yes	
STEC 2410	NZ_LGBG00000000	Yes		Yes	Yes
STEC 2419	NZ_LPWZ00000000				
STEC 2441	NZ_LOHC00000000	Yes		Yes	Yes
STEC 2450	NZ_LPXA00000000	Yes		Yes	Yes
STEC 2499	NZ_LOIE00000000	Yes		Yes	Yes
STEC 2505	NZ_LPXB00000000	Yes		Yes	Yes
STEC 2539	NZ_LOIF00000000				
STEC 2564	NZ_LOIG00000000				
STEC 2573	NZ_LOIH00000000	Yes		Yes	Yes
STEC 2591	NZ_LOII00000000	Yes		Yes	Yes
STEC 2595	NZ_LOIJ00000000	Yes		Yes	
STEC 2620	NZ_LPXC00000000	Yes		Yes	
STEC 2633	NZ_LOIK00000000				

STEC 2667	NZ_LGBH00000000	Yes		Yes	Yes
STEC 2708	NZ_LOIL00000000				
STEC 2743	NZ_LOIM00000000	Yes		Yes	Yes
STEC 2746	NZ_LPXD00000000	Yes		Yes	Yes
STEC 2764	NZ_LOIN00000000	Yes		Yes	
STEC 2770	NZ_LPXF00000000	Yes		Yes	Yes
STEC 2788	NZ_LOIO00000000	Yes		Yes	
STEC 2797	NZ_LOIP00000000	Yes		Yes	
STEC 2820	NZ_LGBQ00000000	Yes		Yes	Yes
STEC 2821	NZ_LGBI00000000	Yes		Yes	Yes
STEC 2826	NZ_LOJA00000000	Yes		Yes	Yes
STEC 2839	NZ_LOJB00000000	Yes		Yes	
STEC 2841	NZ_LOIQ00000000	Yes		Yes	Yes
STEC 2861	NZ_LOIR00000000	Yes		Yes	
STEC 2868	NZ_LGBJ00000000	Yes		Yes	Yes
STEC 2894.1	NZ_LOIS00000000	Yes		Yes	
STEC 2894.2	NZ_LOIT00000000				
STEC 2920	NZ_LOIU00000000	Yes		Yes	Yes
STEC 2938	NZ_LOIV00000000	Yes		Yes	Yes
STEC 2953	NZ_LOIW00000000				
STEC 2954	NZ_LPXE00000000	Yes		Yes	
STEC 2962	NZ_LOIX00000000	Yes		Yes	
STEC 2980	NZ_LOIY00000000	Yes		Yes	Yes
STEC 3031	NZ_LOIZ00000000				
STEC 3039	NZ_LPUH00000000	Yes		Yes	Yes
STEC 3055	NZ_LPUI00000000	Yes		Yes	
STEC 3084	NZ_LPUJ00000000	Yes		Yes	
STEC 3087	NZ_LPUK00000000	Yes		Yes	
STEC 3094	NZ_LPUL00000000	Yes		Yes	Yes
STEC 3098	NZ_LPUM00000000	Yes		Yes	Yes
STEC 3106	NZ_LPUN00000000	Yes		Yes	
upec-2	NZ_JSLN00000000	Yes	Yes		Yes
upec-3	NZ_JSIF00000000	Yes		Yes	Yes
upec-4	NZ_JSHU00000000				
upec-7	NZ_JSGV00000000				
upec-8	NZ_JSGK00000000				
upec-9	NZ_JSFZ00000000				
upec-10	NZ_JSPB00000000				
upec-15	NZ_JSNA00000000				
upec-22	NZ_JSKT00000000				
upec-23	NZ_JSKJ00000000				
upec-24	NZ_JSJZ00000000				
upec-28	NZ_JSIQ00000000				
upec-29	NZ_JSIG00000000				
upec-30	NZ_JSIE00000000	Yes		Yes	Yes

upec-31	NZ_JSID00000000	Yes		Yes	Yes
upec-33	NZ_JSIB00000000	Yes		Yes	
upec-34	NZ_JSIA00000000				
upec-36	NZ_JSHY00000000				
upec-37	NZ_JSHX00000000				
upec-38	NZ_JSHW00000000				
upec-39	NZ_JSHV00000000				
upec-50	NZ_JSHM00000000				
upec-51	NZ_JSHL00000000				
upec-53	NZ_JSHK00000000				
upec-54	NZ_JSHJ00000000				
upec-55	NZ_JSHI00000000				
upec-56	NZ_JSHH00000000	Yes		Yes	
upec-57	NZ_JSHG00000000				
upec-58	NZ_JSHF00000000				
upec-59	NZ_JSHE00000000				
upec-60	NZ_JSHD00000000				
upec-61	NZ_JSHC00000000				
upec-62	NZ_JSHB00000000				
upec-64	NZ_JSHA00000000				
upec-65	NZ_JSGZ00000000				
upec-66	NZ_JSGY00000000	Yes		Yes	Yes
upec-69	NZ_JSGW00000000	Yes		Yes	
upec-70	NZ_JSGU00000000				
upec-72	NZ_JSGS00000000				
upec-73	NZ_JSGR00000000				
upec-74	NZ_JSGQ00000000				
upec-75	NZ_JSGP00000000				
upec-76	NZ_JSGO00000000				
upec-77	NZ_JSGN00000000				
upec-78	NZ_JSGM00000000				
upec-79	NZ_JSGL00000000				
upec-80	NZ_JSGJ00000000				
upec-81	NZ_JSGI00000000	Yes		Yes	Yes
upec-82	NZ_JSGH00000000	Yes		Yes	
upec-83	NZ_JSGG00000000				
upec-84	NZ_JSGF00000000				
upec-85	NZ_JSGE00000000				
upec-87	NZ_JSGC00000000				
upec-88	NZ_JSGB00000000				
upec-89	NZ_JSGA00000000				
upec-90	NZ_JSFY00000000				
upec-91	NZ_JSFX00000000				
upec-93	NZ_JSFV00000000				
upec-94	NZ_JSFU00000000				

upec-95	NZ_JSFT00000000	Yes		Yes	Yes
upec-97	NZ_JSFS00000000				
upec-98	NZ_JSFR00000000				
upec-99	NZ_JSFQ00000000	Yes		Yes	
upec-100	NZ_JSPA00000000				
upec-101	NZ_JSOZ00000000				
upec-103	NZ_JSOX00000000				
upec-104	NZ_JSOW00000000	Yes		Yes	Yes
upec-105	NZ_JSOV00000000				
upec-106	NZ_JSOU00000000				
upec-107	NZ_JSOT00000000				
upec-108	NZ_JSOS00000000				
upec-109	NZ_JSOR00000000				
upec-110	NZ_JSOQ00000000				
upec-114	NZ_JSOM00000000	Yes		Yes	Yes
upec-115	NZ_JSOL00000000				
upec-116	NZ_JSOK00000000				
upec-117	NZ_JSOJ00000000				
upec-118	NZ_JSOI00000000	Yes		Yes	Yes
upec-119	NZ_JSOH00000000	Yes		Yes	Yes
upec-120	NZ_JSOF00000000				
upec-121	NZ_JSOE00000000	Yes		Yes	Yes
upec-123	NZ_JSOC00000000				
upec-124	NZ_JSOB00000000				
upec-125	NZ_JSOA00000000				
upec-126	NZ_JSNZ00000000				
upec-127	NZ_JSNY00000000				
upec-128	NZ_JSNX00000000				
upec-129	NZ_JSNW00000000				
upec-130	NZ_JSNV00000000	Yes		Yes	Yes
upec-131	NZ_JSNU00000000				
upec-132	NZ_JSNT00000000	Yes		Yes	Yes
upec-133	NZ_JSNS00000000	Yes		Yes	
upec-134	NZ_JSNR00000000				
upec-135	NZ_JSNQ00000000				
upec-136	NZ_JSNP00000000				
upec-137	NZ_JS00000000	Yes		Yes	Yes
upec-138	NZ_JSNN00000000				
upec-139	NZ_JSNM00000000				
upec-140	NZ_JSNK00000000				
upec-141	NZ_JSNJ00000000				
upec-142	NZ_JSNI00000000				
upec-143	NZ_JSNH00000000				
upec-144	NZ_JSNG00000000				
upec-145	NZ_JSNF00000000	Yes		Yes	

upec-146	NZ_JSNE00000000	Yes		Yes	Yes
upec-147	NZ_JSND00000000	Yes		Yes	Yes
upec-148	NZ_JSNC00000000				
upec-149	NZ_JSNB00000000				
upec-150	NZ_JSMZ00000000	Yes		Yes	Yes
upec-151	NZ_JSMY00000000	Yes		Yes	Yes
upec-153	NZ_JSMX00000000				
upec-154	NZ_JSMW00000000				
upec-155	NZ_JSMV00000000	Yes		Yes	
upec-156	NZ_JSMU00000000				
upec-157	NZ_JSMT00000000				
upec-158	NZ_JSMS00000000				
upec-159	NZ_JSMR00000000				
upec-161	NZ_JSMQ00000000				
upec-162	NZ_JSMP00000000				
upec-166	NZ_JSMO00000000				
upec-169	NZ_JSMN00000000				
upec-170	NZ_JSML00000000	Yes	Yes		Yes
upec-171	NZ_JSMK00000000				
upec-172	NZ_JSMJ00000000				
upec-173	NZ_JSMI00000000	Yes	Yes		Yes
upec-175	NZ_JSMH00000000	Yes		Yes	Yes
upec-176	NZ_JSMG00000000				
upec-207	NZ_JSLE00000000				
upec-208	NZ_JSLD00000000				
upec-209	NZ_JSLC00000000				
upec-211	NZ_JSLB00000000	Yes		Yes	Yes
upec-212	NZ_JSLA00000000				
upec-213	NZ_JSKZ00000000	Yes		Yes	Yes
upec-219	NZ_JSKU00000000				
upec-220	NZ_JSKS00000000				
upec-221	NZ_JSKR00000000	Yes		Yes	
upec-225	NZ_JSKO00000000				
upec-226	NZ_JSKN00000000				
upec-227	NZ_JSKM00000000				
upec-228	NZ_JSKL00000000				
upec-229	NZ_JSKK00000000				
upec-230	NZ_JSKI00000000				
upec-232	NZ_JSKG00000000				
upec-233	NZ_JSKF00000000				
upec-235	NZ_JSKE00000000				
upec-236	NZ_JSKD00000000				
upec-237	NZ_JSKC00000000				
upec-238	NZ_JSKB00000000	Yes		Yes	Yes
upec-239	NZ_JSKA00000000				

upec-243	NZ_JSJX00000000				
upec-244	NZ_JSJW00000000				
upec-248	NZ_JSJT00000000				
upec-249	NZ_JSJS00000000				
upec-250	NZ_JSJQ00000000				
upec-251	NZ_JSJP00000000				
upec-253	NZ_JSJO00000000				
upec-254	NZ_JSJN00000000	Yes		Yes	
upec-255	NZ_JSJM00000000				
upec-256	NZ_JSJL00000000	Yes		Yes	
upec-257	NZ_JSJK00000000				
upec-258	NZ_JSJJ00000000				
upec-259	NZ_JSJI00000000				
upec-260	NZ_JSJG00000000				
upec-261	NZ_JSJF00000000				
upec-265	NZ_JSJB00000000				
upec-266	NZ_JSJA00000000				
upec-269	NZ_JSIY00000000	Yes	Yes		Yes
upec-271	NZ_JSIV00000000				
upec-273	NZ_JSIU00000000	Yes	Yes		Yes
upec-274	NZ_JSIT00000000	Yes		Yes	
upec-276	NZ_JSIS00000000				
upec-277	NZ_JSIR00000000				
upec-281	NZ_JSIO00000000				
upec-282	NZ_JSIN00000000				
upec-284	NZ_JSIM00000000				
upec-285	NZ_JSIL00000000				
upec-286	NZ_JSIK00000000				
upec-287	NZ_JSIJ00000000				
upec-288	NZ_JSII00000000				
upec-289	NZ_JSIH00000000				
Totals	628	401	25	376	355
Percentages		63.85	6.23	93.77	88.53

Example 14: Expression of LeuO is necessary to elicit CRISPR-Cas lethality

[0282] Most *E. coli* encode the necessary components for Type I CRISPR-Cas3 activity in their genome. However, the *E. coli* Type I-E CRISPR-Cas3 operon is regulated by histone-like nucleoid-structuring (H-NS) repression and is not expressed under normal culture conditions. LeuO acts in opposition to H-NS at overlapping promoter regions and activates gene expression. The interplay between H-NS and LeuO activity has been studied in *S. typhimurium* and *E. coli*, by examining the global transcriptional changes related to LeuO overexpression or knockout. Under conventional culturing conditions, LeuO itself is not expressed but is upregulated during starvation and

stationary phase. However, the *casABCDE* operon in *E. coli* and *S. typhimurium* was significantly upregulated with LeuO overexpression and has predicted H-NS and LeuO binding sequences upstream of CasA. However, in the absence of a LeuO expression cassette, *casABCDE* expression is not sufficient to support lethality via self-targeting crRNAs.

[0283] The primary risks of delivering a LeuO expression cassette to pathogens include its impact on the non-target (non-Cas) genes within its regulon. In *S. typhimurium*, LeuO upregulates some genes related to pathogenicity, but it is unclear if there is a meaningful increase in expression and how this observation applies to bacteria other than *S. typhimurium*. In *E. coli*, LeuO increases or decreases resistance to certain classes of antibiotics.

[0284] To verify functionality of LeuO in CRISPR-mediated lethality in *E. coli*, a phagemid was designed encoding a LeuO expression cassette to overcome the wild-type repression of the endogenous CRISPR-Cas3 operon. The designed phagemid was derived from the M13 bacteriophage, which has been shown to be non-lytic so as not to confound CRISPR-Cas3 based lethality. The phagemid also encodes a CRISPR array targeting the conserved *E. coli ftsA* gene, whereby expression of this array activates and direct self-targeting of Type I-E *E. coli* CRISPR-Cas3 systems to elicit cell death.

[0285] **Figure 17** shows non-lytic M13-derived phagemid delivery of CRISPR constructs using the validated *ftsA* spacer sequence designed to test the dependence on LeuO expression for CRISPR-mediated lethality. Phagemids were produced to titers of 10^9 transducing units per milliliter and maintained in growth media for in vitro studies. The phagemid vector encodes the *ftsA* repeat-spacer array, LeuO expression cassette, and an M13-compatible origin of replication. Lethality of phagemid vectors was tested via transduction of M13 bacteriophages into a range of s including a parent EMG2 containing a wild-type H-NS repressed *E. coli* Type I-E CRISPR-Cas3 operon, a BW25113-derivative lacking the H-NS repression motifs in the CRISPR-Cas3 operon (Δhns), a BW25113-derivative containing an overexpressed CRISPR-Cas3 operon (BW+Cas) and a BW25113-derivative lacking Cas3 genes (BWΔCas).

[0286] Indicated *E. coli* were infected with 10^9 transducing units per milliliter of each M13 phagemid and plated on selective media to recover transduced cells and count surviving colony forming units (transductants). Each was transduced with the following phagemids indicated in the legend: Control, generic M13 transduction control; pCRISPR, phagemid that constitutively expresses non-targeting crRNA; LeuO, phagemid that constitutively expresses the *E. coli* LeuO gene; *ftsA*, phagemid that constitutively expresses crRNA targeting conserved *ftsA* gene present in *E. coli*; *ftsA*::LeuO, phagemid constitutively expresses LeuO gene and crRNA targeting *ftsA*.

[0287] Co-delivery of LeuO and the *ftsA*-targeting spacer resulted in reductions in the range of 3.4-log (± 0.04) to 4.3-log (± 0.06) compared to control across each except the BWΔCas that lacks Cas3 activity, confirming that lethality is dependent on the constructs expressed from the phagemid genome. Notably, CRISPR-Cas3 lethality by expression of a *ftsA*-targeting spacer alone was only observed in the BW+Cas cell line, demonstrating that removal of H-NS repression alone is not sufficient to rescue significant levels of endogenous CRISPR-Cas3 targeting. Based on these data, both the *ftsA* spacer and LeuO are required for cell death in non-engineered, wild-type *E. coli*.

Example 15: LeuO enhanced crPhages have improved lethality kinetics

[0288] Each crPhage was systemically compared to wild-type phage to determine change in potency of CRISPR-enhanced phages compared to their respective wild-type bacteriophage. crPhages and the corresponding wild-type bacteriophage were produced, filtered, and adjusted to the same titer in growth media. Based on existing publicly available sequencing data, the three wild-type phages have significantly different genomic architectures, but are obligate lytic phages (data not shown). Target *E. coli* were incubated for 2 or 5 hours for crT7m, crT7 and crT4, respectively, in growth media at the indicated multiplicity-of-infection (ratio of phage to bacteria) for each phage. After incubation, cultures were immediately collected, serially diluted and plated to count surviving colonies. Significant differences were observed in CFU reduction across all three crPhage: wild-type phage comparisons as seen in **Figure 18A- Figure 18C**, including up to an approximately 4-log improvement of crT7m (**Figure 18A**), approximately 4.5-log improvement of crT4 (**Figure 18B**) and approximately 1-log improvement of crT7 (**Figure 18C**) activities. These data suggest that CRISPR-enhanced phages eliminate the target *E. coli* population, in contrast to the wild-type bacteriophage, at the selected time points.

Example 16: LeuO enhanced crPhages *in vitro* kill curves

[0289] **Figure 19A-Figure 19E** shows the dose-response *in vitro* kill curves for each crPhage. Each crPhage was produced by standard lytic amplification; filtration and left suspended in the original growth media (LB broth). All experiments were conducted in LB broth. *E. coli* MG1655 was grown to mid-log phase and then mixed with the indicated multiplicity-of-infection (MOI) of each crPhage, crPhage cocktail or LB only negative control. Treated populations were grown under aerobic, shaking conditions at 37C for 24 hours in a plate reader to monitor growth of treated populations by optical density (OD 630 nm).

[0290] *E. coli* MG1655 was grown to mid-log phase and treated with multiplicity-of-infection (MOI; ratio of phage to bacteria) as follows: **Figure 19A**, crT7 was incubated at MOIs of 0.0001,

0.01, and 1.0; **Figure 19B**, crT7m was incubated at MOIs of 0.0009, 0.09, and 9.0; and **Figure 19C**, crT4 was incubated at MOIs of 0.0006, 0.06, and 6.0.Each phage was mixed in equal amounts to create a crPhage cocktail ('Cocktail') and was incubated at MOIs (for each crPhage) of 0.0006, 0.06, and 6.0 as seen in **Figure 19D**. **Figure 19E** is a zoomed in graph from **Figure 19D**.

[0291] As expected, all crPhages lysed the target independent of MOI. Notably, an emergent resistant population in the crT7m high-dose population (**Figure 19B**) and in the crT4 low-dose population (MOI = 0.0006, **Figure 19C**) was observed by 24 hours of continuous culture in the presence of the initial phage dose. However, when challenged with a cocktail of crT7, crT7m and crT4, no resistant population was observed (**Figure 19D** and **Figure 19E**).

[0292] A dose-dependent relationship was observed between concentrations of crPhage and observable time-to-lysis as seen in **Figure 20** conducted to quantify time-to-lysis compared to MOI. Time-to-lysis was defined as the time at which the first derivative of the growth curve reaches zero after the bacterial population crashes due to presumed lytic phage amplification. Endonuclease degradation of host genomes is considered to be a non-lytic mechanism for killing bacteria, thus CRISPR-mediated lethality is not expected to be observed by growth curve analysis.

[0293] *E. coli* MG1655 was grown to mid-log phase and treated with multiplicity-of-infection (MOI; ratio of phage to bacteria) as indicated for each crPhage. Growth curves were smoothed and the first derivative of the smoothed lines were determined using the PRISM software suite. Time-to-lysis was calculated as the time where the first derivative reaches zero immediately following the initial observed population decline.

[0294] For all 3 crPhages tested, MOI in excess of 1.0 result in fastest time-to-lysis, presumably being limited by the lytic period of each phage. The observed time-to-lysis of approximately 15-20 minutes for crT7m and crT7 and 45-50 minutes for crT4 largely agree with the values known for the wild-type lytic phages T7 (~17 minutes), T7m (~15-20 minutes) and T4 (35 minutes), respectively.

Example 17: LeuO enhanced crPhages *in vivo* tolerability

[0295] Prior to survival studies in the peritonitis model, the tolerability of each crPhage was determined *in vivo*. crPhages were prepared for tolerability as described in **Table 13** below.

Table 13: crPhages for *in vivo* tolerability studies.

Study information		Batch information					Dosing information		
Study	**Study type**	**Phage**	**Diluent**	**Production notes**	**PFU/mL**	**EU/mL**	**Dose (mL)**	**# doses**	**RoA**
035467	Tolerability	crT7	0.9% saline	CF;TP	2.0E+12	3	0.1	5	i.p.
035467	Tolerability	Control	0.9% saline		N/A	<1	0.1	5	i.p.
035551	Tolerability	crT7m	0.9% saline	CF;TP	3.7E+10	10	0.1	5	i.p.

035551	Tolerability	Control	0.9% saline		N/A	<1	0.1	5	i.p.
035730	Tolerability	crT4	1X TBS, pH 7.4 + C/M	CF;TP	6.0E+09	9.6	0.1	1	i.p.
035730	Tolerability	Control	1X TBS, pH 7.4 + C/M		N/A	<1	0.1	1	i.p.

[0296] The treatment outline for tolerability is schematically shown in **Figure 21A**. In the tolerability study, female CD-1 mice each received one 100-microliter dose per day by intraperitoneal injection for 5 days with $2.0x10^{11}$ PFU/day/mouse of crT7, 5 days with $3.7x10^{9}$ PFU/day/mouse of crT7M or 1 day with $6.0x10^{8}$ PFU/day/mouse of crT4. crT7 and crT7m were suspended in sterile, endotoxin-free 0.9% saline, while crT4 was suspended in sterile, endotoxin-free 1X tris-buffered saline (pH 7.4) supplemented with 10mM of each $CaCl_2$ and $MgCl_2$. No overt toxicity was observed during veterinary observation and no measurable changes in body temperature or body weight were noted after dosing with each crPhage preparation as shown in **Figure 21B- Figure 21G**.

Example 18: LeuO enhanced crPhages *in vivo* peritonitis model study

[0297] crPhages were prepared for the peritonitis model as described in **Table 14** below:

Table 14: crPhages for an *in vivo* peritonitis model studies.

Study information		**Batch information**					**Dosing information**		
Study	**Study type**	**Phage**	**Diluent**	**Production notes**	**PFU/mL**	**EU/mL**	**Dose (mL)**	**# doses**	**RoA**
035552	Peritonitis	crT7m	0.9% saline	CF;TP	3.7E+10	10	0.1	5	i.p.
035552	Peritonitis	Control	0.9% saline		N/A	<1	0.1	5	i.p.
035468	Peritonitis	crT7	0.9% saline	CF;TP	2.0E+12	3	0.1	5	i.p.
035468	Peritonitis	Control	0.9% saline		N/A	<1	0.1	5	i.p.
035730	Peritonitis	crT4	1X TBS, pH 7.4 + C/M	CF;TP	6.0E+09	9.6	0.1	1	i.p.
035730	Peritonitis	Control	1X TBS, pH 7.4 + C/M		N/A	<1	0.1	1	i.p.

[0298] Based on the activity of three crPhages against a fecal *E. coli* isolate (ATCC 8739, crT7M and crT7) or lab (MG1655, crT4), the three crPhages were evaluated in a murine peritonitis model with *E. coli*. The treatment outline for the peritonitis model is schematically shown in **Figure 22A**. Female CD-1 mice were injected intraperitoneally with a lethal dose of *E. coli* (~$5x10^{7}$ CFU/ mouse of ATCC 8739) followed within 30 minutes by intraperitoneal injections of saline or crPhages. As before, crT7 and crT7m were suspended in sterile, endotoxin-free 0.9% saline, while crT4 was suspended in sterile, endotoxin-free 1X tris-buffered saline (pH 7.4) supplemented with 10mM each $CaCl_2$ and $MgCl_2$. Single-dose administration of crPhage ($2.0x10^{11}$ PFU/dose of crT7, $3.7x10^{9}$ PFU/dose of crT7M or $6.0x10^{8}$ PFU/dose of crT4) resulted in significant protection in this acute, highly lethal bacterial challenge as seen in **Figure 22B- Figure 22D**. Control animals were treated with saline injections only. These data demonstrate that bacteriophages are able to infect

and kill sufficient numbers of target bacteria to rescue lethal disease challenge in a relevant animal model of infection.

Example 19: LeuO enhanced crPhages *in vivo* bioburden reduction in a thigh model

[0299] crPhages were prepared for an *in vivo* bioburden reduction in a thigh model as described in **Table 15** below:

Table 15: crPhages for *in vivo* bioburden reduction in a thigh model studies.

Study information		Batch information					Dosing information		
Study	**Study type**	**Phage**	**Diluent**	**Production notes**	**PFU/mL**	**EU/mL**	**Dose (mL)**	**# doses**	**RoA**
035790	Thigh Infection	crT7m	1X TBS, pH 7.4	PE;CC	2.0E+12	<100	0.1	1	i.m.
035790	Thigh Infection	crT4	1X TBS, pH 7.4	CF;CC	2.0E+11	<1000	0.1	1	i.m.
035790	Thigh Infection	crT7	1X TBS, pH 7.4	PE;CC	4.0E+12	<100	0.1	1	i.m.
035790	Thigh Infection	Cocktail (crT7m/T7/T4)	1X TBS, pH 7.4		1.0E+11 each	<1000	0.1	1	i.m.
035790	Thigh Infection	Control	1X TBS, pH 7.4		N/A	<1	0.1	1	i.m.

[0300] As a second demonstration of *in vivo* efficacy, a thigh infection model in mice was conducted to measure bioburden reduction after crPhage treatment. The treatment outline for this thigh model is schematically shown in **Figure 23A**. The original crPhage stocks used in this study have a potency of $4.0x10^{12}$ PFU/mL of crT7, $2.0x10^{12}$ PFU/mL of crT7M, and $2.0x10^{11}$ PFU/mL of crT4 with each phage suspended in sterile, endotoxin-free 1X tris-buffered saline (pH 7.4). The 3 crPhages were pooled into a cocktail with a final concentration of $1x10^{11}$ PFU/mL of each phage containing an estimated endotoxin content of $<10^3$ EU/mL.

[0301] One and four days prior to bacterial inoculation, female CD-1 mice were made neutropenic by intraperitoneal injection of 150 mg/kg cyclophosphamide into the left abdomen. Mice were inoculated with 10^5 CFU of *E. coli* MG1655 by intramuscular injection into the thigh 30 minutes prior to intramuscular injection with the indicated crPhage or 1X tris-buffered saline (phage vehicle). Each individual crPhage or cocktail of 3 crPhages were administered by intramuscular injection into the same thigh with 100 microliters of crPhage solution, corresponding to a dose of $4.0x10^{11}$ PFU/dose of crT7, $2.0x10^{11}$ PFU/dose of crT7M, $2.0x10^{10}$ PFU/dose of crT4 or the cocktail containing $1.0x10^{10}$ PFU/dose of each phage. After injection with each crPhage, whole thigh muscles were excised at the indicated time points, homogenized and immediately diluted and plated to count surviving bacterial colonies per gram of tissue. CFU reductions measured approximately 2-log for crT4, 3-log for crT7M, and >5-log for both crT7and the combined crPhage cocktail as seen in **Figure 23B- Figure 23E**. Taken together with the peritonitis results presented in

Figure 22B- Figure 22D, these data demonstrate that crPhages have the potential to be highly effective antimicrobial agents in vivo.

Example 20: LeuO enhanced crPhages *in vivo* persistence and distribution studies measured by phage titration

[0302] Given the unique PK/PD/ADME considerations of active phage-based products, the persistence and distribution of each crPhage in both target tissues and distal organs was evaluated to understand the kinetics of exposure in healthy animals. crPhages were prepared for an *in vivo* persistence and distribution study by intraurethral administration as described in **Table 16** below:

Table 16: crPhages for in vivo persistence and distribution studies.

Study information		Batch information					Dosing information		
Study	**Study type**	**Phage**	**Diluent**	**Production notes**	**PFU/mL**	**EU/mL**	**Dose (mL)**	**# doses**	**RoA**
036239	Persistence & Distribution	Cocktail (crT7m/T7)	1X TBS, pH 7.4	CF	2.0E+10 each	Not measured	0.05	1	i.u.
036239	Persistence & Distribution	Control	1X TBS, pH 7.4		N/A	<1	0.05	1	i.u.

[0303] Female CD-1 mice were treated with approximately $1.0x10^9$ PFU/dose/phage of a crT7/crT7m cocktail suspended in 1X tris-buffered saline (pH 7.4) by intraurethral instillation to N=3 mice per condition/ time point. Intraurethral instillation was done by placement of a silicone-tipped syringe into the urethra of female mice and solution was injected directly into the bladder. Each mouse was dosed with an approximately 50 µL of vehicle or phage cocktail by intraurethral instillation while under isoflurane anesthesia. At time points of 0 (immediately following intraurethral administration), 0.5, 1, 6, 12, 24 and 72 hours post-inoculation, 3 mice per time point were sacrificed and collected bladder, kidney, blood, liver and spleen whole tissue homogenates were diluted and subjected to phage titration analysis to quantify the total combined amount of crT7 and crT7m. Means ± standard error of the mean (SEM) shown are the result of 3 technical replicates from 3 animals and quantify plaque forming units per gram (bladder, kidney, liver, spleen) or per milliliter (blood) of either crT7 or crT7m (assay is not specific to either crPhage). crPhages were quantified as a pooled measurement using conventional phage titration against a known host that is susceptible to both crT7 and crT7m.

[0304] The presence of active crPhage was detected up to 72 hours after dosing as seen in **Figure 24**. Apparent amplification during this time period was not observed which was expected due to the lack of a target *E. coli* replication host in the normal tissues and the inability to replicate in mammalian cells. Importantly, crPhage levels decreased over time in bladder and were undetectable in kidney, liver, blood and spleen by 72 hours, suggesting the absence of suitable *E.*

coli replication hosts in treated animals results in loss of crPhage over time. Notably, significant phage titers were observed in the kidneys suggesting that intraurethral route of administration, in some cases, results in exposure in the lower and upper urinary tract. Also, significant phage titers were detected in blood, liver and spleen tissues, showing that crPhages appear to enter circulation by crossing the urothelium.

Example 21: LeuO enhanced crPhages *in vivo* persistence and distribution studies measured by quantitative PCR

[0305] A quantitative PCR-based method was developed and validated for the detection of each crPhage within a given cocktail (data not shown) enabling detection levels down to 50 copies per ng of total DNA in complex samples (e.g. commingled mouse blood and whole DNA). Quantitative PCR is a highly specific method to detect and quantify DNA and is theoretically able to measure the total amount of each engineered crPhage within samples as the primers are designed to recognize a specific phage genome containing an identical crRNA cassette insert. crPhages were prepared for an *in vivo* persistence and distribution study by oral administration as described in **Table 17** below:

Table 17: crPhages for *in vivo* persistence and distribution studies.

Study information		**Batch information**					**Dosing information**		
Study	**Study type**	**Phage**	**Diluent**	**Production notes**	**PFU/mL**	**EU/mL**	**Dose (mL)**	**# doses**	**RoA**
035789	Persistence & Distribution	Cocktail (crT7m/T7/T4)	1X TBS, pH 7.4	CF	1.35E+10 each	Not measured	0.2	1	oral
035789	Persistence & Distribution	Control	1X TBS, pH 7.4		N/A	<1	0.2	1	oral

[0306] A small study was conducted in mice to determine the presence of multiple crPhages over time after a single oral administration of a crPhage solution. To mitigate potential phage degradation, mice were gavaged with 0.2mL of 6% sodium bicarbonate to reduce stomach acid levels approximately 30 minutes prior to crPhage dosing. A single dose of $2.7x10^9$ PFU total of each crT7, crT7m and crT4 in 200 µL 1X tris-buffered saline (pH 7.4) was administered by oral gavage to N=3 mice per condition/time point. Animals were sacrificed at various time points and total DNA from whole tissue homogenates was extracted and subjected to qPCR analysis to quantify the amount of crT7 (**Figure 25A**), crT4 (**Figure 25B**) or crT7m (**Figure 25C**) present. Means ± standard error of the mean (SEM) shown are the result of 3 technical replicates from 3 animals.

[0307] As shown in **Figure 25A- Figure 25C**, it was possible to successfully detect each crPhage during transit through the GI tract across and also in feces. By 72 hours, significant quantities of the

crPhage cocktail were not detectable in any tissue, demonstrating that the crPhage is successfully cleared. These data corroborate the observation of loss of crPhages over time after a single intraurethral administration as quantified by phage titration in **Figure 24**. Notably, these data suggest that oral administration of crPhages results in systemic exposure, specifically in blood and liver tissues, as observed following intraurethral administration in **Figure 24**.

Example 22: LeuO enhanced crPhages non-GLP toxicology study

[0308] A test article of crPhage cocktail containing crT7 and crT7m was administered either intravenously ($1.0x10^{11}$ PFU/dose/phage) or by intracatheter instillation into the bladder ($0.5x10^{11}$ PFU/dose/phage) once daily for 7 consecutive days to female Crl:CD-1 mice. crPhages were prepared for an the 7-day toxicology study as described in **Table 18** below:

Table 18: crPhages for 7-day toxicology study.

Study information		Batch information					Dosing information		
Study	**Study type**	**Phage**	**Diluent**	**Production notes**	**PFU/mL**	**EU/mL**	**Dose (mL)**	**# doses**	**RoA**
035938	7-day Toxicology	Cocktail (crT7m/T7)	1X TBS, pH 7.4	PE;CC	1.0E+12 each	Not measured	0.1	7	i.v.
035938	7-day Toxicology	Control	1X TBS, pH 7.4		N/A	<1	0.1	7	i.v.
035938	7-day Toxicology	Cocktail (crT7m/T7)	1X TBS, pH 7.4	PE;CC	1.0E+12 each	Not measured	0.05	7	i.u.
035938	7-day Toxicology	Control	1X TBS, pH 7.4		N/A	<1	0.05	7	i.u.

[0309] The crPhage preparation was prepared using sterile, endotoxin-free 1X tris-buffered saline (pH 7.4). Groups 1 and 2 (9 female mice/group) were dosed with 0.1 mL of vehicle (1X tris-buffered saline, pH 7.4) or test article in a tail vein. Groups 3 and 4 (9 female mice/group) were dosed with 0.05 mL of vehicle or test article into the bladder using a catheter syringe. Group assignment and dosage levels are described in **Table 19** below:

Table 19: Group assignment and dose levels for non-GLP toxicology study.

Dose Group	# of Animals	Test Article	Dose Route	Dose Volume (mL/animal)	# of Animals for Necropsy Day 8
1	9	Vehicle	Intravenous	0.1	6
2	9	crPhage	Intravenous	0.1	6
3	9	Vehicle	Intraurethral	0.05	6
4	9	crPhage	Intraurethral	0.05	6

[0310] Animals were monitored for clinical signs twice daily over the duration of the study. Detailed clinical observations were performed once during the pre-dose period and prior to necropsy on Day 8. Body weights were measured once during the pre-dose period and on Days 1, 3, and 7. Food consumption was measured during the 7-day dosing period. On Day 8, six animals per group were randomly chosen for necropsy, and the remaining three were discarded without

necropsy. At necropsy, body weights were collected from animals fasted for at least 4 hours and were used for calculation of organ weights relative to body weight. Clinical pathology assessments, hematology (3 mice/group) and serum chemistry parameters (3 mice/group), were performed on the day of the scheduled necropsy. Postmortem assessment included necropsy and measurement of selected organ weights. A full tissue list was collected at necropsy. Collected tissues were sectioned with one section frozen in liquid nitrogen (stored frozen at ≤-70 °C) and a second section was preserved in 10% neutral-buffered formalin.

[0311] There were no crPhage-related mortality or morbidity, no effect on body weight, and no abnormal clinical observations for either route of administration. Phage-related effects on hematology were limited to a lower hemoglobin level and decreases in other RBC mass-related parameters, increased reticulocyte counts, and decreased eosinophil count in the crPhage IV-treated group. crPhage-related effects on serum chemistry were limited to higher cholesterol and triglyceride levels in the crPhage IU-treated group. crPhage-related effects on organ weights (absolute and relative to body and brain weights) consisted of increased spleen and kidney weights and decreased lung weights in the crPhage IV-treated group.

[0312] In conclusion, once daily IV or IU administration of crPhage was well tolerated. Possible test article-related effects in the Phage IV-treated group were limited to decreases in red blood cell mass-related parameters, increased reticulocyte counts, decreased eosinophils, and increased spleen, kidney, and decreased lung weights. Possible test article-related effects in the Phage IU-treated group were limited to higher cholesterol and triglyceride levels. The clinical chemistry and organ weight results from this study should be viewed in the context of the small sample sizes used (3 for clinical chemistry and 6 for organ weights) and the possibility that these alterations are artifacts due to small sample size.

Example 23: Identifying a LeuO equivalent in *C. difficile*

[0313] The CRISPR-Cas operon in *C. difficile* is not regulated by LeuO and H-NS as seen in *E. coli*. Rather, regulation is regulated by glucose in ccpA-dependent manner in the absence of ccpA binding site. Notably, the upregulation of CD2983 has been associated with the upregulation of the Cas operon during a nutrient shift. Further, the regulation of CD2983 appears to be controlled by CodY, a global stringent response regulator. The loss of proteins similar in sequence (>40% similarity) in a type I-D system has been shown to result in increased expression off the Cas operon.

[0314] As such, it is possible that CodY and CD2983 are analogous equivalents to HNS and LeuO in *C. difficile*. The similarity between both systems is summarized in **Table 20** below:

Table 20: Summary of characteristics for Cas operon regulation in *E. coli* and *C. difficile.*

Gram Negatives (e.g. *E. coli*)	**Gram Positives (e.g. *C. difficile*)**
Cas regulated by nutrient conditions	Cas regulated by nutrient conditions
HNS global repressor	CodY global repressor
LeuO is regulated by ppGpp	CodY is regulated by GTP, BCAAs
LeuO impacts BCAA biosynthesis	CodY impacts BCAA biosynthesis
LeuO contains N-term HTH	CD2983 contains N-term HTH
LeuO global activator	CD2983 specific regulator?

Example 24: Engineering and validation of a lysogeny module knockout bacteriophage

[0315] Engineering – A plasmid containing homology arms flanking the lysogeny region in φCD24-2 and a counterselective crRNA targeting the lysogeny region was designed in silico and synthesized by BioBasic. The plasmid was transformed into *E. coli* and conjugated into *C. difficile* strain CD19. φCD24-2 was amplified on CD19 carrying the engineering plasmid. The resulting phage population was PCR screened for the presence of engineered phages. If the bulk PCR screen was positive, the lysate was plaqued and individual plaques were screened for the *cI* repressor gene knockout. Pure engineered phage was amplified to high titer for use in validation studies.

[0316] Validation - CD19 culture at mid-log phase was treated with BHI (growth medium, no treatment control), with WT CD24-2 or with ΔcI CD24-2 (i.e. a lysogeny module knockout). **Figure 26A** exemplifies the number of surviving cells (CFU/mL) counted at various time points after the treatment. Surviving cells were further screened for the presence of lysogenized CD24-2. **Figure 26B** exemplifies the % lysogens present at various time points after the treatment.

Example 25: Treatment of a microbiome-related disorder

[0317] To tune a subject's microbiome, a pharmaceutical composition comprising an engineered bacteriophage as described herein can be administered to the subject. The pharmaceutical composition can modulate or kill singular or plural bacterial populations within the microbiome by CRISPR-Cas activity, lytic activity, or a combination thereof.

Example 26: UTI efficacy study illustrating reduction in *E.coli* in bladder and kidney following intraurethral (IU) or intravenous (IV) administration

[0318] Mice colonized with NC101 were treated with saline, a phage cocktail ($2.4x10^{10}$ PFU/mL); or ciprofloxacin. Ciprofloxacin (1-cyclopropyl-6-fluoro-1,4dihydro-4-oxo-7-(1-piperazinyl)-3-quinolinecarboxylic acid hydrochloride) is an antibiotic, while effective, use results in a myriad of side effect and bacterial resistance.

[0319] In this example, tissues were collected after a single dose or after 5 doses and analyzed for CFUs. Phage treatment reduces CFUs in both the bladder (**Figure 30A- Figure 30B**) and kidneys (**Figure 30C – Figure 30D**). The results exemplify that IU and IV delivery of phage reduces CFUs in the bladder. Further, the results exemplify that crPhage cocktail has 1.5- to 3.5-log improved kill over wtPhage cocktail at 120h in the bladder (**Figure 30B**). The results also exemplify that regardless of delivery route, at 120h crPhage cocktail performs comparable with ciprofloxacin in the bladder.

[0320] The study further illustrates route-dependent penetration of phage into different tissues and fluids, such as urine (**Figure 31A**), kidney (**Figure 31B**), bladder (**Figure 31C**), and spleen (**Figure 31D**). The results illustrate that there is measurable phage in the urine regardless of treatment route.

Example 27: UTI efficacy study comparison of research-grade material compared WT versus engineered cocktail via different administration routes

[0321] Mice colonized with NC101 were treated with saline; a phage cocktail ($2.4x10^{10}$ PFU/mL); or ciprofloxacin. Tissues were collected after a single dose or after 5 doses and analyzed for CFUs. Phage treatment reduces CFUs in both the bladder (**Figure 33A- Figure 33B**) and kidneys (**Figure 33C – Figure 33D**). The results exemplify that IU and IV delivery of phage reduces CFUs in the bladder. Further, the results exemplify that high titer crPhage cocktail has 5.1-log improved kill over vehicle at 120 hour in the bladder (**Figure 33B**), and that regardless of the delivery route, a high dose crPhage outperforms ciprofloxacin in the bladder at 120 hours (**Figure 33B**).

Example 28: Clinical trial to assess the safety, pharmacokinetics and potential efficacy of crPhage in E.coli colonized adults

[0322] Phase 1b – Safety, tolerability and PK study is conducted in patients with urinary tract colonization by maximum feasible dose BID via catheter instillation. Pharmacokinetics related to phage persistence time, distribution and elimination is confirmed in the bladder. Bacteriophages that are non-toxic and unable to infect human cells give high therapeutic index.

[0323] Phase 2 – Non-inferiority, 2:1 randomized versus standard of care, double-blind clinical trial is conducted in adult patients with recurrent urinary tract infections (UTI) including MDR and CR strains and in patients with pyelonephritis. IV administration and dosing regimen is evaluated.

[0324] Primary endpoint: resolution of UTI symptoms at end of therapy and test of cure (TOC – 7 days after end of therapy) with demonstration of bacterial pathogen reductions $\leq 10^3$ CFU/mL on urine culture (Microbiological Success) measured at TOC.

[0325] Secondary endpoint: Immunogenicity (presence of anti-phage antibodies), QoL measures (pain/discomfort, etc), durability of response.

[0326] **Figure 34A** is a schematic of an exemplary human study conducted in adults with reoccurring and asymptomatic E.coli colonization of the urinary tract. **Figure 34B** is an exemplary study participant inclusion and exclusion criteria for the UTI Phase 1b study.

Example 29: Phase 1b design in CDAD patients

[0327] Phase 1: Safety, tolerability and PK study is conducted in healthy volunteers (C. diff colonization naturally occurring). 10-14 day oral dosing regimen and 28 day follow-up is evaluated. Safety and tolerability is evaluated. PK analysis of phage and C.difficile in stool is evaluated over time.

[0328] Phase 2: Non-inferiority study, 2:1 randomized, active vs. standard of care (i.e. vancomycin), double blind study in 2nd and 3rd line CDI patients

• Exploratory dose finding

• Active crPhage cocktail vs vancomycin orally for 10-14 days (or, if unresolved, until resolution of symptoms)

• Primary endpoint: time to resolution of diarrhea (TTROD), recurrence of CDAD (time to diarrhea) and grade of diarrhea assessed up to 8 weeks following completion of treatment period, safety and tolerability assessment, PK analysis

• Secondary endpoint: immunogenicity (presence of anti-phage antibodies), additional QoL measures, durability of response

[0329] Phase 3: Efficacy study in larger patient population of first line and recurrent CDI compared to SOC (vancomycin) orally for 10-14 day dosing regimen (40 day study), sub-analysis of front line vs. recurrent patient treatment

• Placebo-controlled double-blinded study targeting initial FDA interactions will determine what expanded data sets are necessary

• If Phase 2 data provides sufficient evidence of efficacy and safety smaller data sets may be sufficient

Example 30: Engineered phages show increased killing against both Type IE and Type IF *E.coli* strains

[0330] For all host range and CFU reduction experiments, the strains and phages were prepared as follows. All experiments were performed in 96 well flat bottom clear plates with total final volume of 200uL in LB with salts (10mM $MgCl_2$ and $CaCl_2$). Three strains were selected to compare WT

and CRISPR phages. Each *E. coli* strain was placed in a microtiter plate either alone, with WTphage (p33s; p46), or with the crPhage (p33s-6; p46cr) at MOI 0.1. For host range experiments, the cultures were incubated at 37°C for 20 hours in a plate reader to monitor growth of populations by optical density (OD; 600 nm). For CFU reduction experiments, the cultures were incubated at 37°C for 24 hours in a shaking incubator and aliquots were taken at (0, 3, 6, and 24 hours).Results are exemplified in **Figure 35A** – **Figure 35F**, and **Figure 36A** – **Figure 36F**.

Example 31: Switching phage cocktails overcomes target bacterial resistance in *E.coli*

[0331] Host range experiments were setup as described above for crCocktail (cr33s, cr46, cr4k) against strains 508 and 527. After 24 hours only strain 508 yielded viable colonies. The colonies were re-streaked 3x to remove any phage. The 508 resistant strains were reevaluated with the crCocktail and WT cocktail 2 (pF0, pJ0, pJc, pE8, pE4, and pJ4) using the same host range protocol. Results are exemplifies in **Figure 37A** – **Figure 37C**.

Example 32: Comparison of wild type phage PB1 and CRISPR-enhanced PB1 against Pseudomonas aeruginosa strains

[0332] A panel of 44 *P. aeruginosa* strains was mixed with either LB, PB1 or cr-PB1 at an MOI of 0.01 (~5 x 10^5 bacteria and 5 x 10^3 phage or LB). Strains + phage or LB were grown for 20 hours at 37 °C and the optical density at 600 nm (OD_{600}) was measured every hour to generate a growth curve. A Riemann sum of the values from t= 4hrs to t=12 hrs were calculated for uninfected, PB1-infected and cr-PB1-infected cultures. Results are exemplified in **Table 21**. The values in the table represent the ratio of the area under the curve (AUC) for PB1 or cr-PB1 (PB.Engineered) compared to the uninfected control. A smaller number represents a larger decrease in optical density. Gray boxes indicate AUC ratios < 0.7. Hit percentage is the percent of strains with an AUC ratio < 0.7.

Table 21

	PB1	cr-PB1
b1031	0.963297	0.870552
b1045	1.717988	1.011726
b1066	0.96017	0.98204
b1046	0.971213	0.969247
b1073	1.024263	1.017944
b1048	0.976147	0.998046
b1074	0.83458	1.072608

b1050	0.999267	1.001961
b1075	0.998144	1.021623
b1051	0.954393	0.890835
b1055	0.860689	1.192755
b1076	0.966672	0.99161
b1052	0.982928	0.947011
b1079	**0.350342**	**0.440958**
b1054	0.879394	0.987756
b1084	1.025938	0.987852
b1034	1.007372	1.028967
b1058	0.953205	1.006118
b1059	0.925737	0.983083
b1035	**0.229912**	**0.20969**
b1061	0.931473	0.993693
b1041	0.970653	0.970961
b1085	1.023776	1.001058
b1126	0.99979	0.969054
b1102	1.013489	0.998923
b1127	**0.136604**	**0.13795**
b1128	**0.183435**	**0.164191**
b1109	0.948729	0.742165
b1138	0.982688	0.967086
b1110	**0.261029**	**0.258299**
b1111	1.035309	0.969425
b1118	1.084256	1.036543
b1233	**0.423587**	**0.253027**
b1112	1.05943	1.041742
b1033	0.989803	1.002004
b1117	1.023928	1.026984
b1099	1.006169	1.150375

b1086	1.019048	1.014256
b1121	**0.129781**	**0.126992**
b1090	1.068717	1.064457
b1122	**0.180161**	**0.181129**
b1092	**0.54725**	**0.532722**
b1125	1.05666	0.986049
b1100	0.763823	0.768105
Hit Percentage	18.75	18.75

[0333] Growth curves: A stationary phase culture of LFP805 was diluted to a concentration of ~10^7 CFU/ml in supplemented LB (LB + 10 mM $MgCl_2$ and 10 mM $CaCl_2$). Stocks of wild-type PB1 and cr-PB1 phage were diluted to a concentration of 10^5 PFU/ml in supplemented LB. Bacterial and phage stocks were mixed 1:1 (MOI of 0.01) and grown in a plate reader with aeration at 37 °C for 20 hours. The OD_{600} was measured every 10 min. Growth curves are exemplified for PB1 and cr-PB1 in **Figure 38A**.

[0334] CFU Reductions: Stationary phase cultures of LFP805 were diluted to an optical density of 1.0 and 10 µL of diluted cultures were added to 180 µL of LB (~10^5 bacteria). PB1 and cr-PB1 were diluted to 10^8 PFU/ml and either 10 µL of phage (10^6 PFU) or 10 µL of LB were added. Cultures were grown with aeration at 37 °C and 10-fold dilutions were plated on LB agar at 4 hrs and 8 hours post-inoculation to determine the CFU/ml in each culture. Calculated CFU/ml at 4 hours and 8 hours are exemplified for uninfected (black bars), wild-type PB1 infected (light gray bars) or cr-PB1 phage infected cultures (medium gray bars) in **Figure 38B**. CFU were compared between PB1 and cr-PB1 using a t test with Holm-Sidak comparison. * = $p < 0.05$, *** = $p < 0.001$

Example 33: Plasmid based killing of *E.coli* and *P.aeruginosa* by Type I CRISPR-Cas systems

[0335] E.coli Type I testing: Five Type-I Cas systems were cloned from bacteria gDNA into pUCP19. Corresponding E.coli (BL21) targeting spacers were cloned into a second compatible plasmid (pRSF1b). Bl21 electrocompetent cells were transformed with each Cas system plasmid and targeting spacer or pRSF1b control. The transformations were diluted and spot plated on Kan/Carb LB plates. CFUs were counted after overnight incubation at 37C. Results are exemplified in **Figure 39A**.

[0336] P. aeruginosa Type-I Testing: Four Type-I Cas systems were cloned from bacteria gDNA into pUCP19 along with a pseudomonas targeting spacer. Electrocompetent PA01 cells were made

competent using the Locus lab protocol. The cells were transformed with the Cas + spacer plasmid or Cas plasmid. The transformations were diluted and spot plated on Carb300 LB plates. CFUs were counted after overnight incubation at 37C. Results are exemplified in **Figure 39B**. Results exemplify that 3 of 4 systems were able to successfully target PA01 with a minimum 3-log reduction. 1 system showed no CFUs for either plasmid.

[0337] While preferred embodiments of the present disclosure have been shown and described herein, it will be obvious to those skilled in the art that such embodiments are provided by way of example only. Numerous variations, changes, and substitutions will now occur to those skilled in the art without departing from the disclosure. It should be understood that various alternatives to the embodiments of the disclosure described herein are employed in practicing the disclosure. It is intended that the following claims define the scope of the disclosure and that methods and structures within the scope of these claims and their equivalents be covered thereby.

CLAIMS

WHAT IS CLAIMED IS:

1. A method for killing a target bacterium comprising:

introducing into a target bacterium a bacteriophage comprising:

(a) a first nucleic acid sequence encoding a spacer sequence or a crRNA transcribed therefrom, wherein the spacer sequence is complementary to a target nucleotide sequence from a target gene in the target bacterium; and

(b) a gene that is capable of inducing lysis of the target bacterium,

wherein the target bacterium is killed by lytic activity of the bacteriophage or activity of a CRISPR-Cas system using the spacer sequence or the crRNA transcribed therefrom.

2. The method of claim 1, wherein the first nucleic acid sequence is a CRISPR array further comprising at least one repeat sequence.
3. The method of claim 1 or 2, wherein the bacteriophage further comprises a second nucleic acid encoding a transcriptional activator for the CRISPR-Cas system.
4. The method of any one of claims 1-3, wherein the gene is endogenous to the bacteriophage.
5. The method of any one of claims 1-3, wherein the gene is exogenous to the bacteriophage.
6. The method of any one of claims 3-5, wherein the transcriptional activator is regulated by Quorum Sensing (QS) signals.
7. The method of any one of claims 3-5, wherein the transcriptional activator is a protein involved in sensing stress of a bacterium membrane.
8. The method of claim 7, wherein the protein involved in sensing stress is response regulator BaeSR.
9. The method of any one of claims 3-5, wherein the transcriptional activator is a protein that stabilizes Cas.
10. The method of claim 9, wherein the protein that stabilizes Cas is heat shock protein G (HtpG).
11. The method of any one of claims 3-5, wherein the transcriptional activator is a metabolic sensing protein.
12. The method of claim 11, wherein the metabolic sensing protein is cAMP receptor protein (CRP).
13. The method of claim 12, wherein the CRP is sensitive to cyclic AMP (cAMP).
14. The method of claim 11, wherein the metabolic sensing protein is a sigma factor.
15. The method of claim 14, wherein the sigma factor is RpoN (σ^{54}).
16. The method of any one of claims 3-5, wherein the transcriptional activator disrupts the activity of an inhibitory element.

17. The method of claim 16, wherein the inhibitory element comprises heat-stable nucleoid-structuring protein (H-NS), leucine responsive regulatory protein (LRP), or CodY.
18. The method of claim 16, wherein the inhibitory element is a transcriptional repressor.
19. The method of claim 18, wherein the transcriptional repressor is a global transcriptional repressor.
20. The method of any one of claims 3-19, wherein the transcriptional activator comprises LeuO or a polypeptide having at least 75% sequence homology with SEQ ID NO: 1.
21. The method of any one of claims 3-19, wherein the transcriptional activator comprises CD2983 or a polypeptide having at least 75% sequence homology with SEQ ID NO: 2.
22. The method of any one of claims 1-21, wherein the CRISPR-Cas system is endogenous to the target bacterium.
23. The method of any one of claims 1-21, wherein the CRISPR-Cas system is exogenous to the target bacterium.
24. The method of any one of claims 1-23, wherein the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system.
25. The method of claim 24, wherein the CRISPR-Cas system comprises the type I CRISPR-Cas system.
26. The method of any one of claims 1-25, wherein the target nucleotide sequence comprises all or a part of a promoter sequence for the target gene.
27. The method of any one of claims 1-26, wherein the target nucleotide sequence comprises all or a part of a nucleotide sequence located on a coding strand of a transcribed region of the target gene.
28. The method of any one of claims 1-27, wherein the target nucleotide sequence is at least a portion of an essential gene that is needed for the survival of the target bacterium.
29. The method of claim 28, wherein the essential gene is *Tsf*, a*cpP*, *gapA*, *infA*, *secY*, *csrA*, *trmD*, *ftsA*, *fusA*, *glyQ*, *eno*, or *nusG*.
30. The method of any one of claims 2-29, wherein the at least one repeat sequence is operably linked to the spacer sequence at either its 5' end or its 3' end.
31. The method of any one of claims 1-30, wherein the target bacterium is killed solely by the lytic activity of the bacteriophage.
32. The method of any one claims of 1-30, wherein the target bacterium is killed solely by the activity of the CRISPR-Cas system.
33. The method of any one of claims 1-30, wherein the target bacterium is killed by both the lytic activity of the bacteriophage and the activity of the CRISPR-Cas system in combination.
34. The method of any one of claims 1-30, wherein the target bacterium is killed by the activity of the CRISPR-Cas system independently of the lytic activity of the bacteriophage.

35. The method of claim 33, wherein the activity of the CRISPR-Cas system supplements or enhances the lytic activity of the bacteriophage.
36. The method of any one of claims 1-35, wherein the spacer sequence overlaps with a second spacer sequence.
37. The method of any one of claims 1-36, wherein the lytic activity of the bacteriophage and the activity of the CRISPR-Cas system are synergistic.
38. The method of any one of claims 1-37, wherein the lytic activity of the bacteriophage, the activity of the CRISPR-Cas system, or both is modulated by a concentration of the bacteriophage.
39. The method of any one of claims 1-38, wherein the bacteriophage infects multiple bacterial strains.
40. The method of any one of claims 1-39, wherein the bacteriophage is an obligate lytic bacteriophage.
41. The method of any one of claims 1-39, wherein the bacteriophage is a temperate bacteriophage that is rendered lytic.
42. The method of any one of claims 1-41, wherein the bacteriophage does not confer any new properties onto the target bacterium beyond cellular death caused by the lytic activity of the bacteriophage and/or the activity of the CRISPR-Cas array.
43. The method of any one of claims 1-42, wherein the target bacterium is *C. difficile*.
44. The method of claim 43, wherein the bacteriophage is ϕCD146 or ϕCD24-2.
45. The method of any one of claims 1-42, wherein the target bacterium is *E. coli*.
46. The method of claim 45, wherein the bacteriophage is T4, T7, or T7m.
47. The method of any one of claims 1-46, wherein the first nucleic acid encoding a spacer sequence or a crRNA is inserted into a non-essential bacteriophage gene.
48. The method of claim 47, wherein the non-essential gene is *gp49*.
49. The method of claim 47, wherein the non-essential gene is *gp75*.
50. The method of claim 47, wherein the non-essential gene is *hoc*.
51. The method of claim 47, wherein the non-essential gene is *gp0.7, gp4.3, gp4.5,* or *gp4.7*.
52. The method of claim 47, wherein the non-essential gene is *gp0.6, gp0.65, gp0.7, gp4.3,* or *gp4.5*.
53. A bacteriophage comprising:

(a) a first nucleic acid encoding a spacer sequence or a crRNA transcribed therefrom, wherein the spacer sequence is complementary to a target nucleotide sequence from a target gene in a target bacterium; and

(b) a gene that is capable of inducing lysis of the target bacterium,

wherein the target bacterium is killed by the lytic activity of the bacteriophage or activity of a CRISPR-Cas system using the spacer sequence or the crRNA transcribed therefrom.

54. The bacteriophage of claim 53, further comprising a second nucleic acid encoding a transcriptional activator for the CRISPR-Cas system.
55. The bacteriophage of claim 54, wherein the transcriptional activator is regulated by Quorum Sensing (QS) signals.
56. The bacteriophage of claim 54, wherein the transcriptional activator is a protein involved in sensing stress of a bacterium membrane.
57. The bacteriophage of claim 56, wherein the protein is response regulator BaeSR.
58. The bacteriophage of claim 54, wherein the transcriptional activator is a protein that stabilizes Cas.
59. The bacteriophage of claim 58, wherein the protein that stabilizes Cas is heat shock protein G (HtpG).
60. The bacteriophage of claim 54, wherein the transcriptional activator is a metabolic sensing protein.
61. The bacteriophage of claim 60, wherein the metabolic sensing protein is cAMP receptor protein (CRP).
62. The bacteriophage of claim 61, wherein the CRP is sensitive to cyclic AMP (cAMP).
63. The bacteriophage of claim 60, wherein the metabolic sensing protein is a sigma factor.
64. The bacteriophage of claim 63, wherein the sigma factor is RpoN (σ^{54}).
65. The bacteriophage of claim 54, wherein the transcriptional activator disrupts the activity of an inhibitory element of the target bacterium.
66. The bacteriophage of claim 65, wherein the inhibitory element is heat-stable nucleoid-structuring protein (H-NS), leucine responsive regulatory protein (LRP), or CodY.
67. The bacteriophage of claim 65, wherein the inhibitory element is a transcriptional repressor.
68. The bacteriophage of claim 67, wherein the transcriptional repressor is a global transcriptional repressor.
69. The bacteriophage of any one of claims 54-68, wherein the transcriptional activator comprises LeuO or a polypeptide having at least 75% sequence homology with SEQ ID NO: 1.
70. The bacteriophage of any one of claims 54-68, wherein the transcriptional activator comprises CD2983 or a polypeptide having at least 75% sequence homology with SEQ ID NO: 2.
71. The bacteriophage of any one of claims 53-70, wherein the CRISPR-Cas system is endogenous to the target bacterium.
72. The bacteriophage of any one of claims 53-70, wherein the CRISPR-Cas system is exogenous to the target bacterium.
73. The bacteriophage of any one of claims 53-72, wherein the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system.
74. The bacteriophage of claim 73, wherein the CRISPR-Cas system is a type I CRISPR-Cas system.

75. The bacteriophage of any one of claims 53-74, wherein the target nucleotide sequence comprises all or a part of a promoter sequence for the target gene.
76. The bacteriophage of any one of claims 53-75, wherein the target nucleotide sequence comprises all or a part of a nucleotide sequence located on a coding strand of a transcribed region of the target gene.
77. The bacteriophage of any one of claims 53-76, wherein the target nucleotide sequence is in an essential gene.
78. The bacteriophage of claim 77, wherein the essential gene is *Tsf*, a*cpP*, *gapA*, *infA*, *secY*, *csrA*, *trmD*, *ftsA*, *fusA*, *glyQ*, *eno*, or *nusG*.
79. The bacteriophage of any one of claims 53-76, wherein the target nucleotide sequence is a non-essential gene.
80. The bacteriophage of any one of claims 53-79, wherein the first nucleic acid sequence is a CRISPR array comprising at least one repeat sequence.
81. The bacteriophage of claim 80, wherein the at least one repeat sequence is operably linked to the spacer sequence at either its 5' end or its 3' end.
82. The bacteriophage of any one of claims 53-81, wherein the bacteriophage infects multiple bacterial strains.
83. The bacteriophage of any one of claims 53-82, wherein the bacteriophage is an obligate lytic bacteriophage.
84. The bacteriophage of any one of claims 53-82, wherein the bacteriophage is a temperate bacteriophage that is rendered lytic.
85. The bacteriophage of claim 84, wherein the temperate bacteriophage is rendered lytic by the removal, replacement, or inactivation of one or more lysogeny genes.
86. The bacteriophage of any one of claims 53-85, wherein the target bacterium is *C. difficile*.
87. The bacteriophage of claim 86, wherein the bacteriophage is ϕCD146 or ϕCD24-2.
88. The bacteriophage of any one of claims 53-85, wherein the target bacterium is *E. coli*.
89. The bacteriophage of claim 88, wherein the bacteriophage is T4, T7, or T7m.
90. The bacteriophage of any one of claims 53-89, wherein the first nucleic acid encoding a spacer sequence or a crRNA is inserted into a non-essential gene.
91. The bacteriophage of any one of claims 53-90, wherein the non-essential gene is *gp49*.
92. The bacteriophage of any one of claims 53-90, wherein the non-essential gene is *gp75*.
93. The bacteriophage of any one of claims 53-90, wherein the non-essential gene is *hoc*.
94. The bacteriophage of any one of claims 53-90, wherein the non-essential gene is *gp0.7*, *gp4.3*, *gp4.5*, or *gp4.7*.

95. The bacteriophage of any one of claims 53-90, wherein the non-essential gene is *gp0.6, gp0.65, gp0.7, gp4.3,* or *gp4.5*.

96. A method for modulating the activity of a CRISPR-Cas system in a target bacterium, comprising: introducing a bacteriophage comprising a nucleic acid encoding a transcriptional activator for the CRISPR-Cas system in the target bacterium.

97. The method of claim 96, wherein the transcriptional activator is regulated by Quorum Sensing (QS) signals.

98. The method of claim 96, wherein the transcriptional activator is a protein involved in sensing stress to a bacterium membrane.

99. The method of claim 98, wherein the protein involved in sensing stress is response regulator BaeSR.

100. The method of claim 96, wherein the transcriptional activator is a protein that stabilizes Cas.

101. The method of claim 100, wherein the protein that stabilizes Cas is heat shock protein G (HtpG).

102. The method of claim 96, wherein the transcriptional activator is a metabolic sensing protein.

103. The method of claim 102, wherein the metabolic sensing protein is cAMP receptor protein (CRP).

104. The method of claim 103, wherein the CRP is sensitive to cyclic AMP (cAMP).

105. The method of claim 102, wherein the metabolic sensing protein is a sigma factor.

106. The method of claim 105, wherein the sigma factor is RpoN (σ54).

107. The method of any one of claims 96-106, wherein the transcriptional activator disrupts the activity of an inhibitory element.

108. The method of claim 107, wherein the inhibitory element is heat-stable nucleoid-structuring protein (H-NS), leucine responsive regulatory protein (LRP), or CodY.

109. The method of any one of claims 107-108, wherein the inhibitory element is a transcriptional repressor.

110. The method of claim 109, wherein the transcriptional repressor is a global transcriptional repressor.

111. The method of any one of claims 96-110, wherein the transcriptional activator comprises LeuO or a polypeptide having at least 75% sequence homology with SEQ ID NO: 1.

112. The method of any one of claims 96-110, wherein the transcriptional activator comprises CD2983 or a polypeptide having at least 75% sequence homology with SEQ ID NO: 2.

113. The method of any one of claims 96-112, wherein the CRISPR-Cas system is endogenous to the target bacterium.

114. The method of any one of claims 96-112, wherein the CRISPR-Cas system is exogenous to the target bacterium.

115. The method of any one of claims 96-114, wherein the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system.

116. The method of claim 115, wherein the CRISPR-Cas system is a type I CRISPR-Cas system.

117. The method of any one of claims 96-116, wherein the bacteriophage infects multiple bacterial strains.

118. The method of any one of claims 96-117, wherein the bacteriophage is an obligate lytic bacteriophage.

119. The method of any one of claims 96-117, wherein the bacteriophage is a temperate bacteriophage that is rendered lytic.

120. The method of any one of claims 96-119, wherein the target bacterium is *C. difficile*.

121. The method of claim 120, wherein the bacteriophage is ϕCD146 or ϕCD24-2.

122. The method of any one of claims 96-119, wherein the target bacterium is *E. coli*.

123. The method of claim 122, wherein the bacteriophage is T4, T7, or T7m.

124. The method of any one of claims 96-123, wherein the nucleic acid encoding a transcriptional activator is inserted into a non-essential bacteriophage gene.

125. The method of any one of claims 96-124, wherein the non-essential gene is *gp49*.

126. The method of any one of claims 96-124, wherein the non-essential gene is *gp75*.

127. The method of any one of claims 96-124, wherein the non-essential gene is *hoc*.

128. The method of any one of claims 96-124, wherein the non-essential gene is *gp0.7, gp4.3, gp4.5,* or *gp4.7*.

129. The method of any one of claims 96-124, wherein the non-essential gene is *gp0.6, gp0.65, gp0.7, gp4.3,* or *gp4.5*.

130. A bacteriophage comprising a nucleic acid encoding a transcriptional activator for a CRISPR-Cas system in a target bacterium.

131. The bacteriophage of claim 130, wherein the transcriptional activator is regulated by Quorum Sensing (QS) signals.

132. The bacteriophage of claim 130, wherein the transcriptional activator is a protein involved in sensing stress to a bacterium membrane.

133. The bacteriophage of claim 132, wherein the protein involved in sensing stress is response regulator BaeSR.

134. The bacteriophage of claim 130, wherein the transcriptional activator is a protein that stabilizes Cas.

135. The bacteriophage of claim 134, wherein the protein that stabilizes Cas is heat shock protein G (HtpG).

136. The bacteriophage of claim 130, wherein the transcriptional activator is a metabolic sensing protein.

137. The bacteriophage of claim 136, wherein the metabolic sensing protein is cAMP receptor protein (CRP).

138. The bacteriophage of claim 137, wherein the CRP is sensitive to cyclic AMP (cAMP).

139. The bacteriophage of claim 136, wherein the metabolic sensing protein is a sigma factor.

140. The bacteriophage of claim 139, wherein the sigma factor is RpoN (σ54).

141. The bacteriophage of any one of claims 130-140, wherein the transcriptional activator disrupts the activity of an inhibitory element.

142. The bacteriophage of claim 141, wherein the inhibitory element is heat-stable nucleoid-structuring protein (H-NS), leucine responsive regulatory protein (LRP), or CodY.

143. The bacteriophage of any one of claims 141-142, wherein the inhibitory element is a transcriptional repressor.

144. The bacteriophage of claim 143, wherein the transcriptional repressor is a global transcriptional repressor.

145. The bacteriophage of any one of claims 130-144, wherein the transcriptional activator comprises LeuO or a polypeptide having at least 75% sequence homology with SEQ ID NO: 1.

146. The bacteriophage of any one of claims 130-144, wherein the transcriptional activator comprises CD2983 or a polypeptide having at least 75% sequence homology with SEQ ID NO: 2.

147. The bacteriophage of any one of claims 130-146, wherein the CRISPR-Cas system is endogenous to the target bacterium.

148. The bacteriophage of any one of claims 130-146, wherein the CRISPR-Cas system is exogenous to the target bacterium.

149. The bacteriophage of any one of claims 130-148, wherein the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system.

150. The bacteriophage of claim 149, wherein the CRISPR-Cas system is a type I CRISPR-Cas system.

151. The bacteriophage of any one of claims 130-150, wherein the bacteriophage infects multiple bacterial strains.

152. The bacteriophage of any one of claims 130-151, wherein the bacteriophage is an obligate lytic bacteriophage.

153. The bacteriophage of any one of claims 130-151, wherein the bacteriophage is a temperate bacteriophage that is rendered lytic.

154. The bacteriophage of any one of claims 130-153, wherein the target bacterium is *C. difficile.*

155. The bacteriophage of claim 154, wherein the bacteriophage is ϕCD146 or ϕCD24-2.

156. The bacteriophage of any one of claims 130-153, wherein the target bacterium is *E. coli.*

157. The bacteriophage of claim 156, wherein the bacteriophage is T4, T7, or T7m.

158. The bacteriophage of any one of claims 130-157, wherein the nucleic acid encoding a transcriptional activator is inserted into a non-essential bacteriophage gene.

159. The bacteriophage of any one of claims 130-158, wherein the non-essential gene is *gp49.*

160. The bacteriophage of any one of claims 130-158, wherein the non-essential gene is *gp75.*

161. The bacteriophage of any one of claims 130-158, wherein the non-essential gene is *hoc.*

162. The bacteriophage of any one of claims 130-158, wherein the non-essential gene is *gp0.7, gp4.3, gp4.5*, or *gp4.7.*

163. The bacteriophage of any one of claims 130-158, wherein the non-essential gene is *gp0.6, gp0.65, gp0.7, gp4.3*, or *gp4.5.*

164. A method of killing a target bacterium, comprising introducing into a target bacterium a bacteriophage comprising:

(a) lytic activity, and

(b) a first nucleic acid sequence encoding an anti-CRISPR polypeptide,

wherein the anti-CRISPR polypeptide enhances the lytic activity of the bacteriophage.

165. The method of claim 164, wherein the anti-CRISPR polypeptide inactivates a CRISPR-Cas system.

166. The method of claim 165, wherein the anti-CRISPR polypeptide inactivates the CRISPR-Cas system using a process comprising gene regulation interference.

167. The method of any one of claims 165-166, wherein the anti-CRISPR polypeptide inactivates the CRISPR-Cas system using a process comprising nuclease recruitment interference.

168. The method of any one of claims 165-167, wherein the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system.

169. The method of claim 168, wherein the CRISPR-Cas system is a type I CRISPR-Cas system.

170. The method of any one of claims 164-169, wherein the anti-CRISPR polypeptide binds directly or indirectly to a Cascade or Cascade-like complex.

171. The method of any one of claims 164-170, wherein the anti-CRISPR polypeptide is a truncated protein, a fusion protein, a dimer protein, or a mutated protein.

172. The method of any one of claims 164-171, wherein the bacteriophage further comprises a second nucleic acid encoding a CRISPR array.

173. The method of claim 172, wherein the CRISPR array comprises at least one repeat sequence and at least one spacer sequence that is complementary to a target nucleotide sequence from a target gene in the target bacterium.

174. A bacteriophage comprising:

(c) lytic activity, and

(d) a first nucleic acid sequence encoding an anti-CRISPR polypeptide,

wherein the anti-CRISPR polypeptide enhances the lytic activity of the bacteriophage.

175. The bacteriophage of claim 174, wherein the anti-CRISPR polypeptide inactivates a CRISPR-Cas system.

176. The bacteriophage of claim 175, wherein the anti-CRISPR polypeptide inactivates the CRISPR-Cas system using a process comprising gene regulation interference.

177. The bacteriophage of claim 175 or 176, wherein the anti-CRISPR polypeptide inactivates the CRISPR-Cas system using a process comprising nuclease recruitment interference.

178. The bacteriophage of any one of claims 175-177, wherein the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system.

179. The bacteriophage of claim 178, wherein the CRISPR-Cas system is a type I CRISPR-Cas system.

180. The bacteriophage of any one of claims 174-179, wherein the anti-CRISPR polypeptide binds directly or indirectly to a Cascade or Cascade-like complex.

181. The bacteriophage of any one of claims 174-180, wherein the anti-CRISPR polypeptide is a truncated protein, a fusion protein, a dimer protein or mutated protein.

182. The bacteriophage of any one of claims 174-180, wherein the bacteriophage further comprises a second nucleic acid encoding a CRISPR array.

183. The bacteriophage of claim 182, wherein the CRISPR array comprises at least one repeat sequence and at least one spacer sequence that is complementary to a target nucleotide sequence from a target gene in a target bacterium.

184. A method of treating a disease in a subject comprising administering the bacteriophage of any one of claims 53-95, 130-163 or 174-183 to the subject.

185. The method of claim 184, wherein the subject is a mammal.

186. The method of any one of claims 184-185, wherein the disease is a bacterial infection.

187. The method of claim 186, wherein a bacterium causing the bacterial infection is an *Acinetobacter* species, an *Actinomyces* species, *Burkholderia cepacia* complex, a *Campylobacter*

species, a *Candida* species, *Clostridium difficile*, *Corynebacterium minutissium, Corynebacterium pseudodiphtheriae, Corynebacterium stratium, Corynebacterium group G1, Corynebacterium group G2, Enterobacteriaceae*, an *Enterococcus* species, *Escherichia coli, Haemophilus influenzae, Klebsiella pneumoniae,* a *Moraxella* species, *Mycobacterium tuberculosis* complex, *Neisseria gonorrhoeae*, *Neisseria meningitidis*, a non-tuberculous mycobacteria species, a *Porphyromonas* species, *Prevotella melaninogenicus,* a *Pseudomonas* species, *Salmonella typhimurium, Serratia marcescens, Staphylococcus aureus*, *Streptococcus agalactiae*, *Staphylococcus epidermidis, Staphylococcus salivarius, Streptococcus mitis, Streptococcus sanguis, Streptococcus pneumoniae*, *Streptococcus pyogenes*, *Vibrio cholerae*, a *Coccidioides* species, a *Cryptococcus* species, *Helicobacter felis, Helicobacter pylori*, *Clostridium bolteae,* and any combination thereof.

188. The method of claim 187, wherein the bacterium is a drug resistant bacterium that is resistant to at least one antibiotic.

189. The method of claim 187 or 188, wherein the bacterium is a multi-drug resistant bacterium that is resistant to at least one antibiotic.

190. The method of claim 188 or 189, wherein the antibiotic comprises a cephalosporin, a fluoroquinolone, a carbapenem, a colistin, an aminoglycoside, vancomycin, streptomycin, or methicillin.

191. The method of any one of claims 184-190, wherein the administering is intra-arterial, intravenous, intramuscular, oral, subcutaneous, inhalation, or any combination thereof.

192. A pharmaceutical composition comprising:

a. a bacteriophage of any one of claims 53-95, 130-163 or 174-183; and

b. a pharmaceutically acceptable excipient.

193. The pharmaceutical composition of claim 192, that is in a form of a tablet, a liquid, a syrup, an oral formulation, an intravenous formulation, an intranasal formulation, an ocular formulation, an otic formulation, a subcutaneous formulation, an inhalable respiratory formulation, a suppository, and any combination thereof.

194. A method of killing a target bacterium, the method comprising:

introducing a temperate bacteriophage into the target bacterium,

wherein a lysogeny gene of the temperate bacteriophage is removed, replaced, or inactivated, and the temperate bacteriophage is rendered lytic, thereby killing the target bacterium.

195. The method of claim 194, wherein the lysogeny gene comprises *cI* phage repressor gene.

196. The method of claim 194 or 195, wherein the bacteriophage infects multiple bacterial strains.

197. The method of any one of claims 194-196, wherein the target bacterium is *C. difficile.*

198. The method of claim 197, wherein the bacteriophage is ϕCD146 or ϕCD24-2.

199. The method of any one of claims 194-198, wherein the bacteriophage further comprises a first nucleic acid encoding a spacer sequence or a crRNA transcribed therefrom.

200. The method of claim 199, wherein the spacer sequence is complementary to a target nucleotide sequence from a target gene in the target bacterium.

201. The method of claim 199 or 200, wherein the first nucleic acid sequence is a CRISPR array further comprising at least one repeat sequence.

202. The method of claim 201, wherein the at least one repeat sequence is operably linked to the spacer sequence at either its 5' end or its 3' end.

203. The method of any one of claims 199-202, wherein the bacteriophage further comprises a second nucleic acid encoding a transcriptional activator for a CRISPR-Cas system.

204. The method of claim 203, wherein the CRISPR-Cas system is endogenous to the target bacterium.

205. The method of claim 203, wherein the CRISPR-Cas system is exogenous to the target bacterium.

206. The method of any one of claims 203-205, wherein the CRISPR-Cas system is a type I CRISPR-Cas system, a type II CRISPR-Cas system, or a type III CRISPR-Cas system.

207. The method of any one of claims 203-205, wherein the CRISPR-Cas system comprises a type I CRISPR-Cas system.

208. The method of any one of claims 200-207, wherein the target nucleotide sequence comprises all or a part of a promoter sequence for the target gene.

209. The method of any one of claims 200-207, wherein the target nucleotide sequence comprises all or a part of a nucleotide sequence located on a coding strand of a transcribed region of the target gene.

210. The method of any one of claims 200-207, wherein the target nucleotide sequence comprises at least a portion of an essential gene that is needed for survival of the target bacterium.

211. The method of any one of claims 203-210, wherein the target bacterium is killed by both lytic activity of the bacteriophage and activity of the CRISPR-Cas system in combination.

212. The method of claim 211, wherein activity of the CRISPR-Cas system supplements or enhances lytic activity of the bacteriophage.

213. The method of any one of claims 203-210, wherein the target bacterium is killed by activity of the CRISPR-Cas system independently of lytic activity of the bacteriophage.

214. The method of any one of claims 203-210, wherein lytic activity of the bacteriophage and activity of the CRISPR-Cas system are synergistic.

215. The method of any one of claims 194-214, wherein lytic activity of the bacteriophage, activity of the CRISPR-Cas system, or both is modulated by a concentration of the bacteriophage.

216. A pharmaceutical composition comprising: (a) a temperate bacteriophage, wherein a lysogeny gene of the temperate bacteriophage is removed, replaced, or inactivated; and (b) a pharmaceutically acceptable excipient.

217. The pharmaceutical composition of claim 216, that is in a form of a tablet, a liquid, a syrup, an oral formulation, an intravenous formulation, an intranasal formulation, an ocular formulation, an otic formulation, a subcutaneous formulation, an inhalable respiratory formulation, a suppository, and any combination thereof.

218. A method of tuning the microbiome of a subject, the method comprising: administering to the subject the pharmaceutical composition of any one of claims 192-193 or 216-217.

219. The method of any one of claims 187-191, wherein the bacterium is Pseudomonas.

220. The method of any one of claims 187-191, wherein the bacterium is staphylococcus.

221. The method of any one of claims 187-191 or 219-220, wherein the bacterium is methicillin resistant.

222. The method of any one of claims 187-191 or 221, wherein the bacterium is methicillin resistant staphylococcus aureus.

223. The method of any one of claims 187-191, wherein the bacterium is multidrug resistant Pseudomonas Aeruginosa.

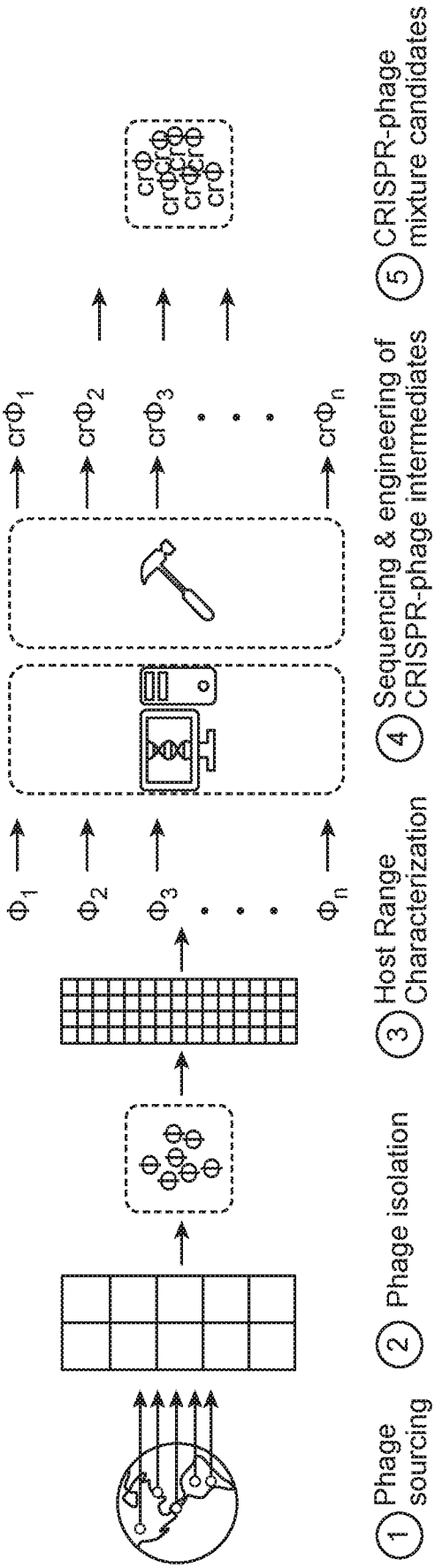
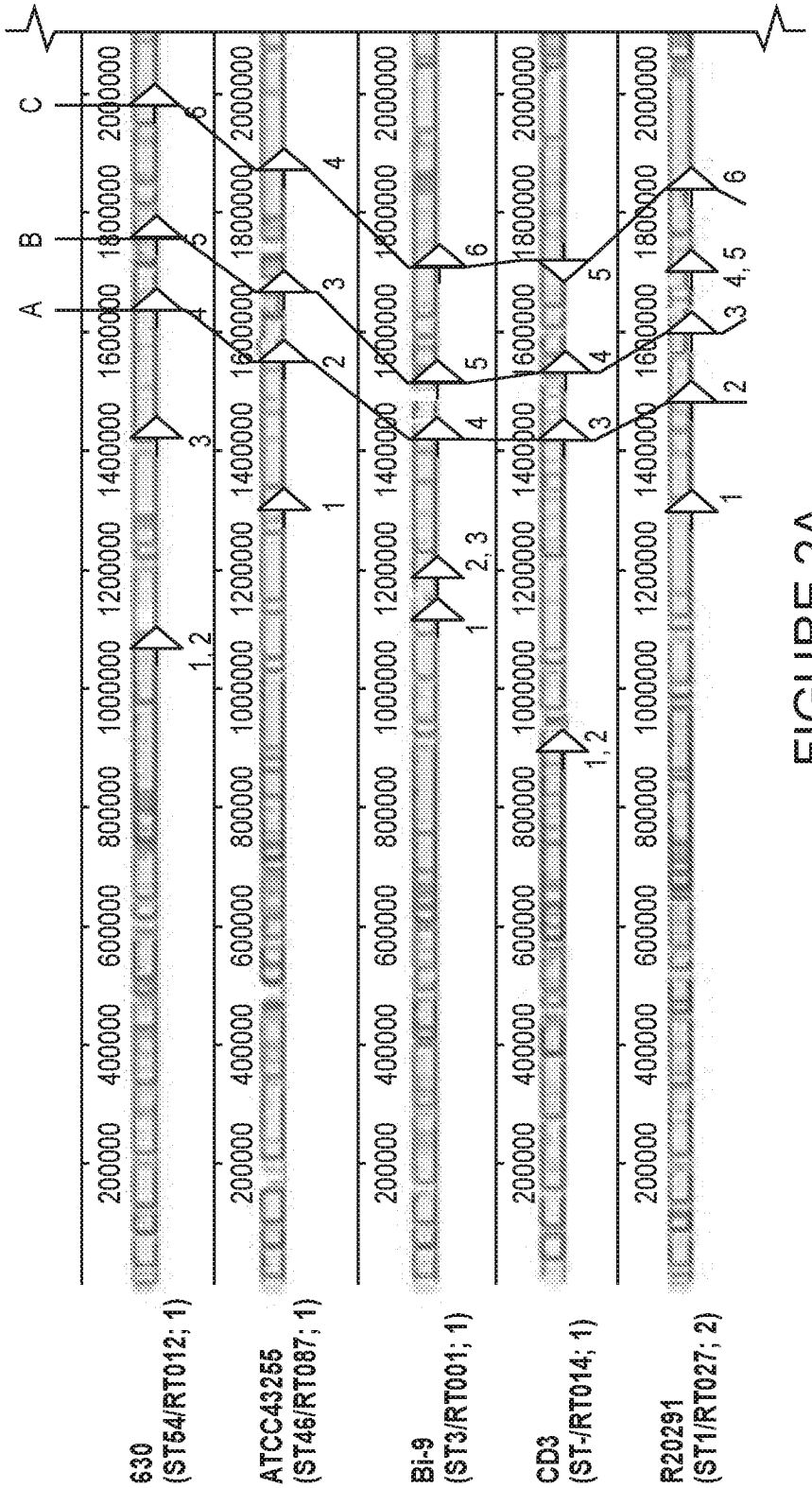

1 Phage sourcing
2 Phage isolation
3 Host Range Characterization
4 Sequencing & engineering of CRISPR-phage intermediates
5 CRISPR-phage mixture candidates
Φ1
Φ2
Φ3
Φn
crΦ1
crΦ2
crΦ3
crΦn

FIGURE 1


A
B
C
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400000
600000
800000
1000000
1200000
1400000
1600000
1800000
2000000
630
(ST54/RT012; 1)
ATCC43255
(ST46/RT087; 1)
Bi-9
(ST3/RT001; 1)
CD3
(ST-/RT014; 1)
R20291
(ST1/RT027; 2)

FIGURE 2A

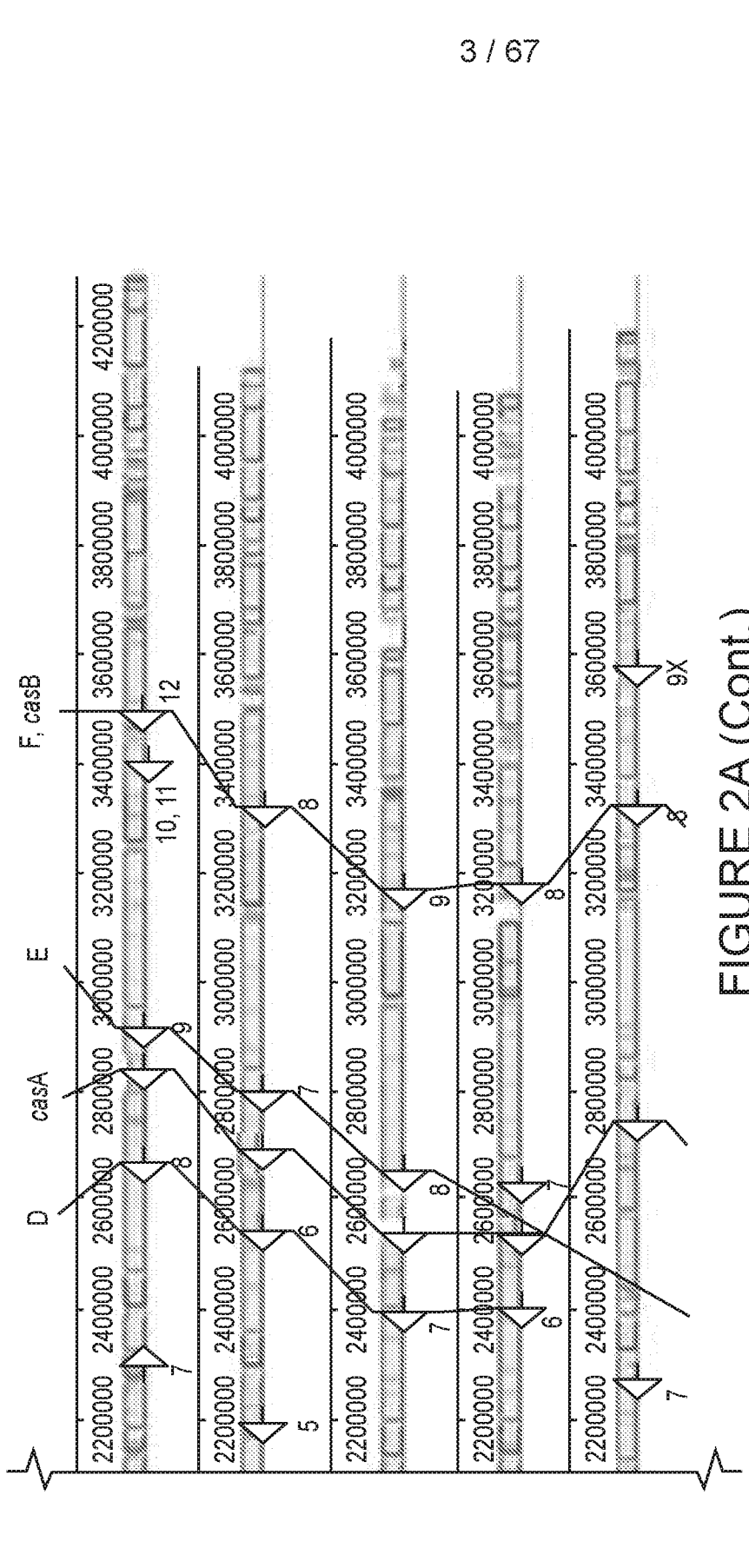


FIGURE 2A (Cont.)

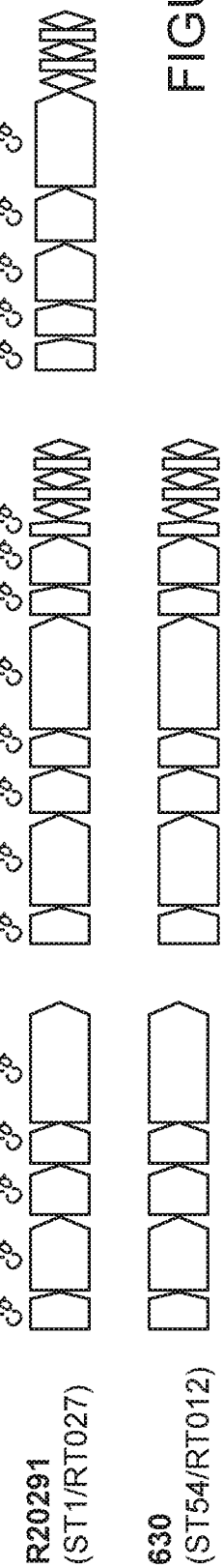


FIGURE 2B

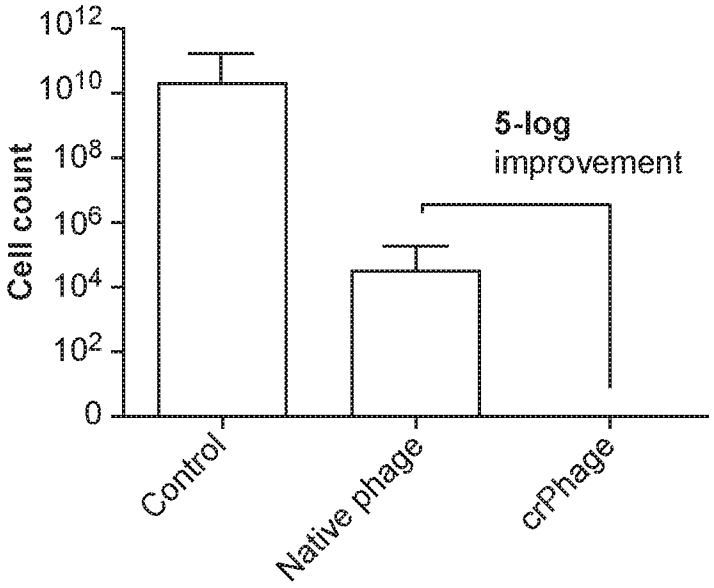


FIGURE 3A

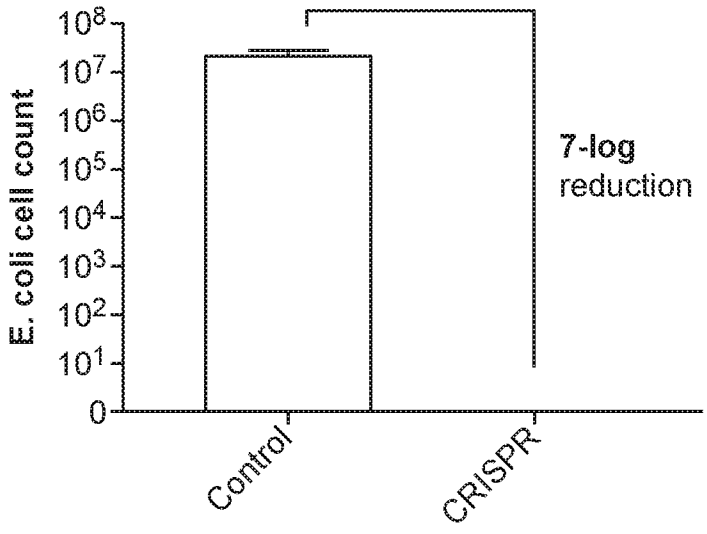


FIGURE 3B

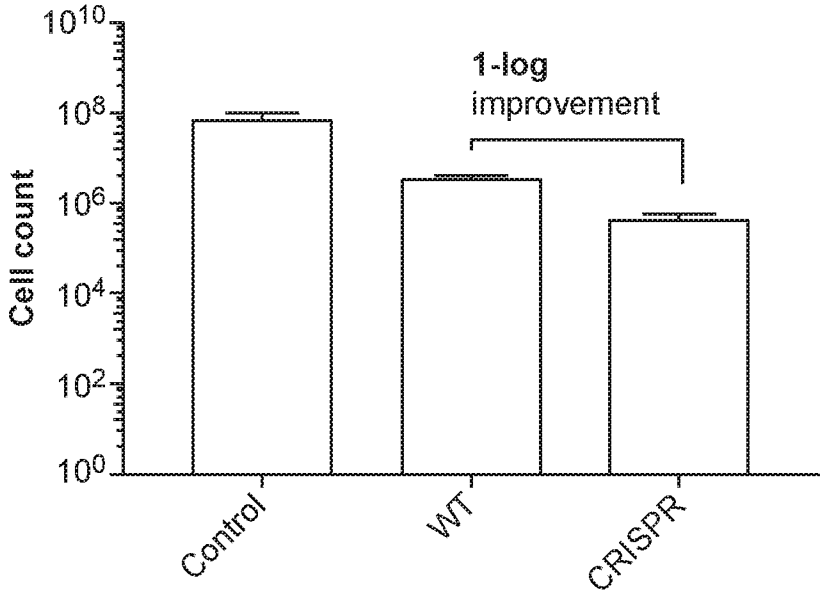


FIGURE 4A

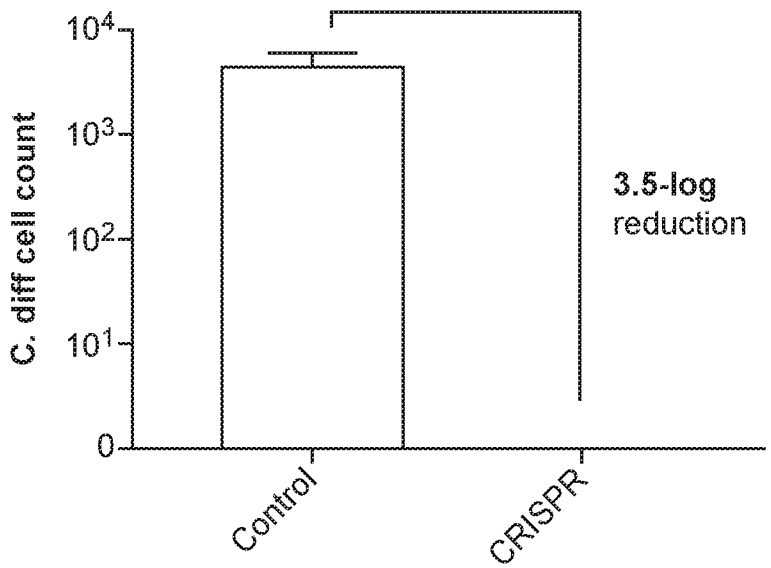


FIGURE 4B

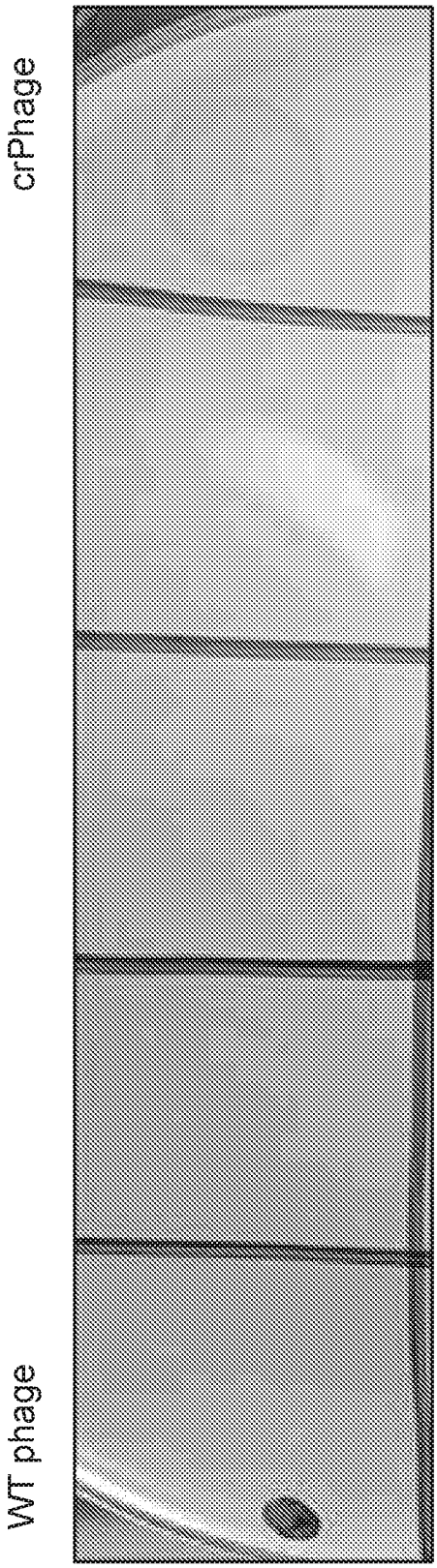


FIGURE 5

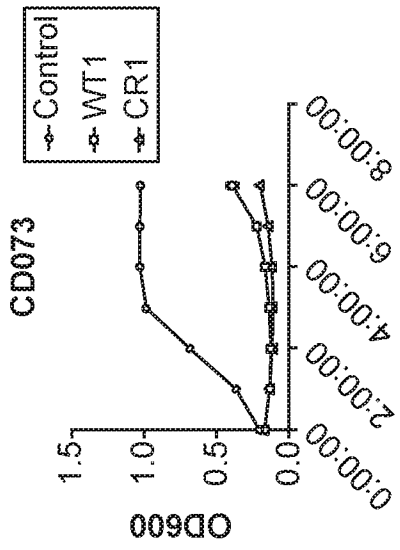


FIGURE 6A

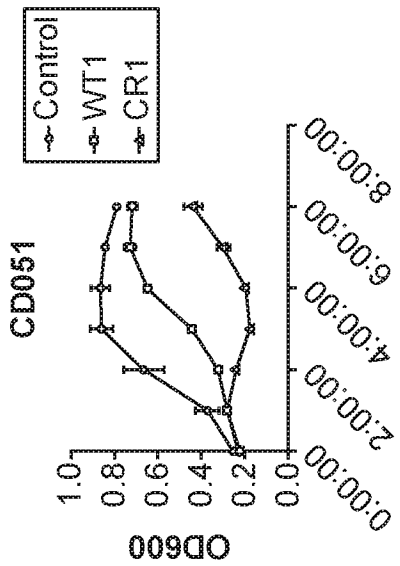


FIGURE 6B

FIGURE 6C

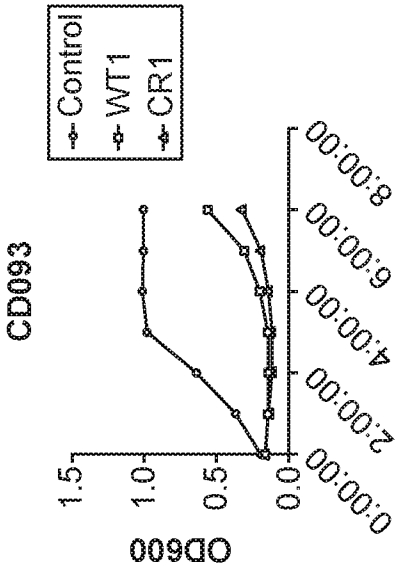


FIGURE 6D

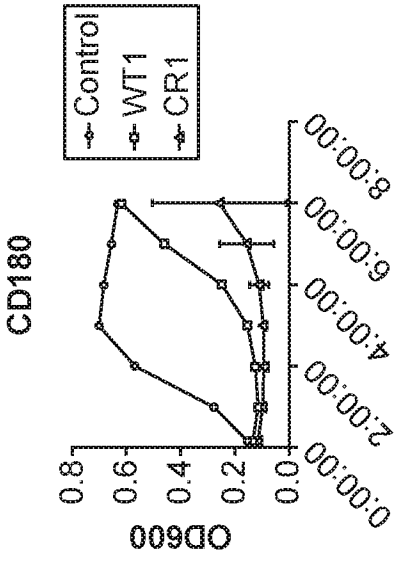


FIGURE 6E

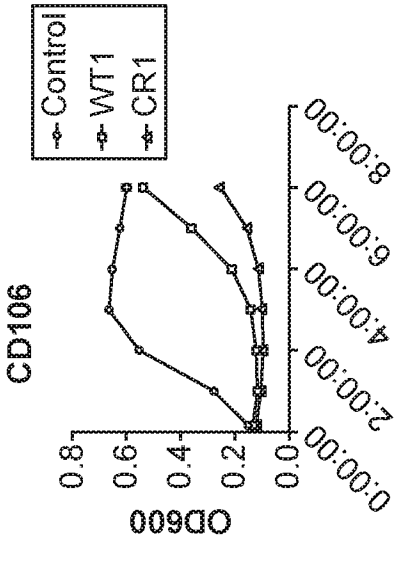


FIGURE 6F

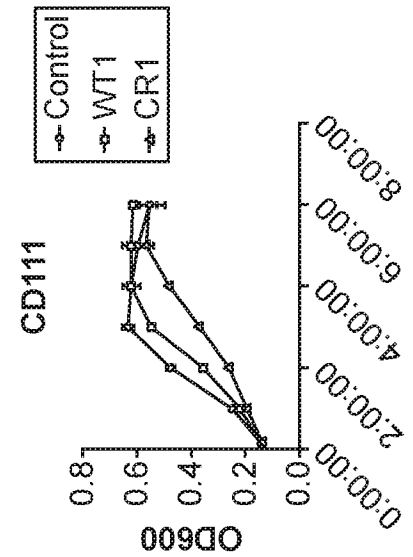


FIGURE 6I

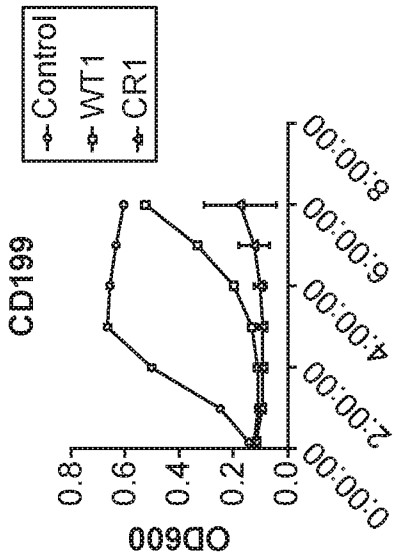


FIGURE 6H

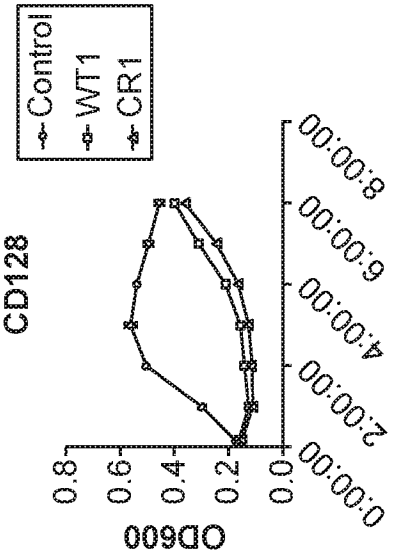


FIGURE 6G

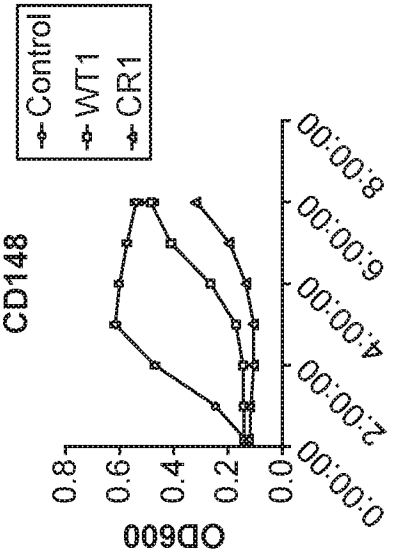


FIGURE 6L

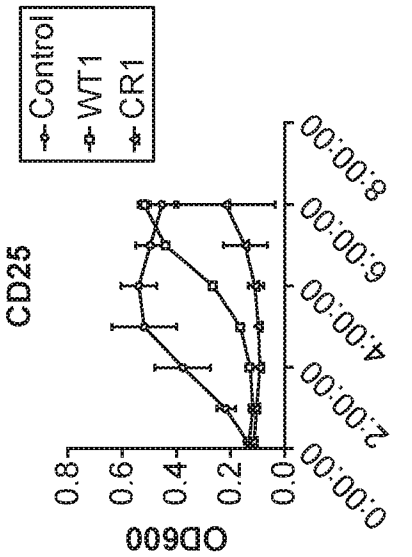


FIGURE 6K

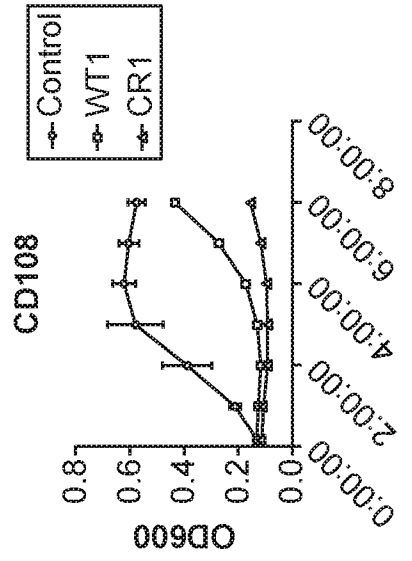


FIGURE 6J

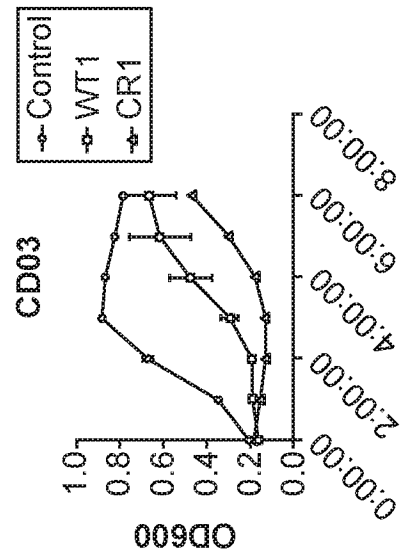


FIGURE 6O

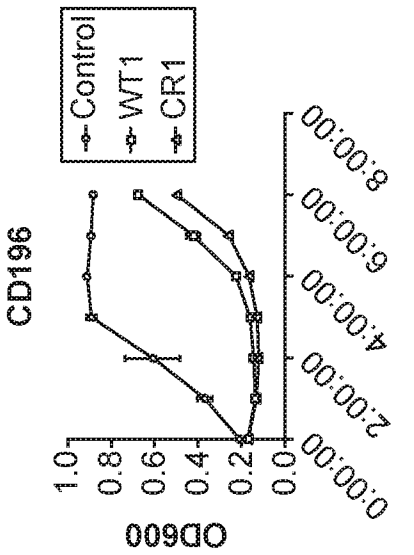


FIGURE 6R

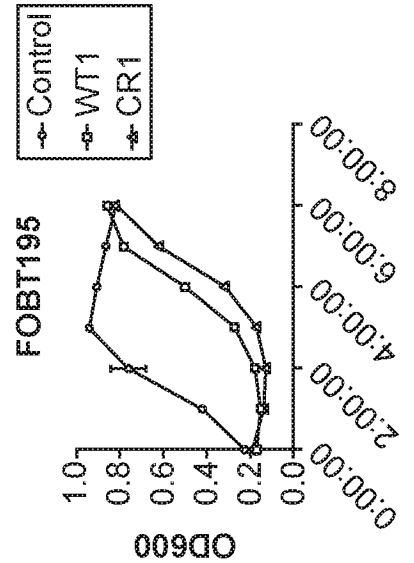


FIGURE 6N

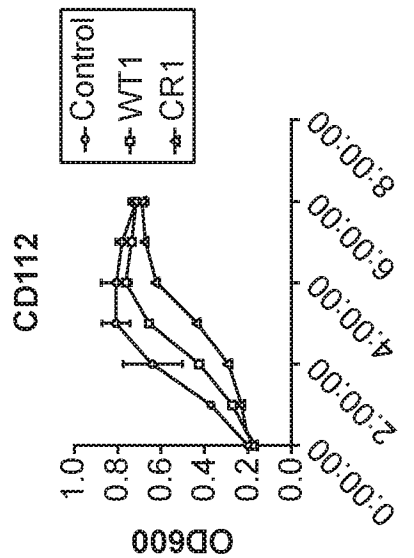


FIGURE 6Q

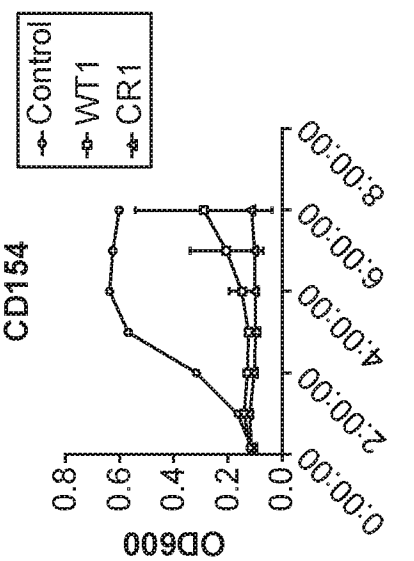


FIGURE 6M

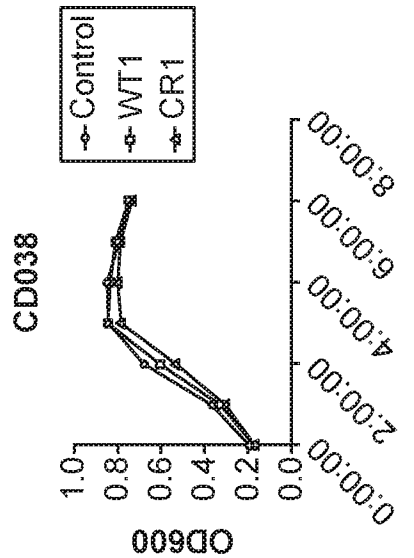


FIGURE 6P

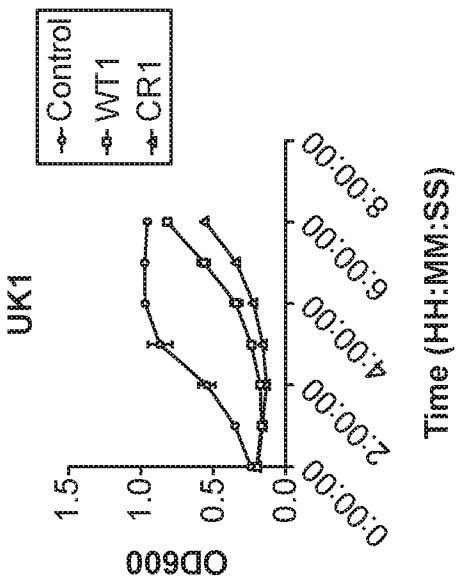


FIGURE 6T

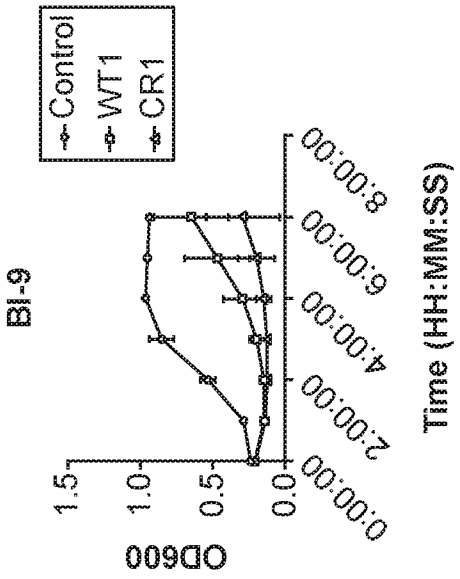


FIGURE 6V

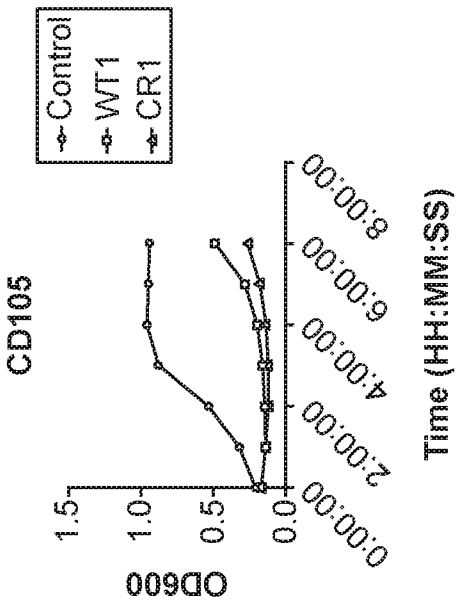


FIGURE 6S

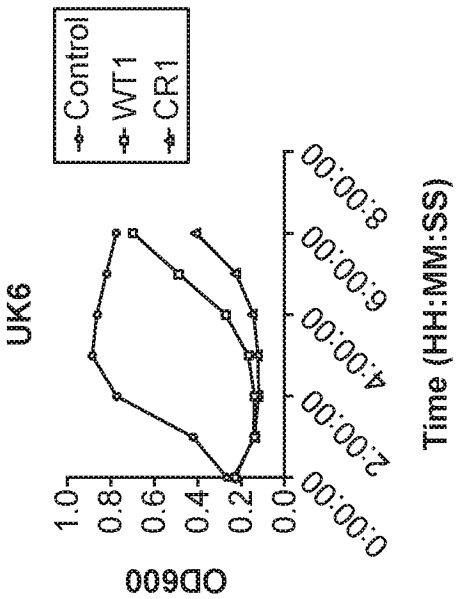


FIGURE 6U

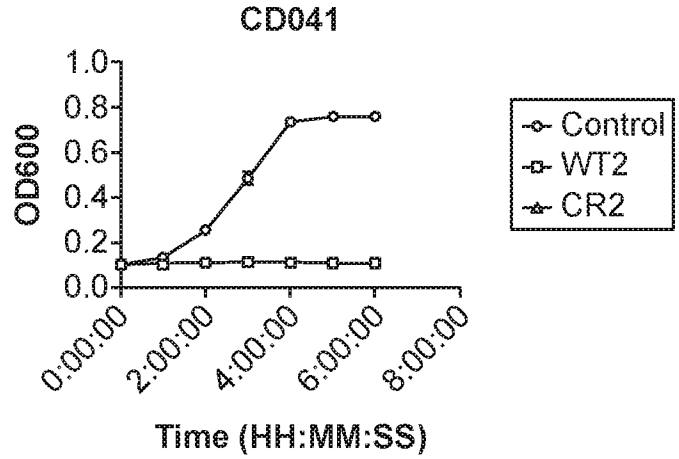


FIGURE 7A

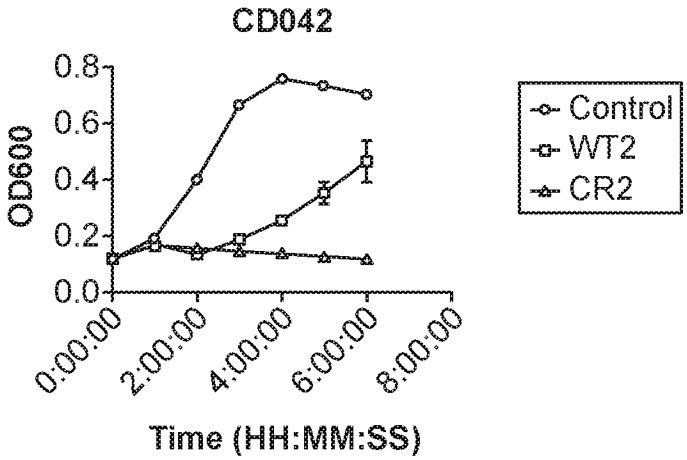


FIGURE 7B

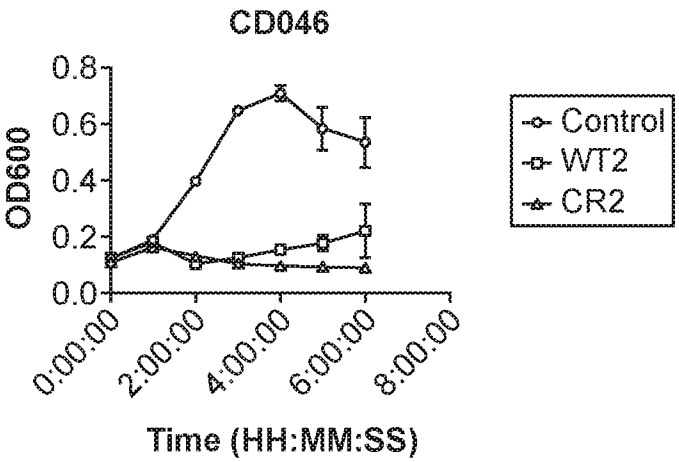


FIGURE 7C

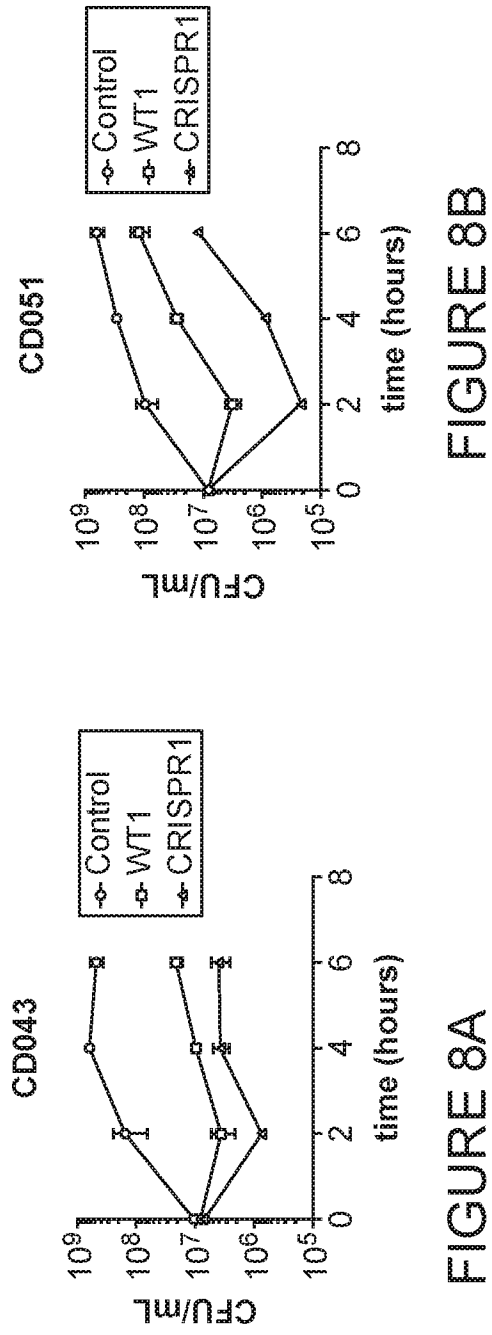


FIGURE 8A

FIGURE 8B

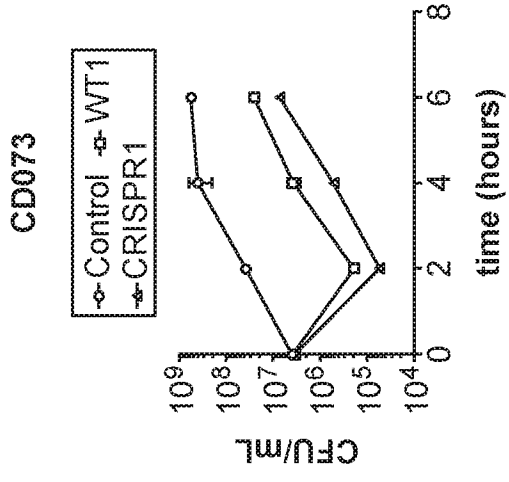


FIGURE 8C

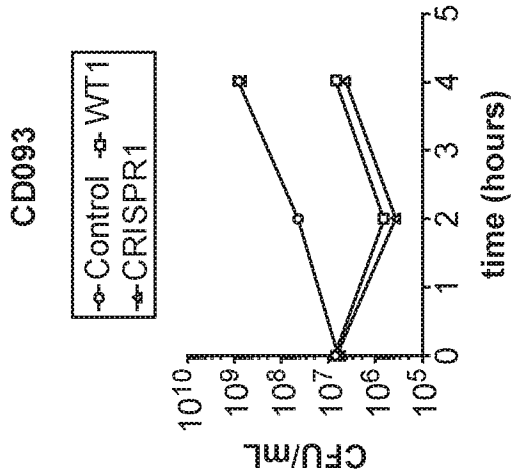


FIGURE 8D

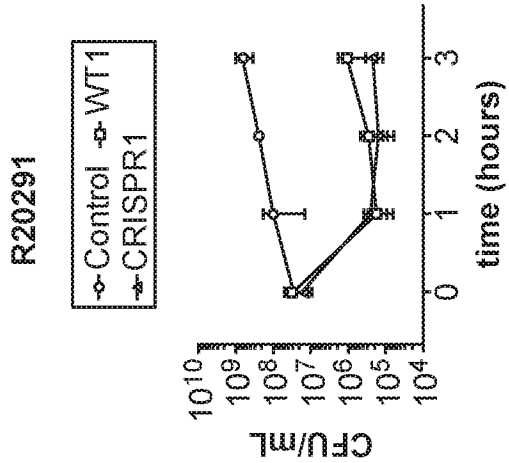


FIGURE 8E

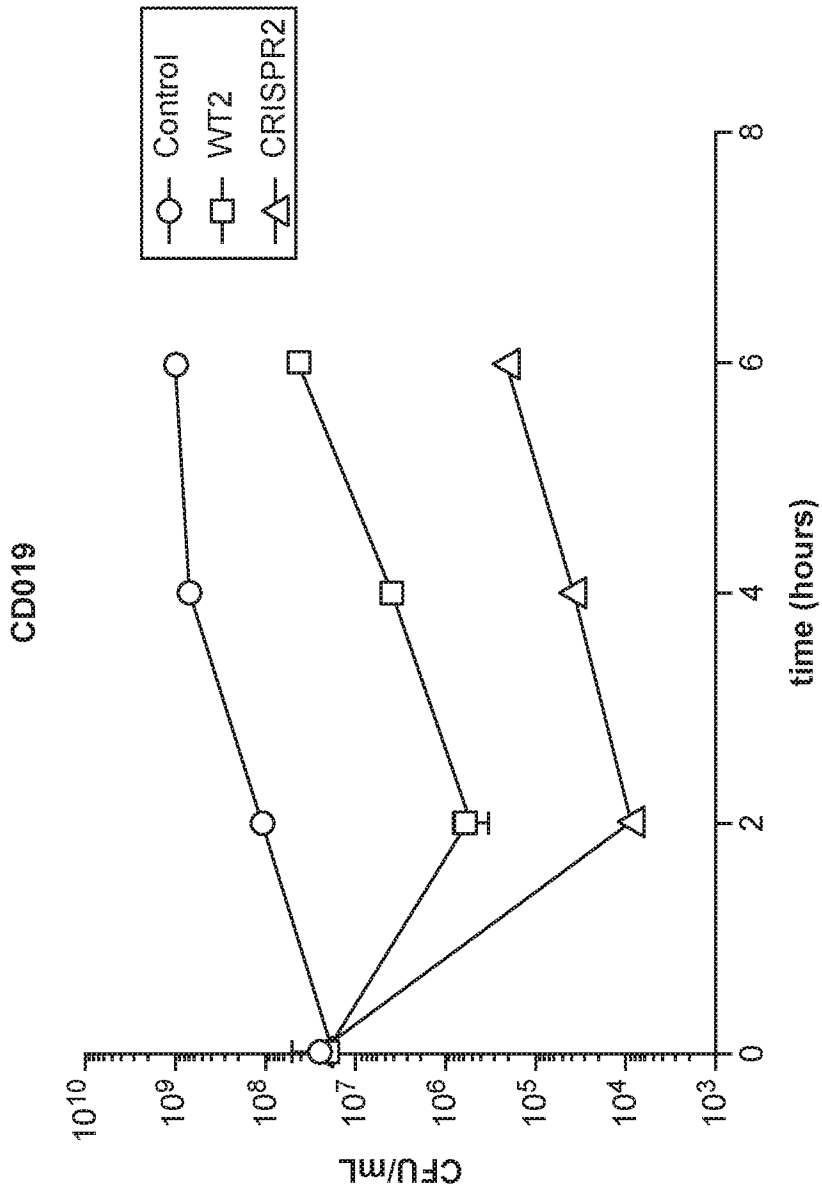

CD019
Control
WT2
CRISPR2
CFU/mL
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
0
2
4
6
8
time (hours)

FIGURE 9

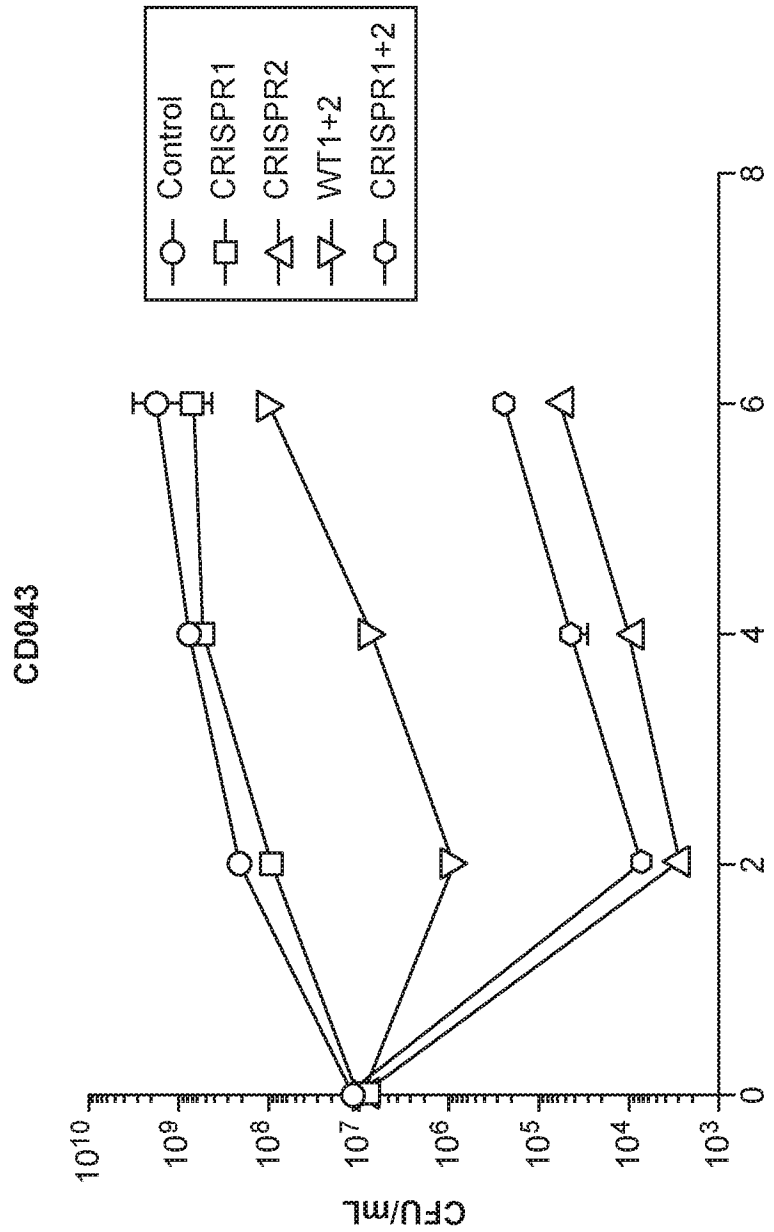

CD043
Control
CRISPR1
CRISPR2
WT1+2
CRISPR1+2
CFU/mL
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
0
2
4
6
8
time (hours)

FIGURE10

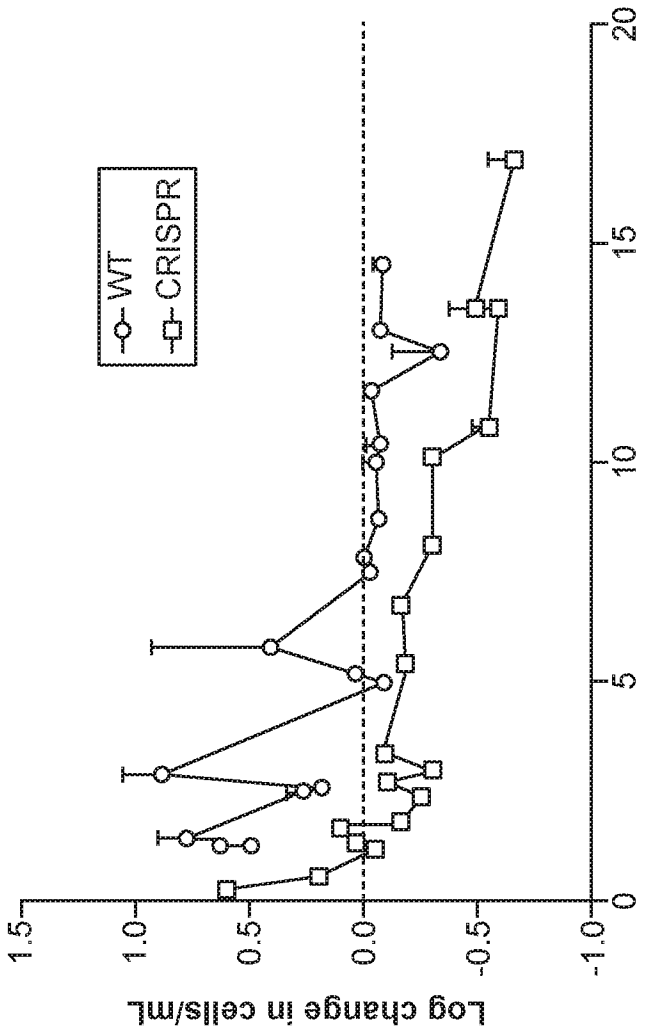


FIGURE 11

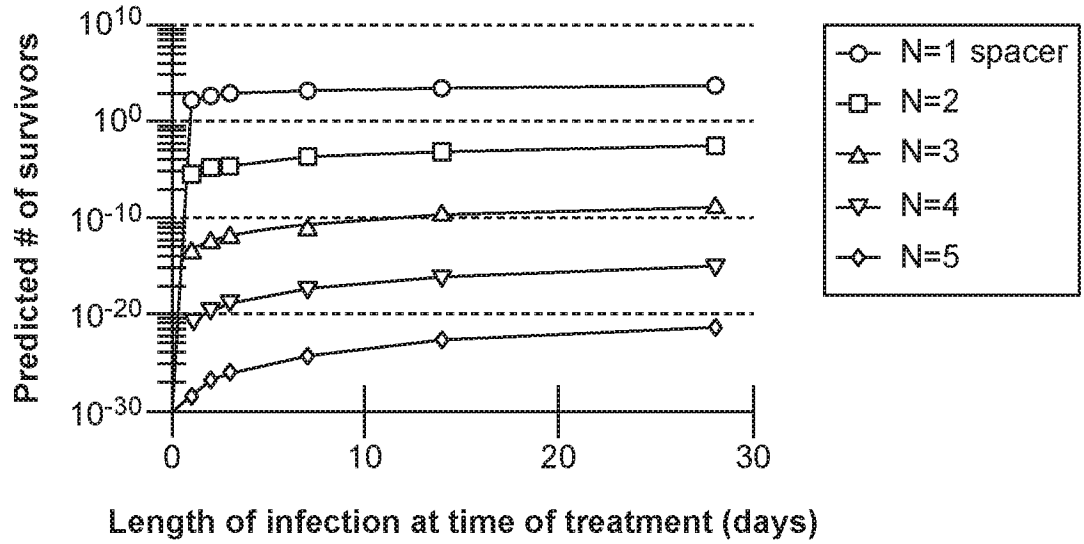


FIGURE 12A

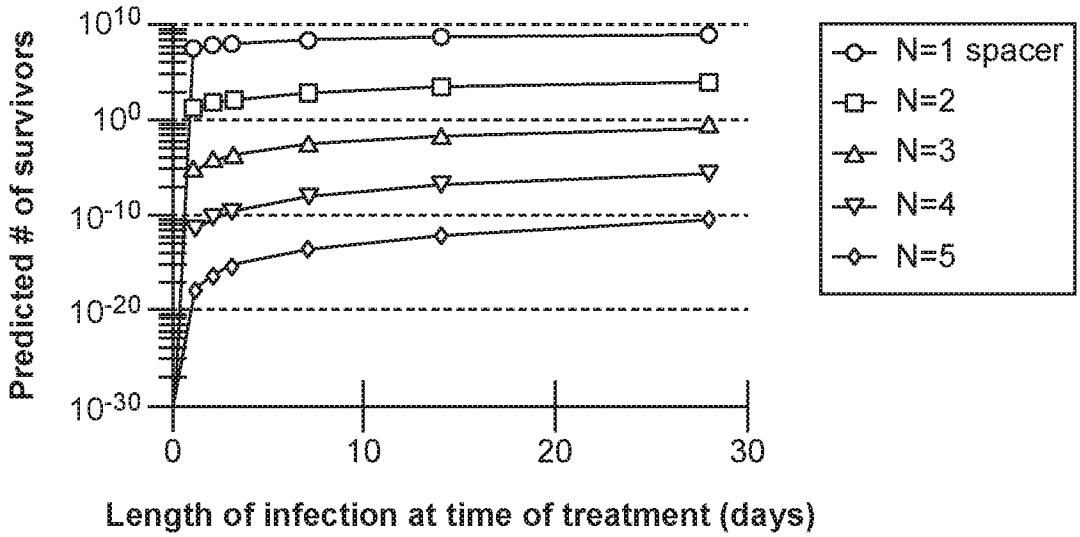
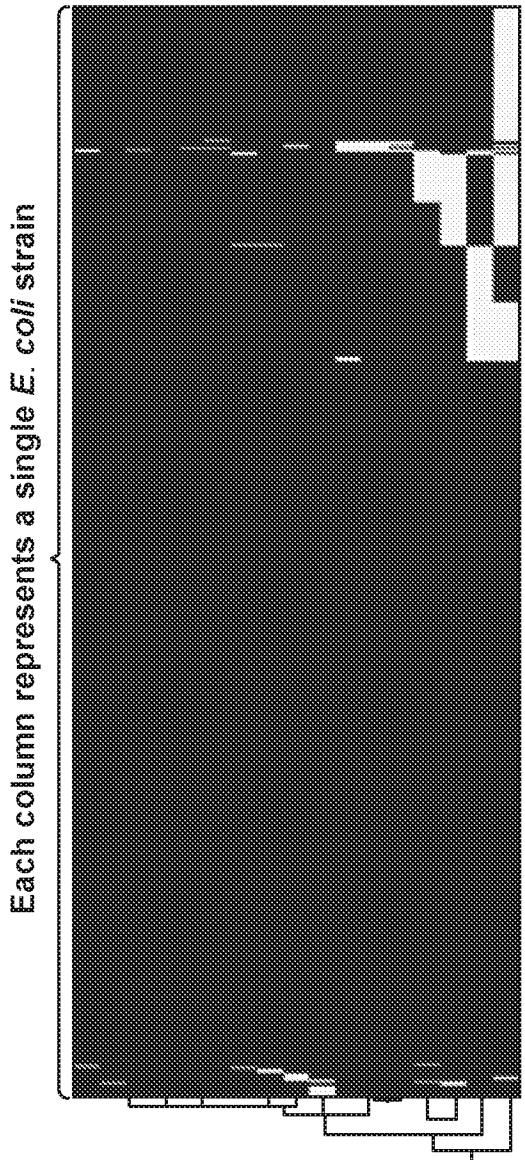


FIGURE 12B



crRNA target	Strains covered	% coverage
acpP	622/625	99%
gapA	624/625	99%
InfA	624/625	99%
Tsf	625/625	100%
secY	624/625	99%
secY2	623/625	99%
csrA	621/625	99%
trmD	621/625	99%
ftsA	620/625	99%
glyQ	617/625	98%
fusA	616/625	98%
fusA2	619/625	99%
nusG	621/625	99%
eno	593/625	95%
eno2	556/625	89%
gapA2	569/625	91%
nusG2	456/625	73%

FIGURE 13

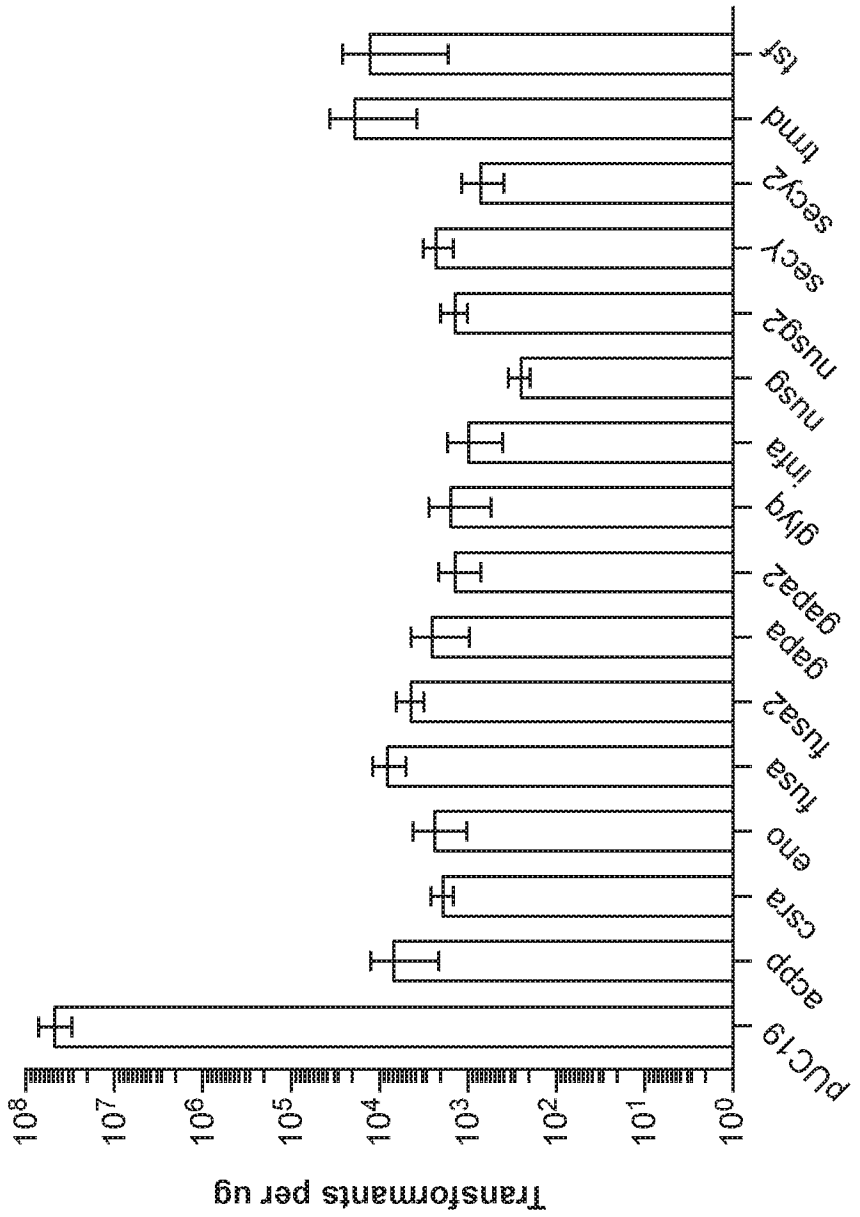

Transformants per ug
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
pUC19
acpp
csra
eno
fusa
fusa2
gapa
gapa2
glyq
infa
nusg
nusg2
secy
secy2
tmd
tsf
crRNA target

FIGURE 14

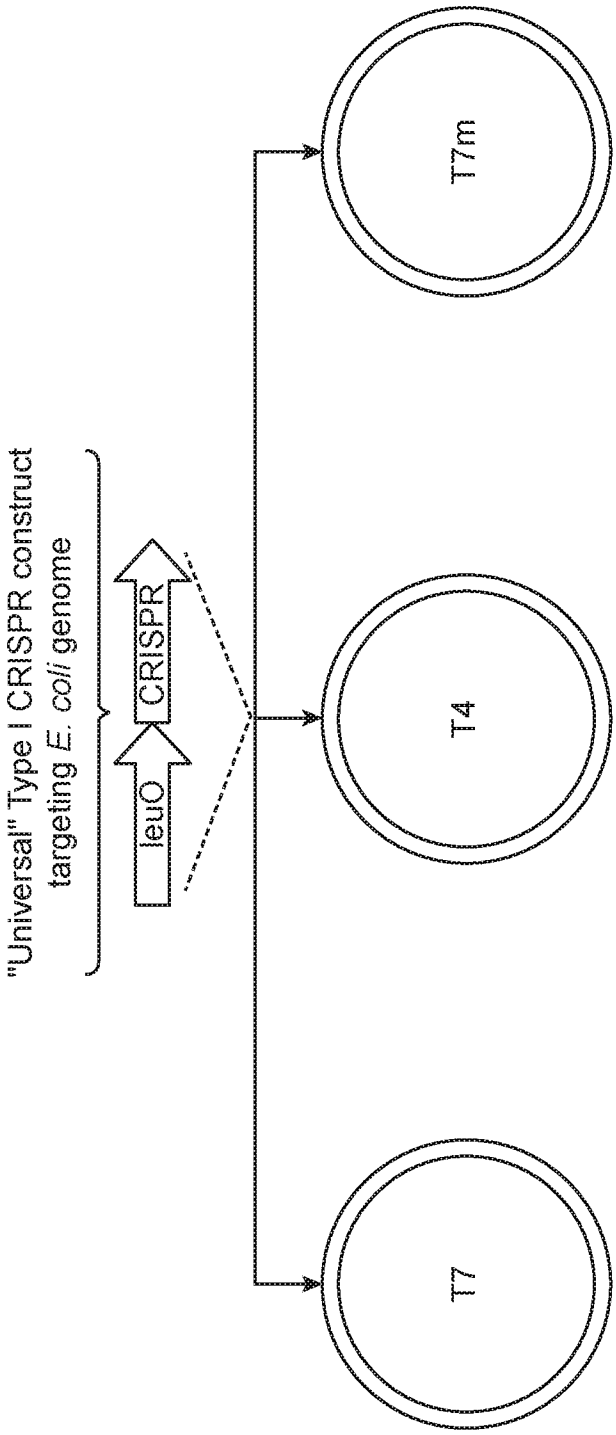

"Universal" Type I CRISPR construct
targeting E. coli genome
leuO
CRISPR
T7m
T4
T7

FIGURE 15

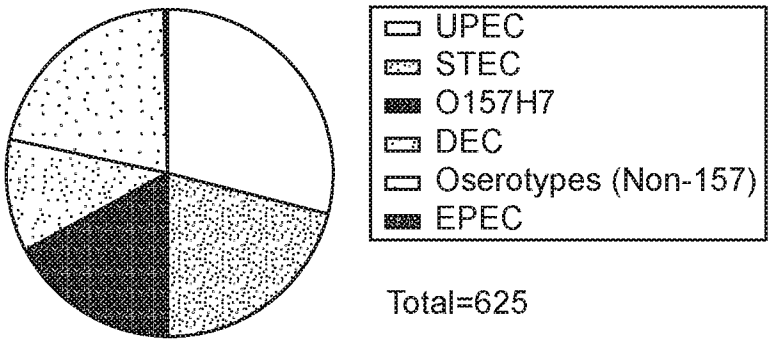


FIGURE 16A

System type	Strains	% coverage
I-E	442/625	70.7%
I-F	45/625	7.2%
Total	487/625	77.9%

FIGURE 16B

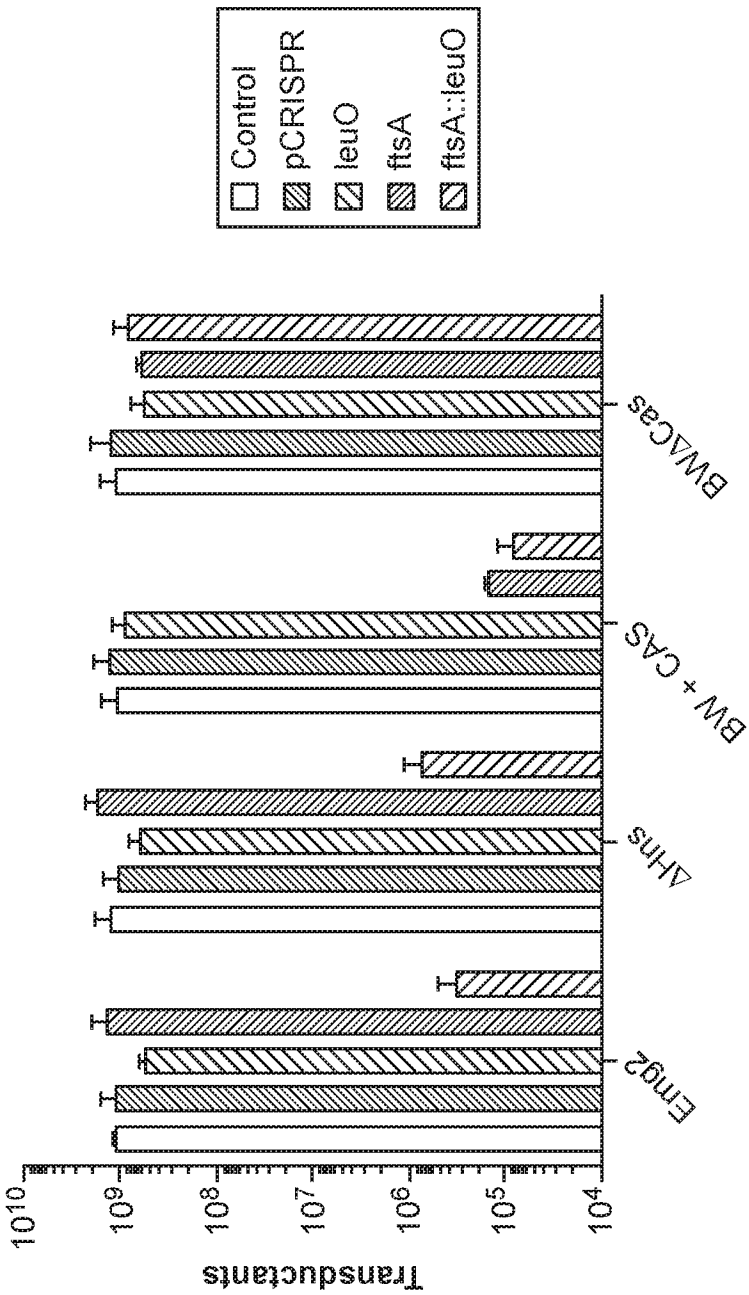

Control
pCRISPR
leuO
ftsA
ftsA::leuO
Transductants
10^10
10^9
10^8
10^7
10^6
10^5
10^4
Emg2
Δhns
BW + CAS
BWΔCas

FIGURE 17

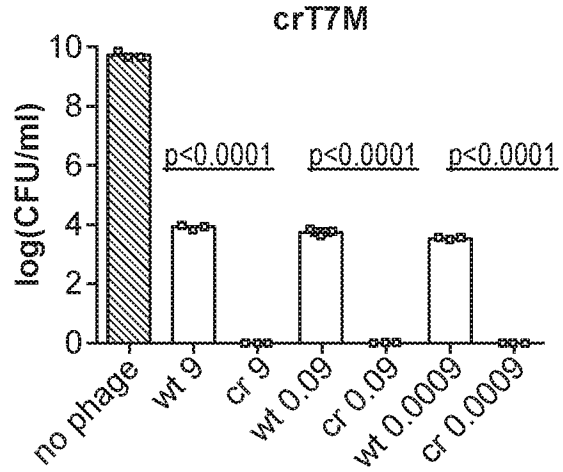


FIGURE 18A

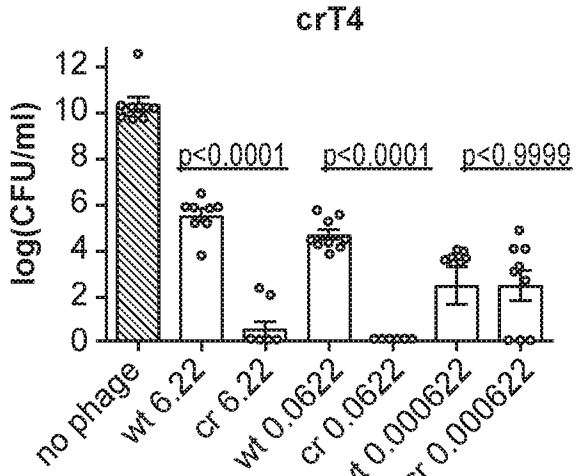


FIGURE 18B

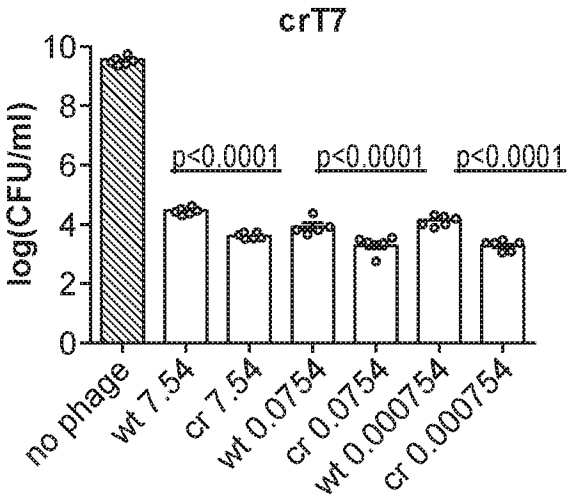


FIGURE 18C

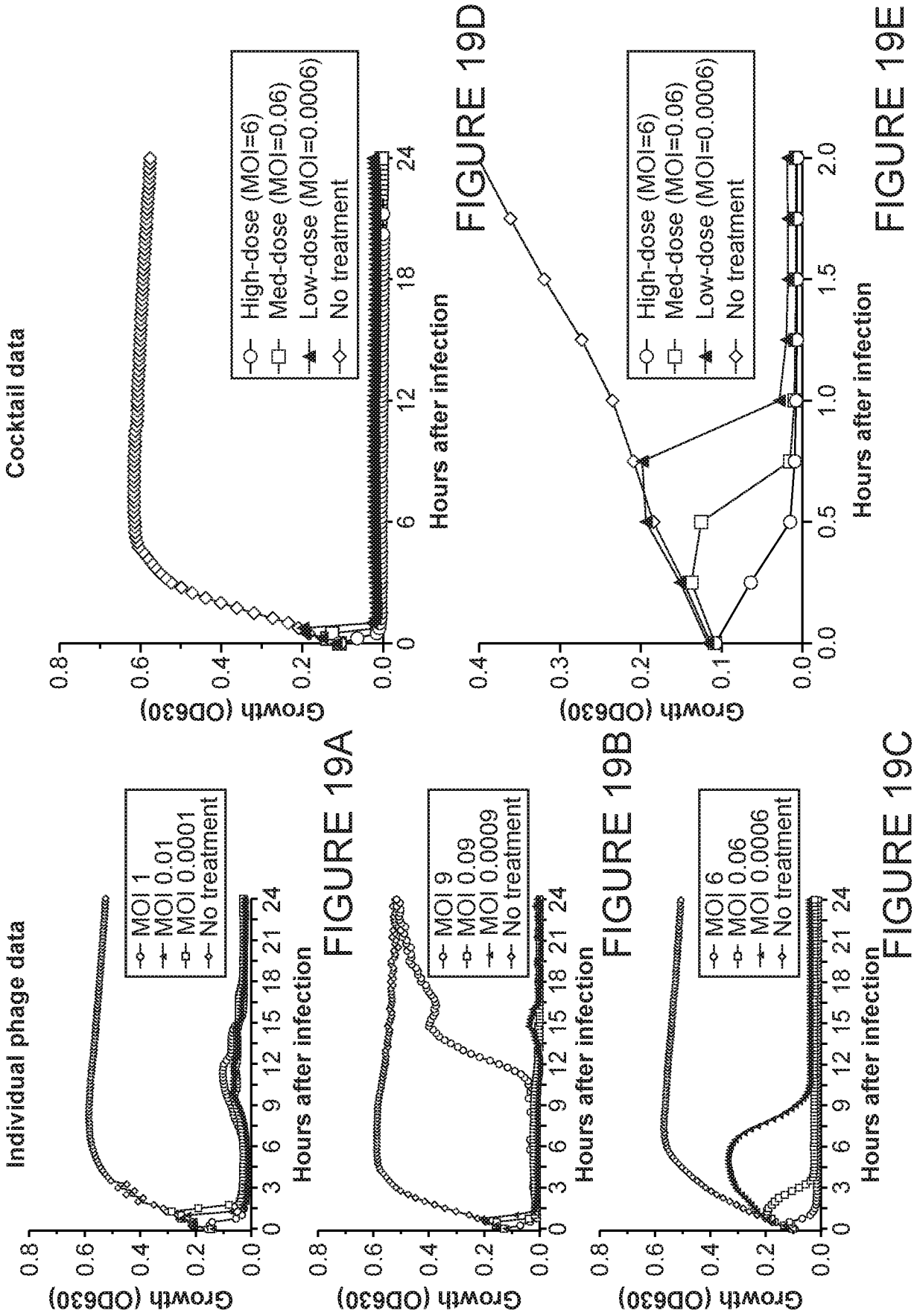


FIGURE 19A

FIGURE 19B

FIGURE 19C

FIGURE 19D

FIGURE 19E

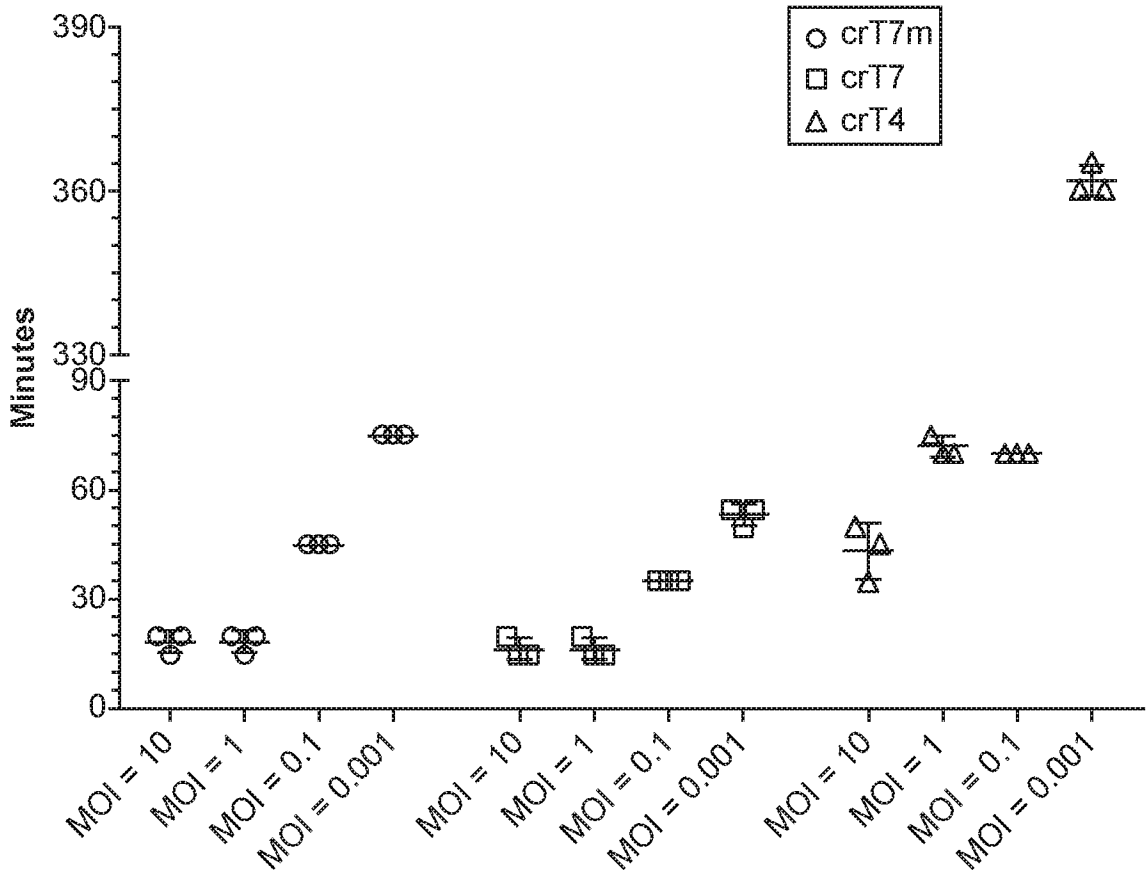

crT7m
crT7
crT4
Minutes
390
360
330
90
60
30
0
MOI = 10
MOI = 1
MOI = 0.1
MOI = 0.001
MOI = 10
MOI = 1
MOI = 0.1
MOI = 0.001
MOI = 10
MOI = 1
MOI = 0.1
MOI = 0.001

FIGURE 20

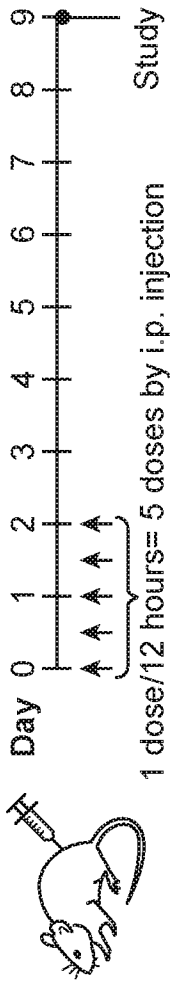


FIGURE 21A

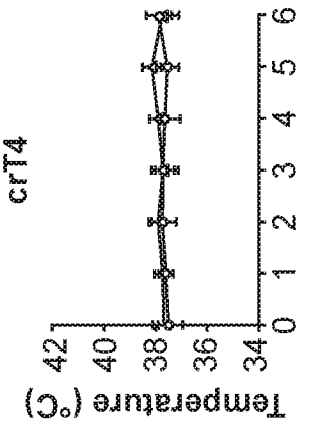


FIGURE 21B

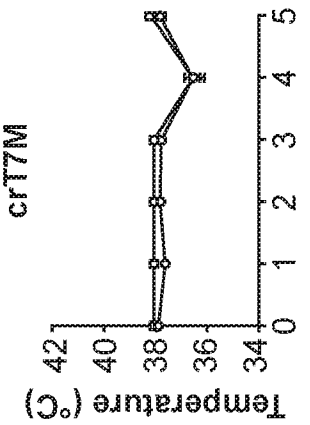


FIGURE 21C

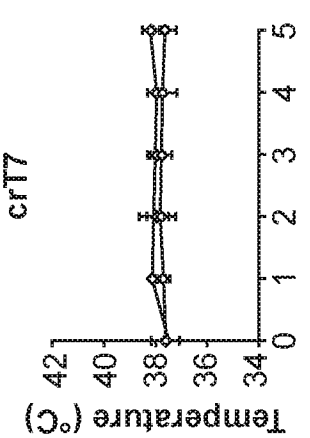


FIGURE 21D

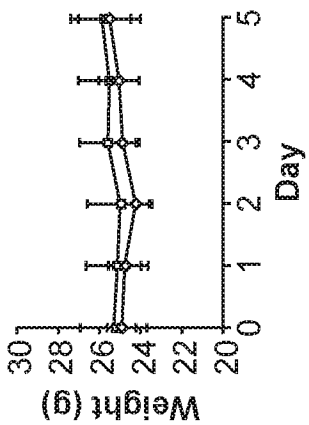


FIGURE 21E

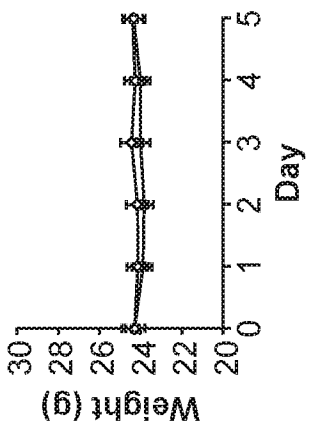


FIGURE 21F

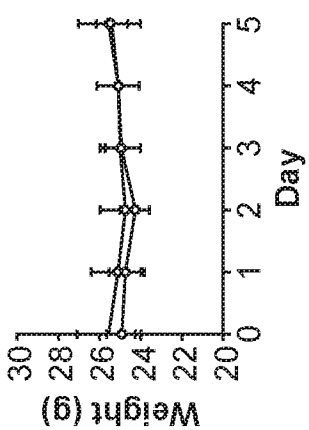


FIGURE 21G

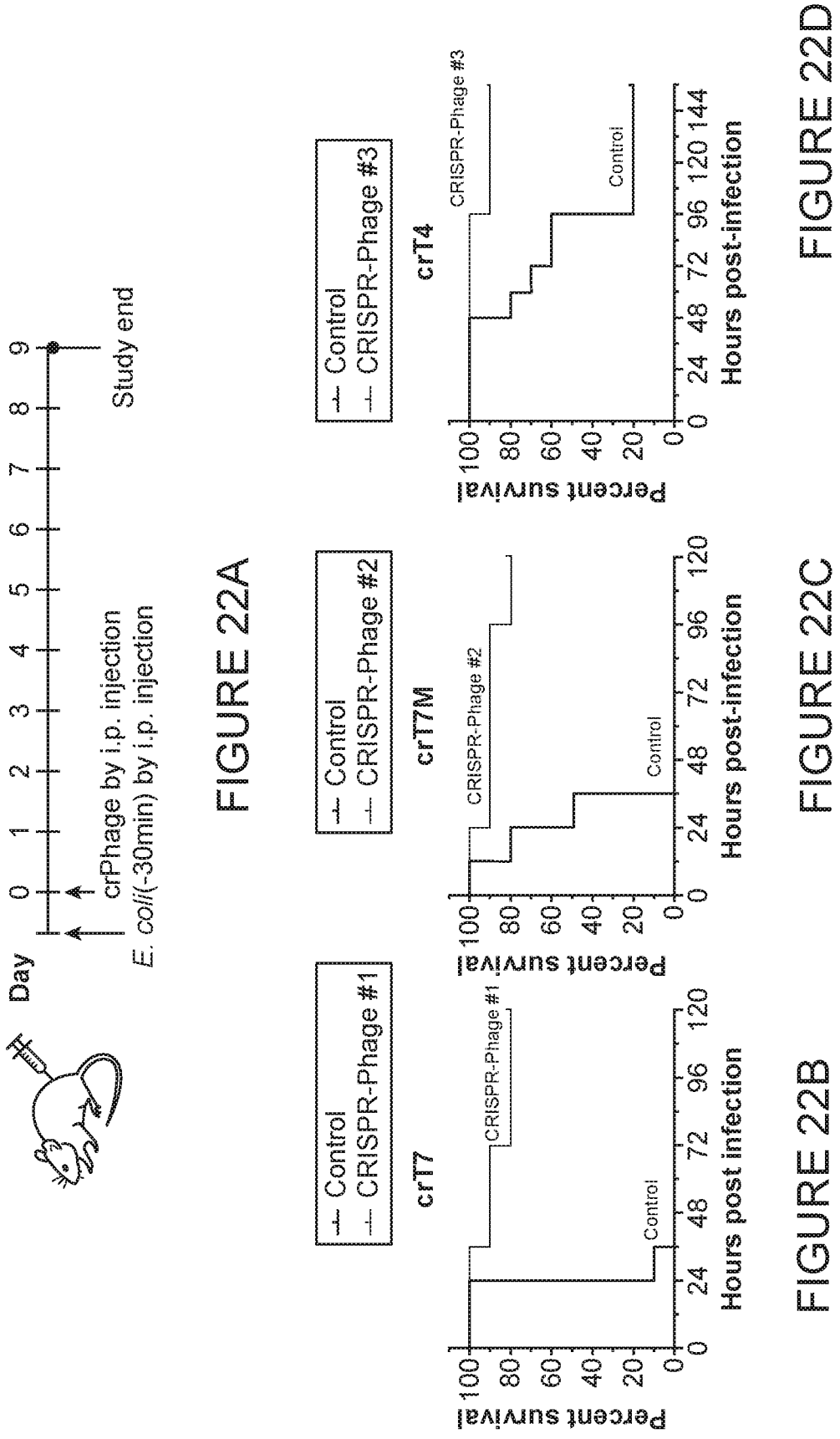


FIGURE 22A

FIGURE 22B

FIGURE 22C

FIGURE 22D

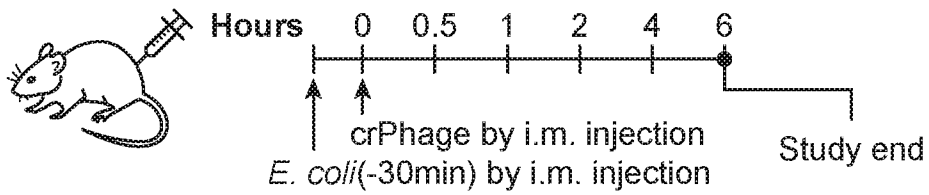


FIGURE 23A

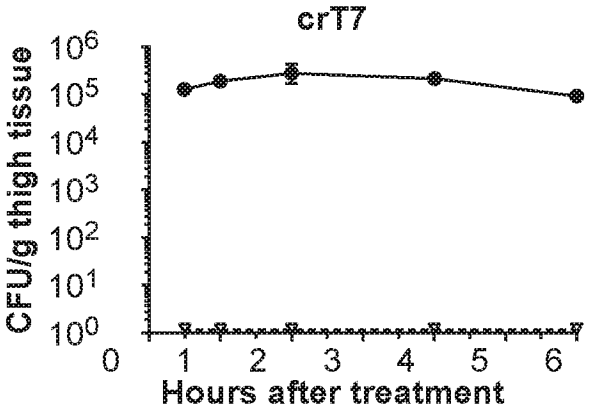


FIGURE 23B

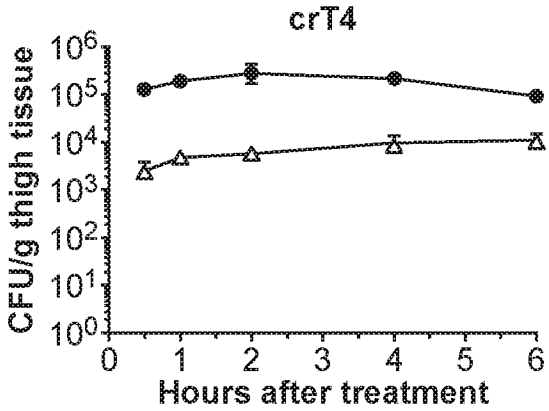
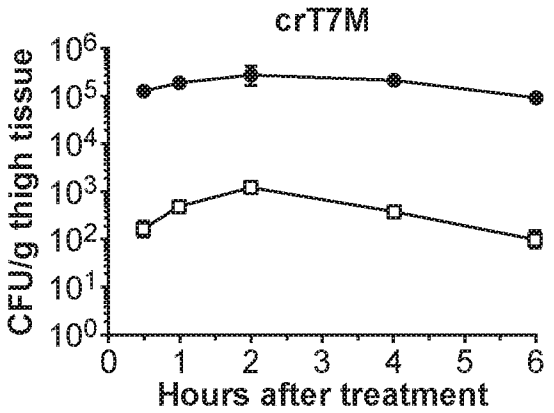
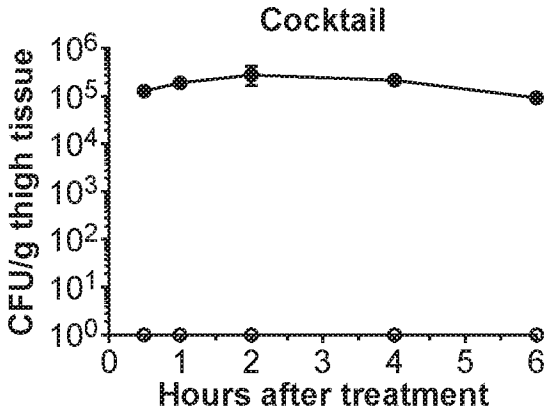


FIGURE 23C





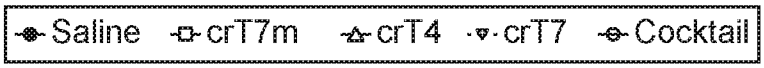


FIGURE 23D

FIGURE 23E

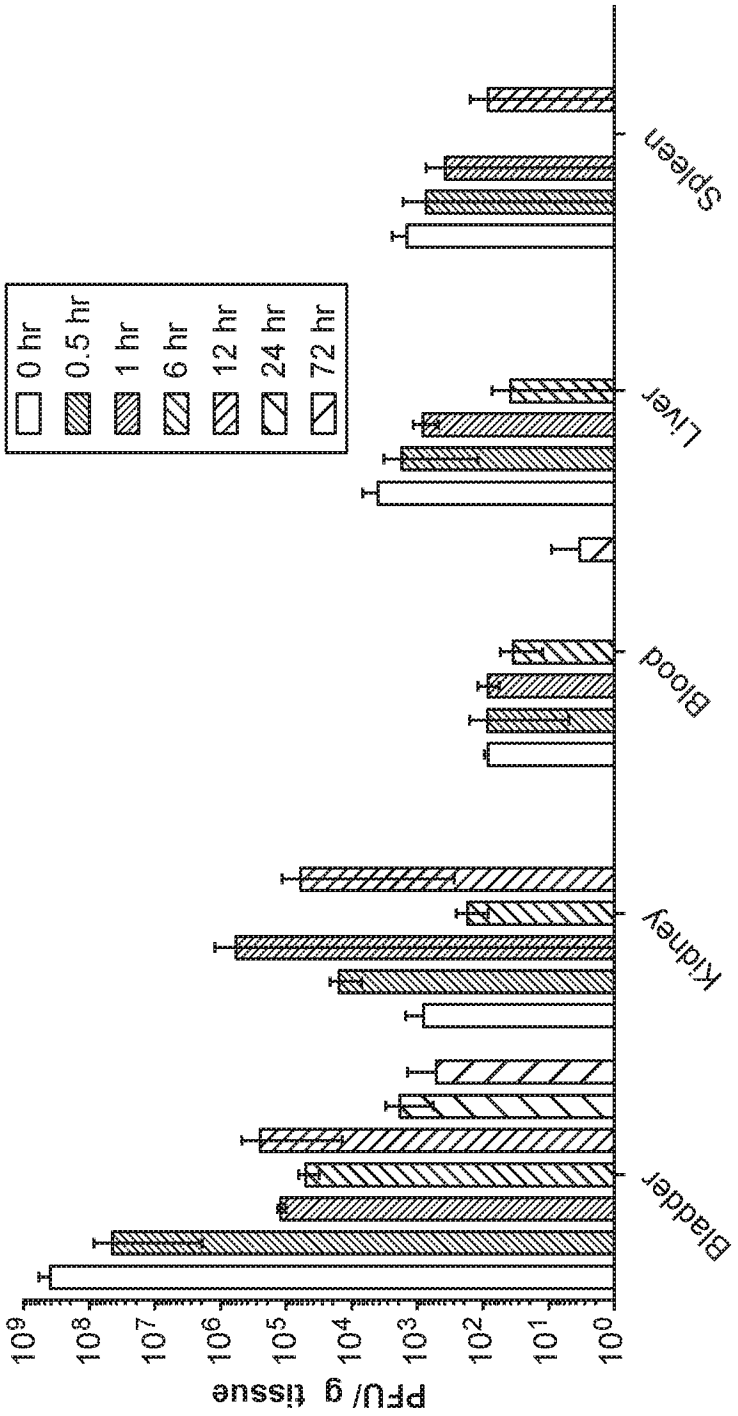

0 hr
0.5 hr
1 hr
6 hr
12 hr
24 hr
72 hr
Spleen
Liver
Blood
Kidney
Bladder
PFU/ g tissue
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0

FIGURE 24

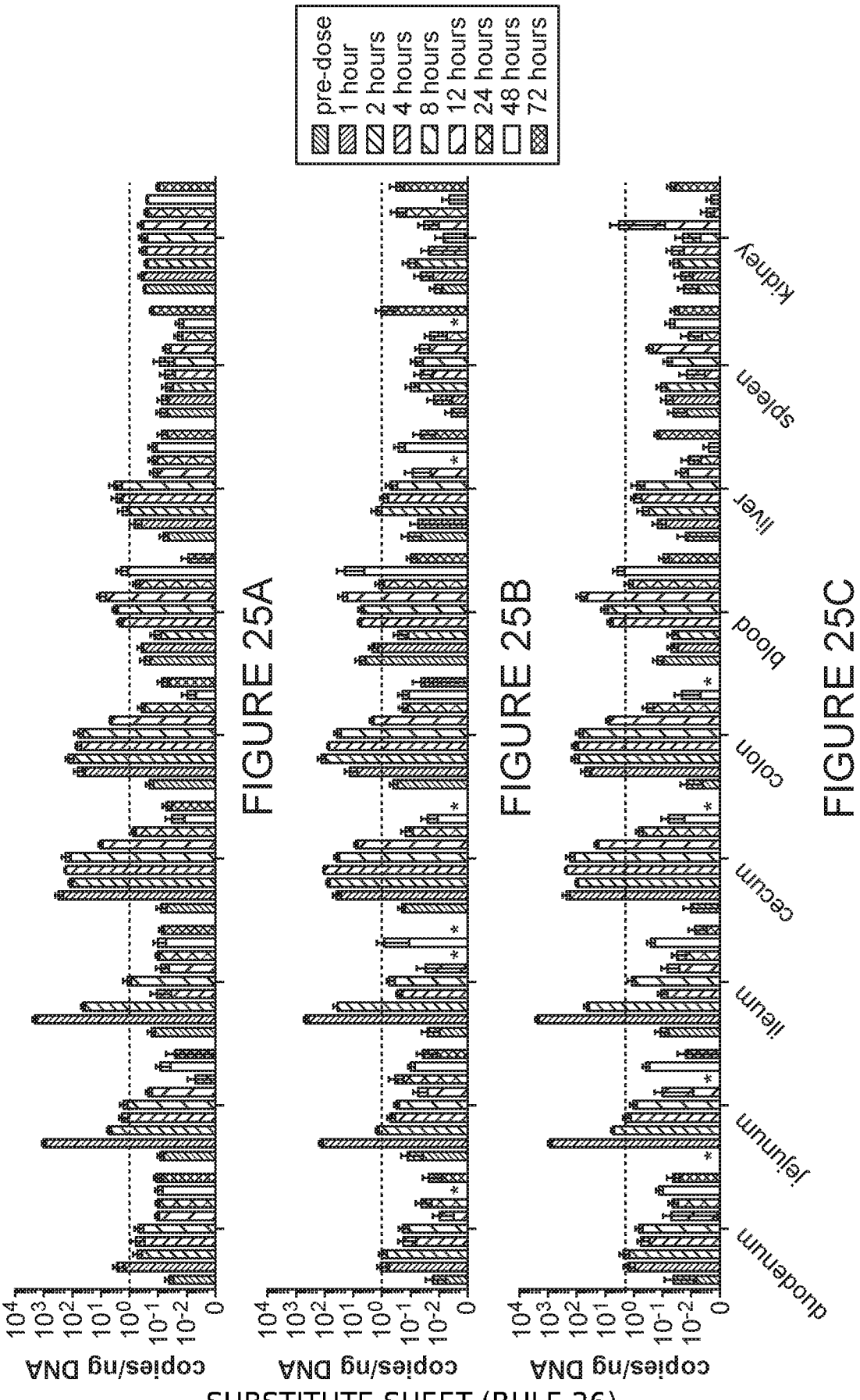


FIGURE 25A

FIGURE 25B

FIGURE 25C

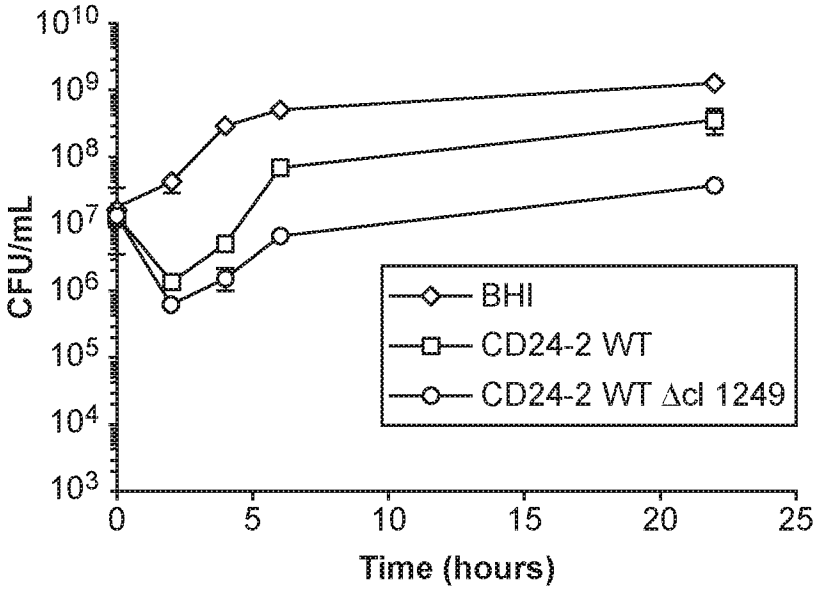


FIGURE 26A

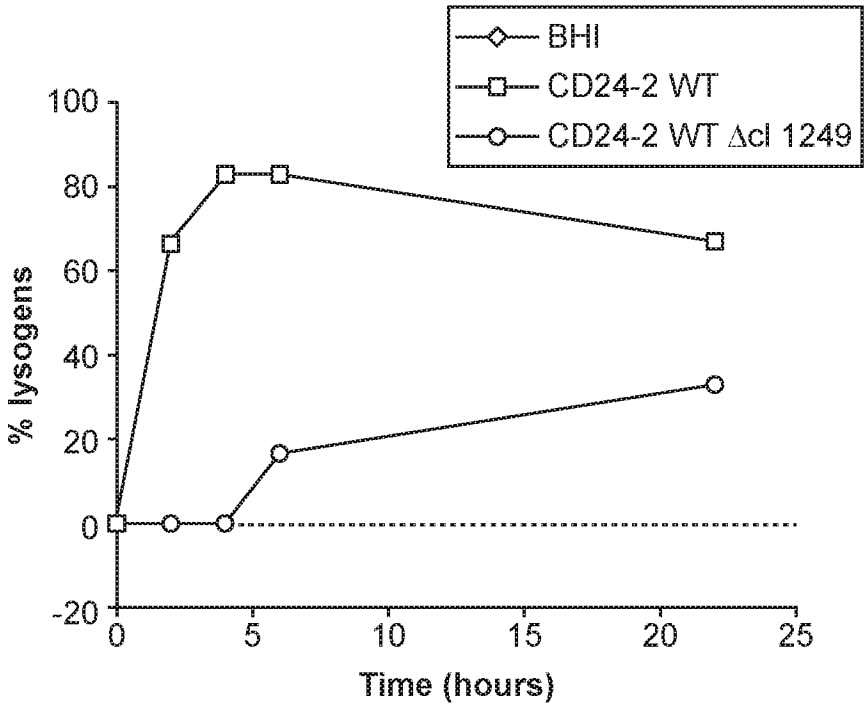


FIGURE 26B

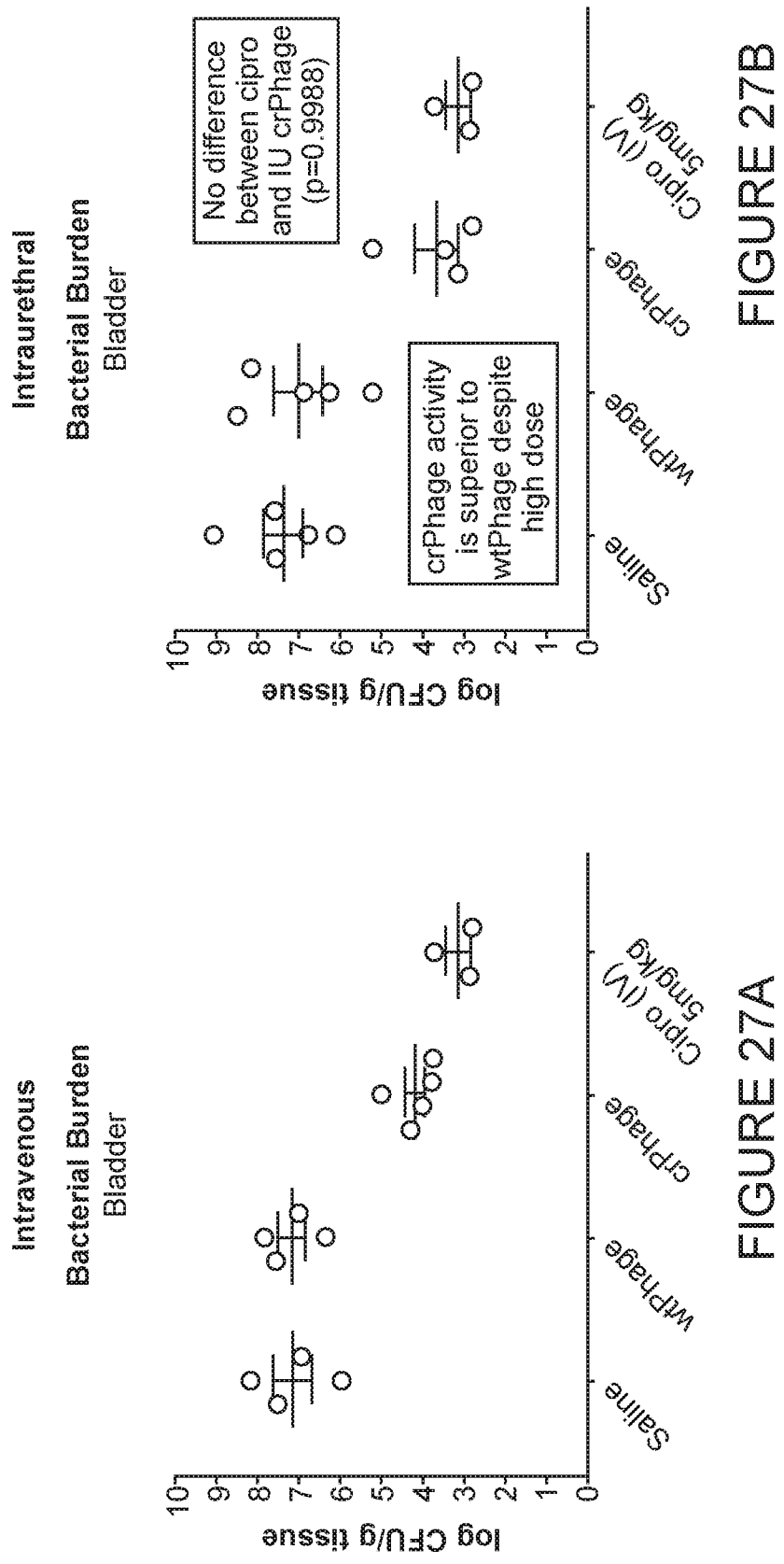


FIGURE 27A

FIGURE 27B

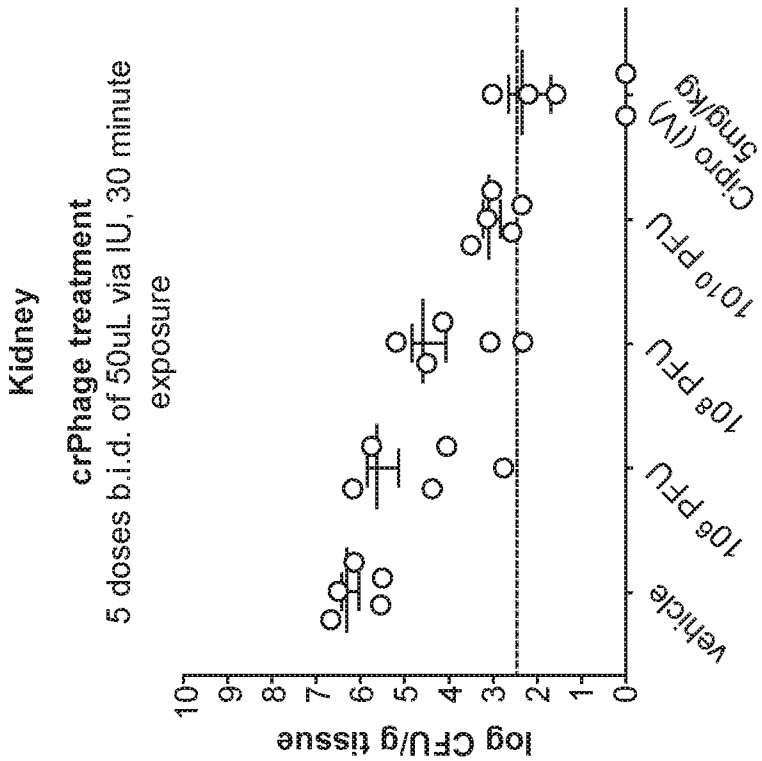


FIGURE 28B

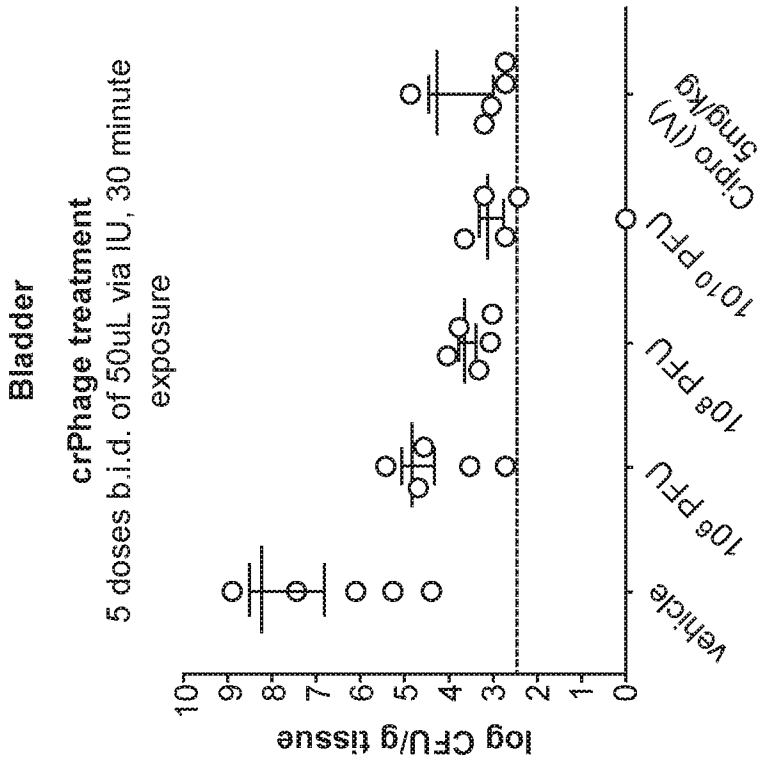


FIGURE 28A

Group	N	Treatment	Route	Dose
1	10	None	N/A	N/A
2	10	Saline	IV	N/A
3	10		IU	N/A
4	10		IV+IU	N/A
5	10	wtPhage	IV	8×10^8 PFU/phage
6	10		IU	4×10^8 PFU/phage
7	10		IV+IU	8×10^8 PFU (IV) + 4×10^8 PFU (IU)/phage
8	10	crPhage	IV	8×10^8 PFU/phage
9	10		IU	4×10^8 PFU/phage
10	10		IV+IU	8×10^8 PFU (IV) + 4×10^8 PFU (IU)/phage
11	10	Ciprofloxacin	IV	5 mg/kg body weight

FIGURE 29

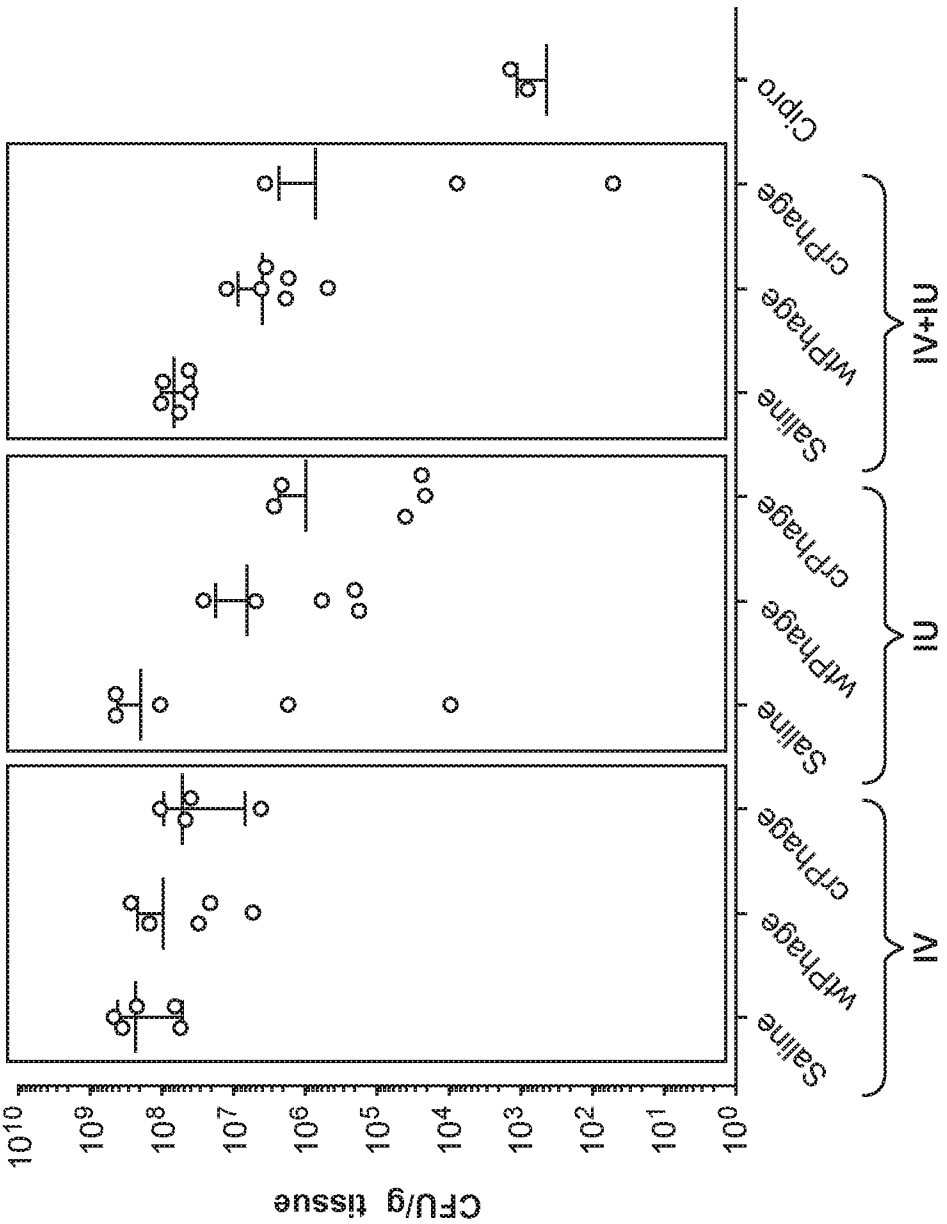

CFU/g tissue
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Saline
wtPhage
crPhage
Cipro
IV
Saline
wtPhage
crPhage
IU
Saline
wtPhage
crPhage
IV+IU

FIGURE 30A

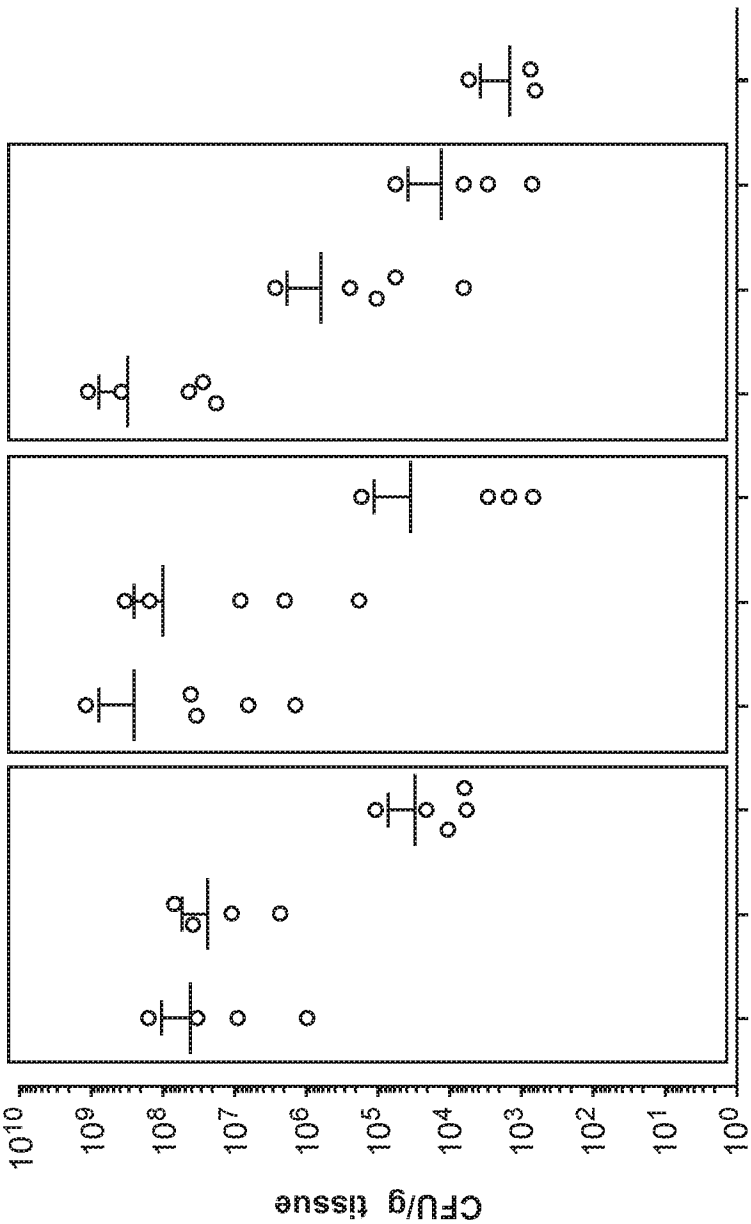

CFU/g tissue
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Saline
wtPhage
crPhage
Cipro
IV
IU
IV+IU

FIGURE 30B

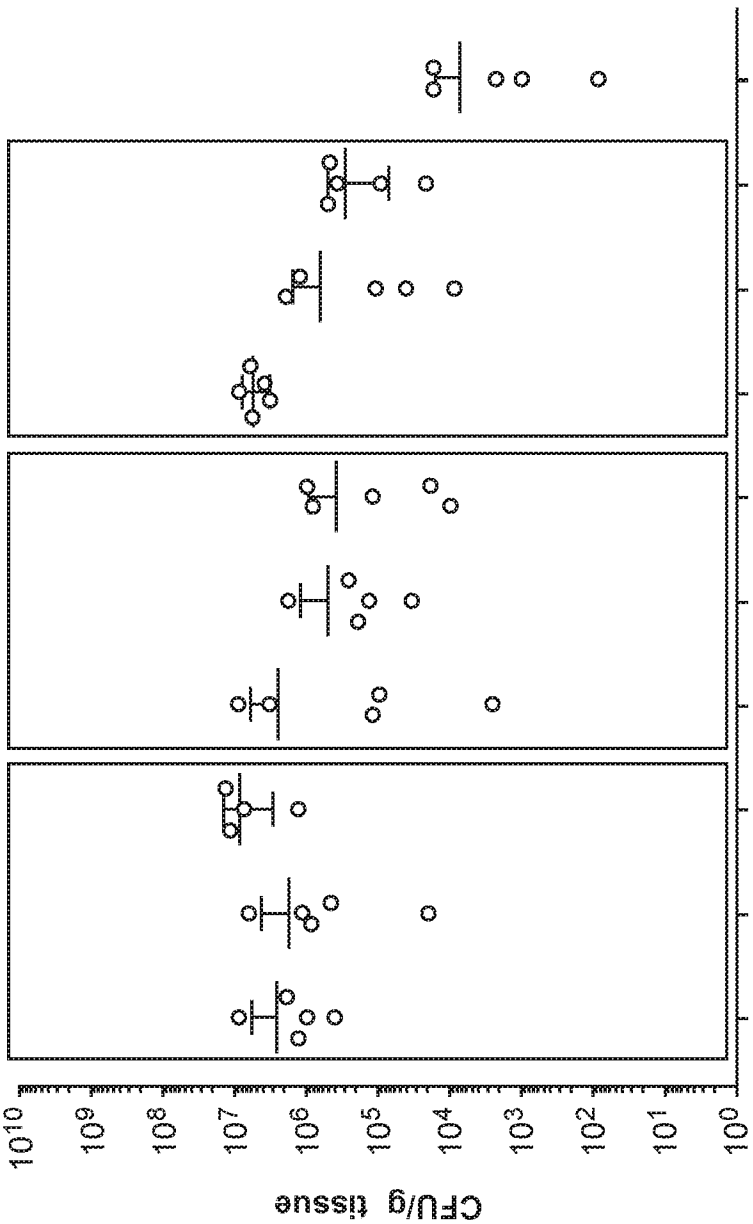

CFU/g tissue
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Saline
wtPhage
crPhage
Cipro
IV
Saline
wtPhage
crPhage
IU
Saline
wtPhage
crPhage
IV+IU

FIGURE 30C

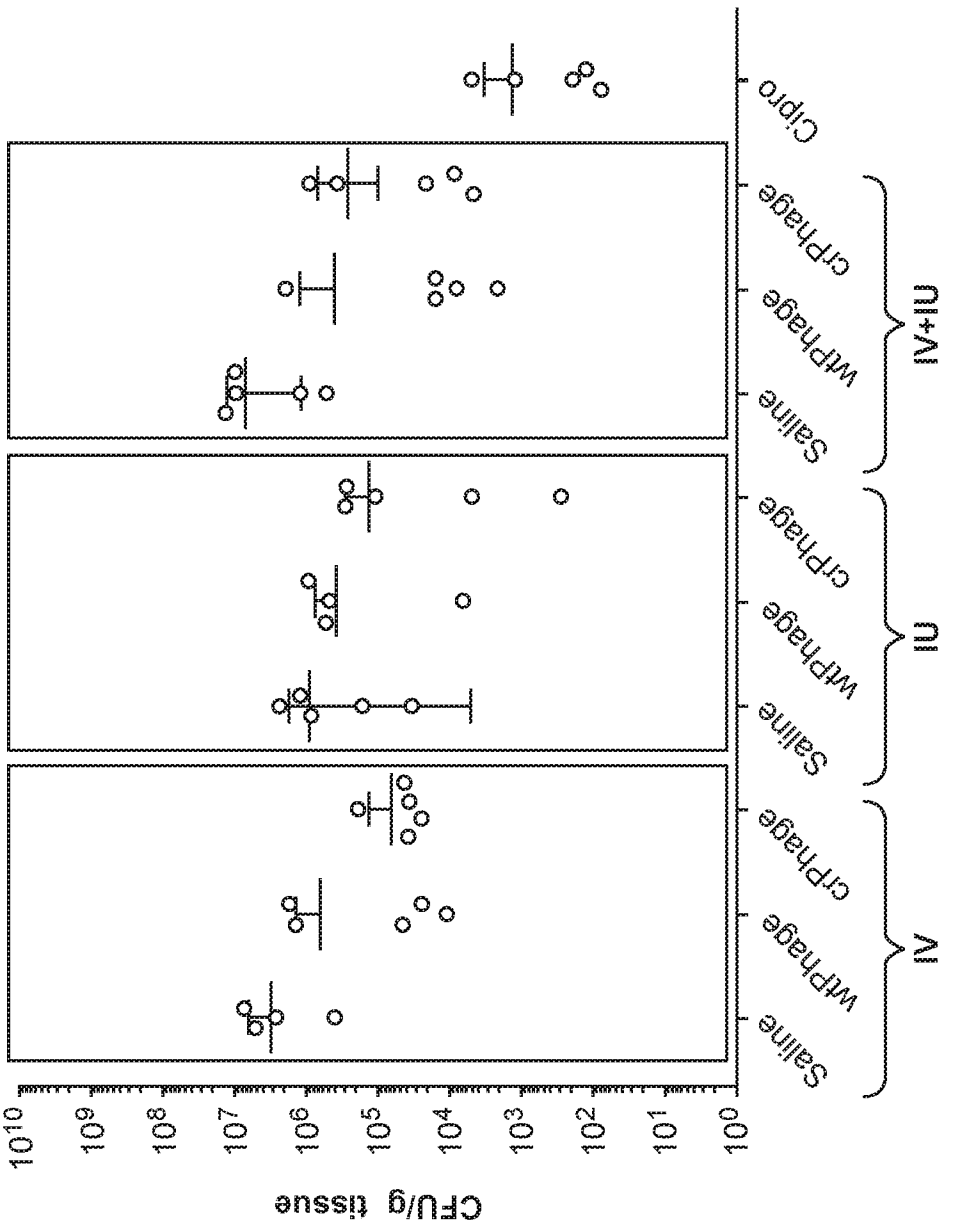

CFU/g tissue
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Saline
wtPhage
crPhage
Cipro
IV
IU
IV+IU

FIGURE 30D

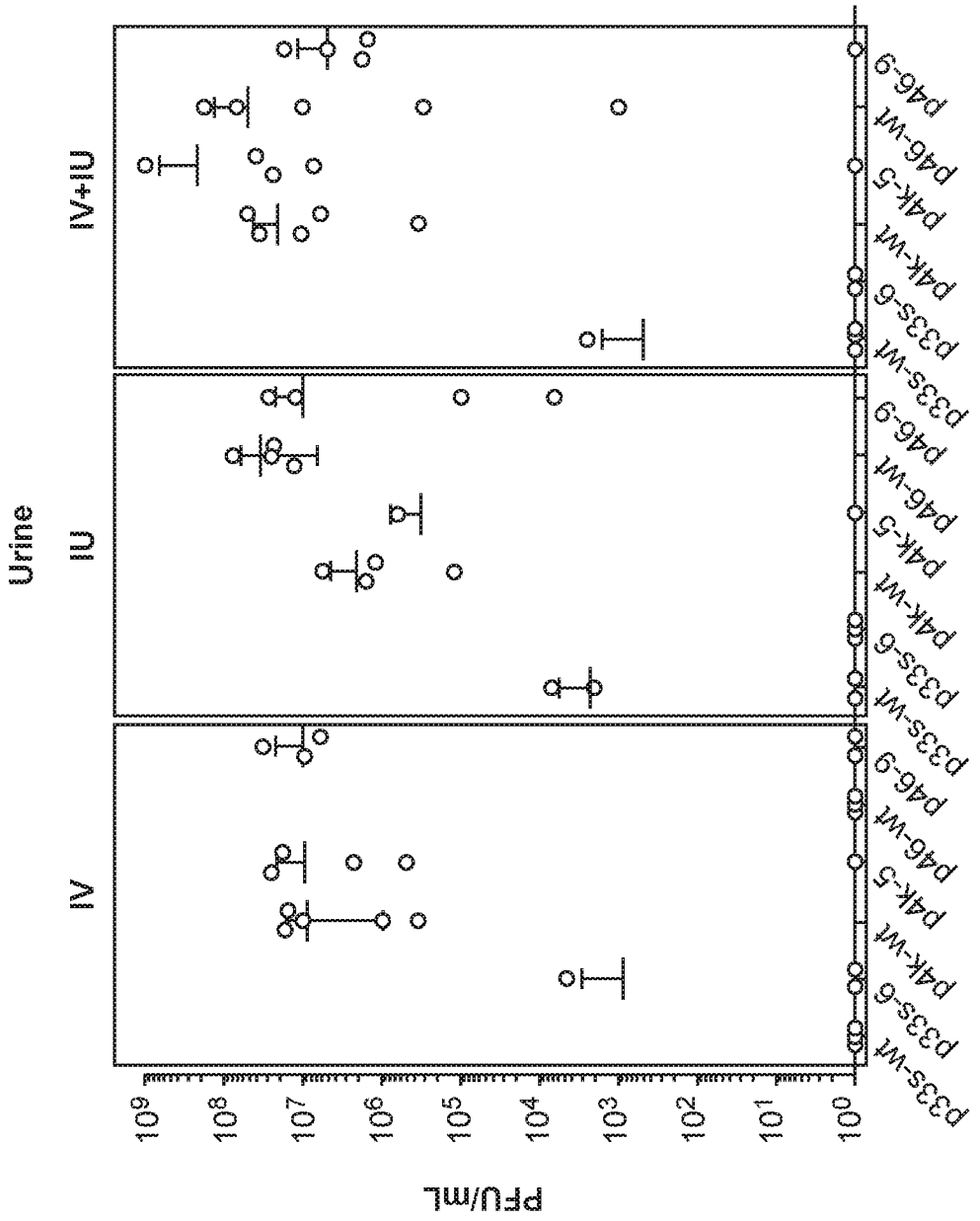

Urine
IV
IU
IV+IU
PFU/mL
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
p33s-wt
p33s-6
p4k-wt
p4k-5
p46-wt
p46-9

FIGURE 31A

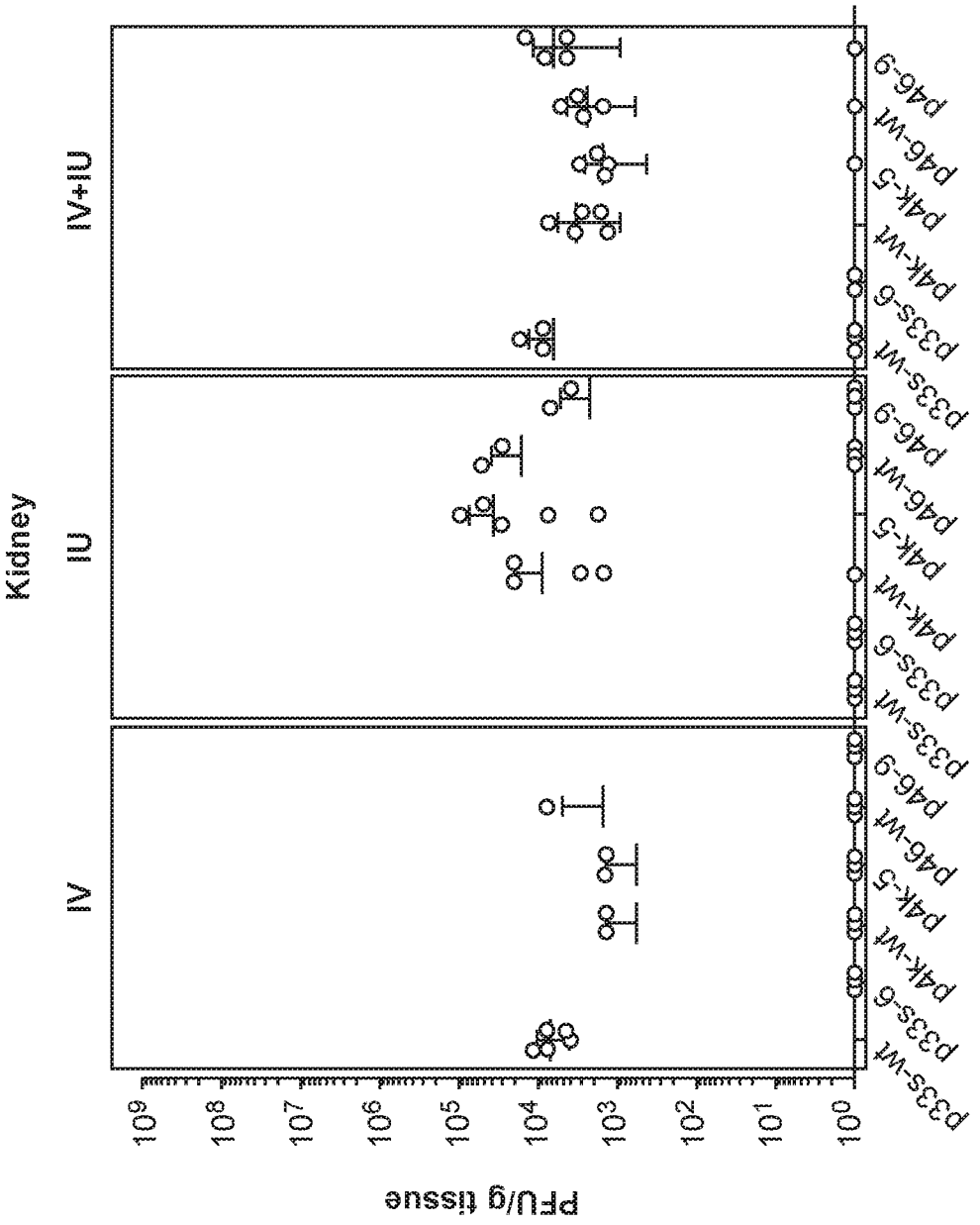

Kidney
IV
IU
IV+IU
PFU/g tissue
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
p33s-wt
p33s-6
p4k-wt
p4k-5
p46-wt
p46-9

FIGURE 31B

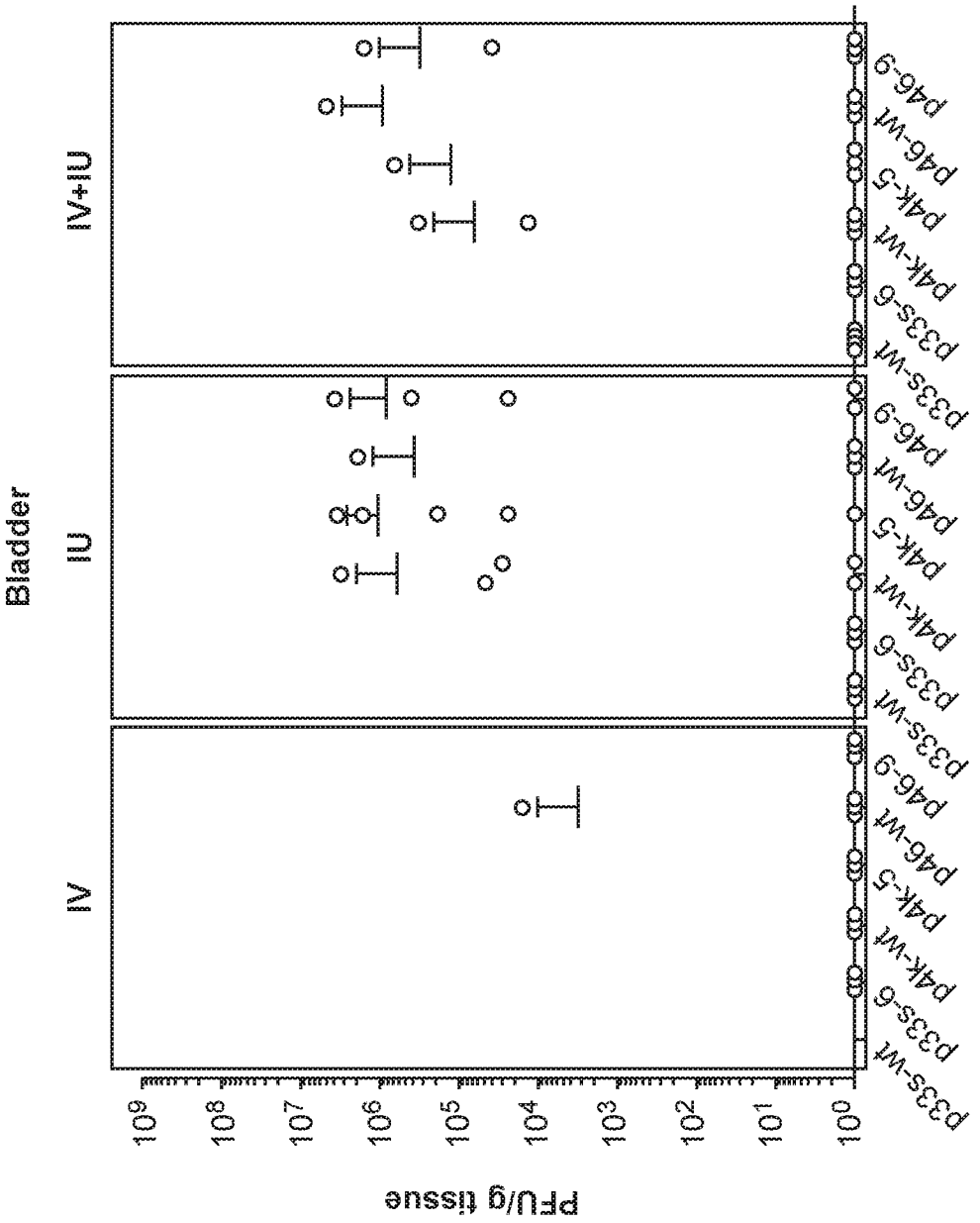

Bladder
IV
IU
IV+IU
PFU/g tissue
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
p33s-wt
p33s-6
p4k-wt
p4k-5
p46-wt
p46-9

FIGURE 31C

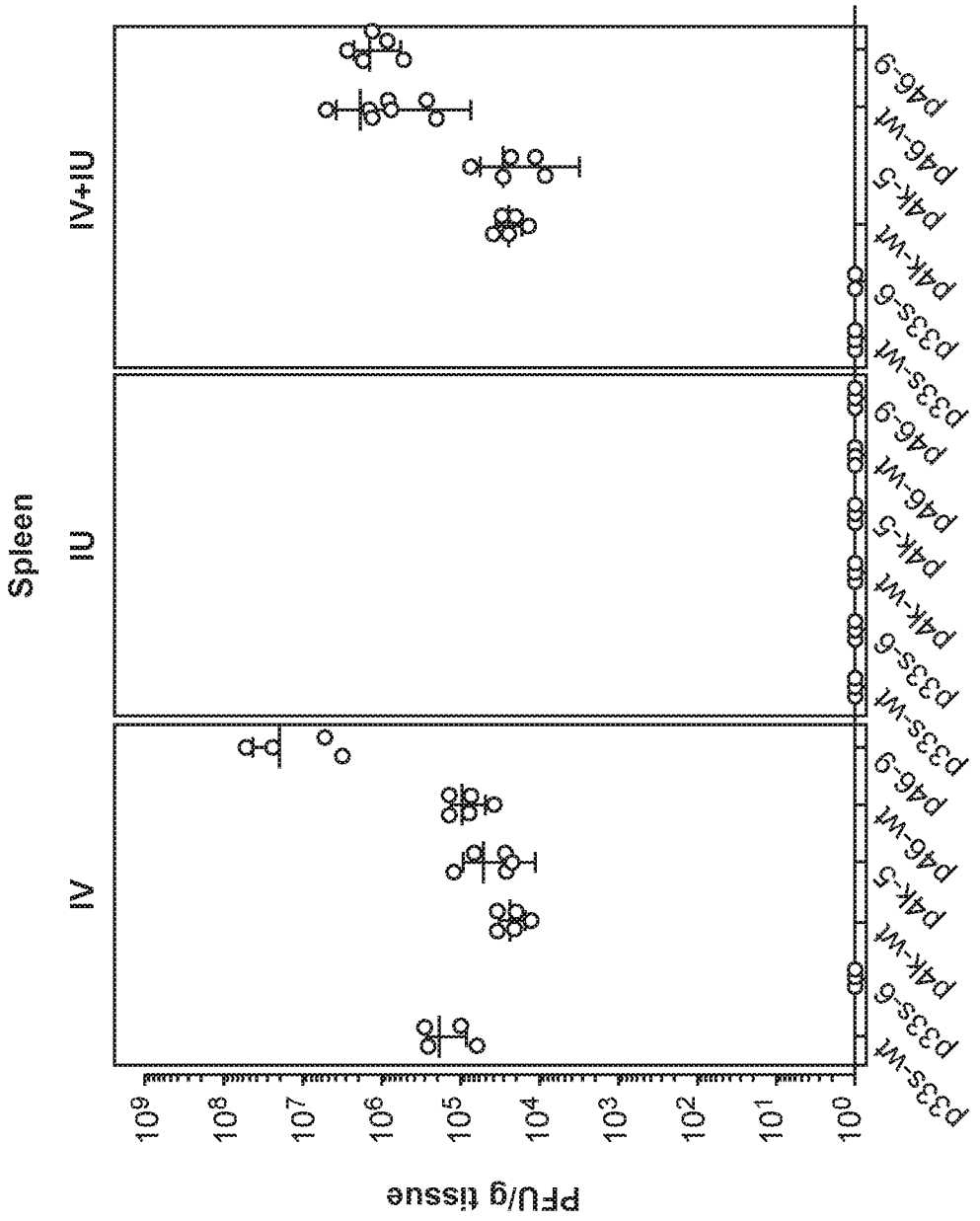
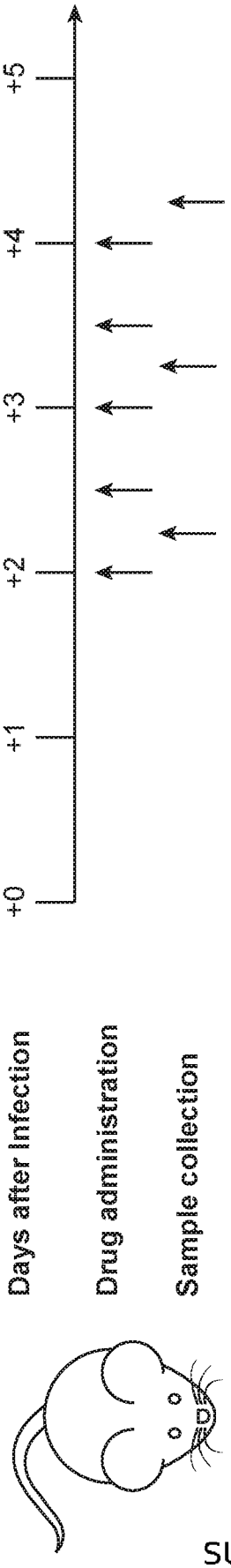

Spleen
IV
IU
IV+IU
PFU/g tissue
10^9
10^8
10^7
10^6
10^5
10^4
10^3
10^2
10^1
10^0
p33s-wt
p33s-6
p4k-wt
p4k-5
p46-wt
p46-9

FIGURE 31D



Group	N	Treatment	Route	Dose
1	10	None	N/A	N/A
2	10	Saline	IV	N/A
3	10		IU	N/A
4	10	crPhage high	IV	1 x 10^{10} PFU/phage
5	10		IU	1 x 10^{10} PFU/phage
6	10	crPhage med	IV	1 x 10^{8} PFU/phage
7	10		IU	1 x 10^{8} PFU/phage
8	10	crPhage low	IV	1 x 10^{6} PFU/phage
9	10		IU	1 x 10^{6} PFU/phage
10	10	Pyophage	IU	~7.5 x 10^{4} PFU (commercial dose)
11	10	Ciprofloxacin	IV	5 mg/kg body weight

FIGURE 32

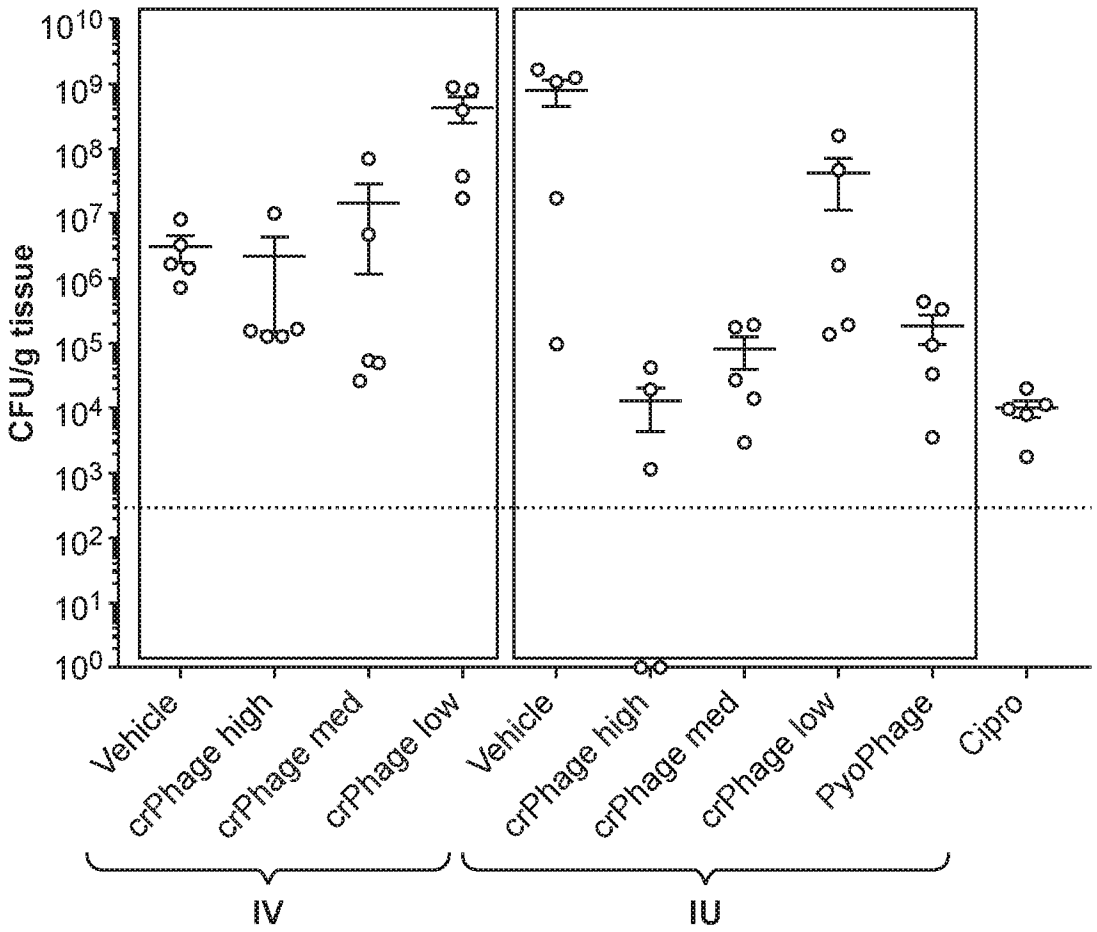

CFU/g tissue
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Vehicle
crPhage high
crPhage med
crPhage low
Vehicle
crPhage high
crPhage med
crPhage low
PyoPhage
Cipro
IV
IU

FIGURE 33A

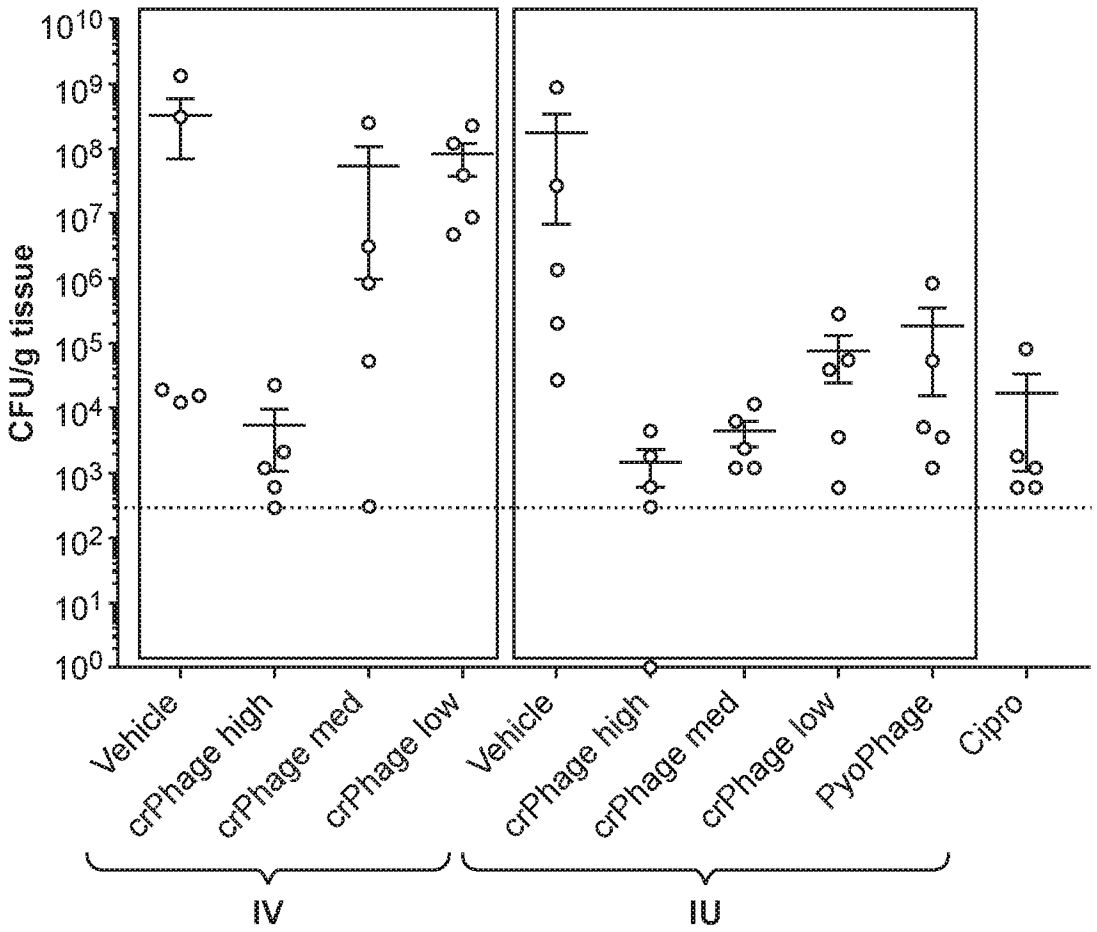

CFU/g tissue
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Vehicle
crPhage high
crPhage med
crPhage low
Vehicle
crPhage high
crPhage med
crPhage low
PyoPhage
Cipro
IV
IU

FIGURE 33B

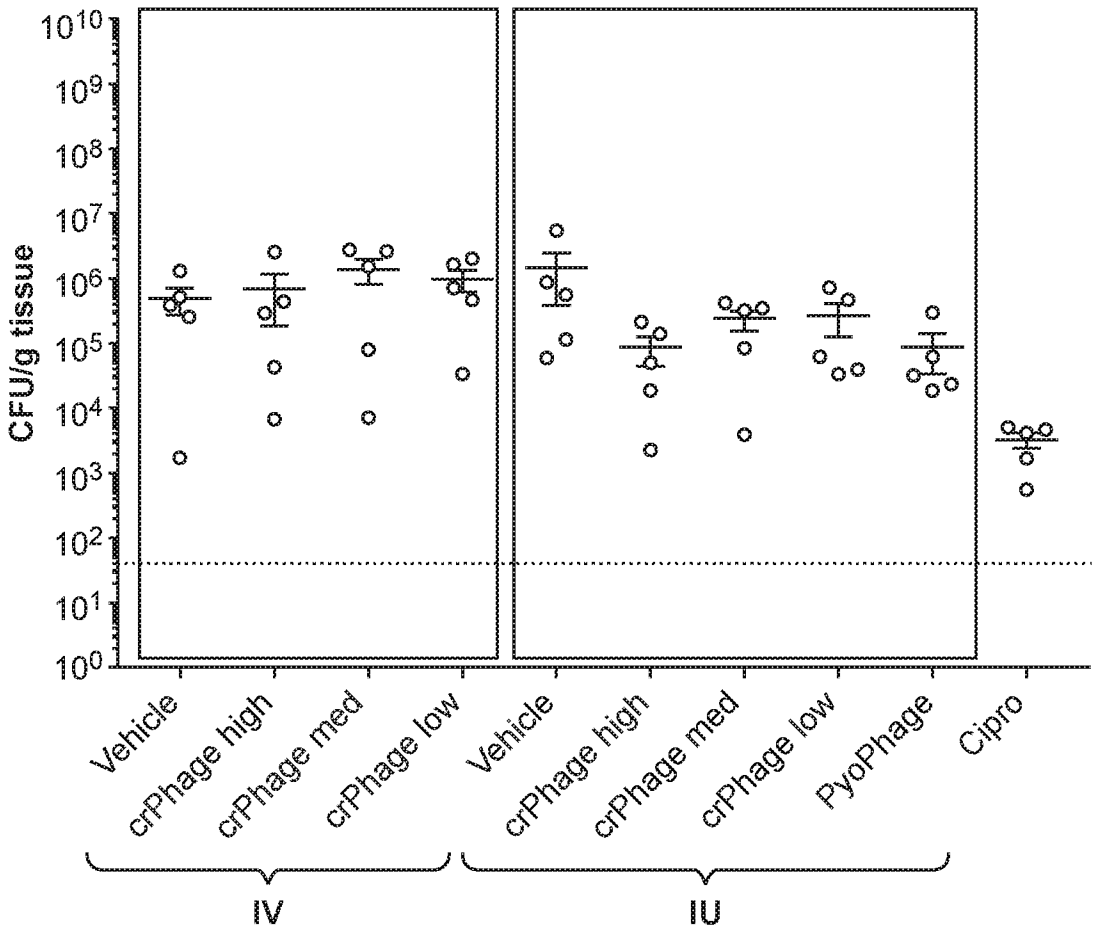

CFU/g tissue
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Vehicle
crPhage high
crPhage med
crPhage low
Vehicle
crPhage high
crPhage med
crPhage low
PyoPhage
Cipro
IV
IU

FIGURE 33C

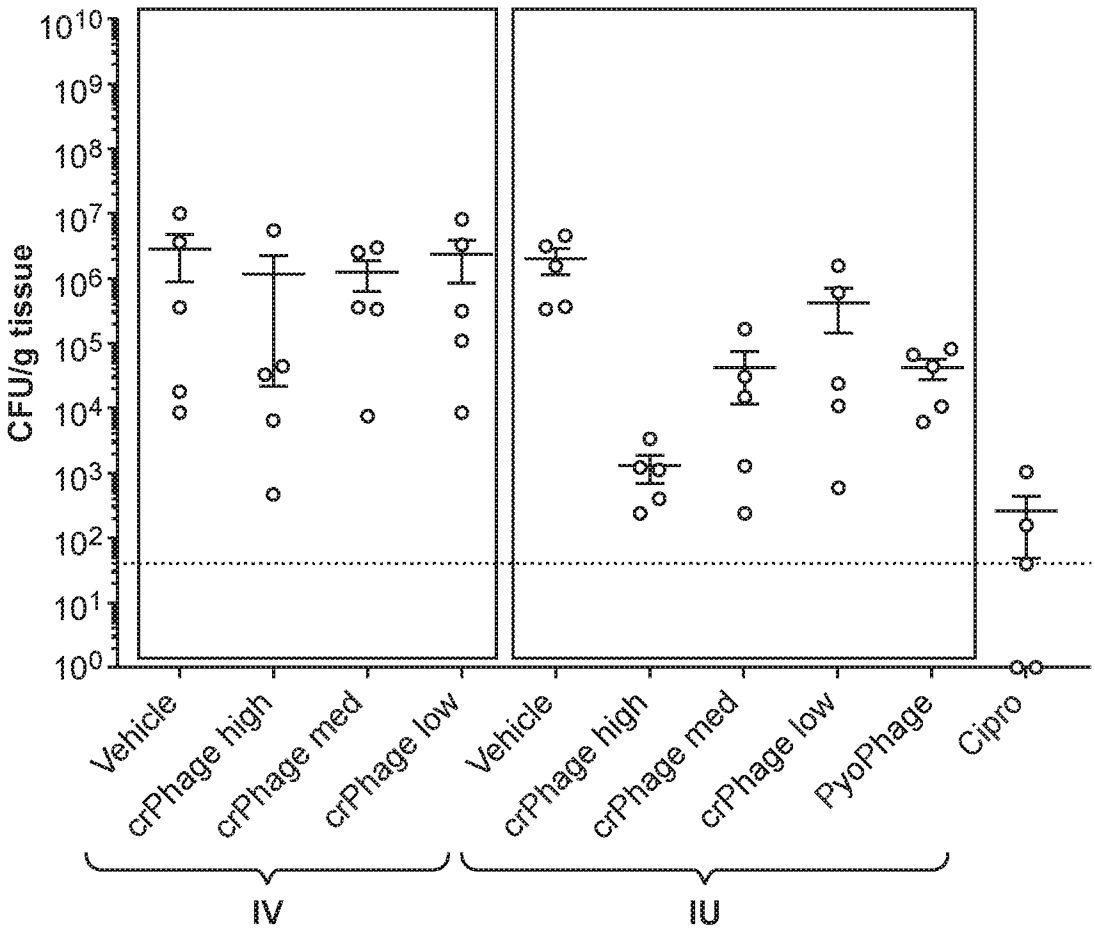

CFU/g tissue
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
Vehicle
crPhage high
crPhage med
crPhage low
Vehicle
crPhage high
crPhage med
crPhage low
PyoPhage
Cipro
IV
IU

FIGURE 33D

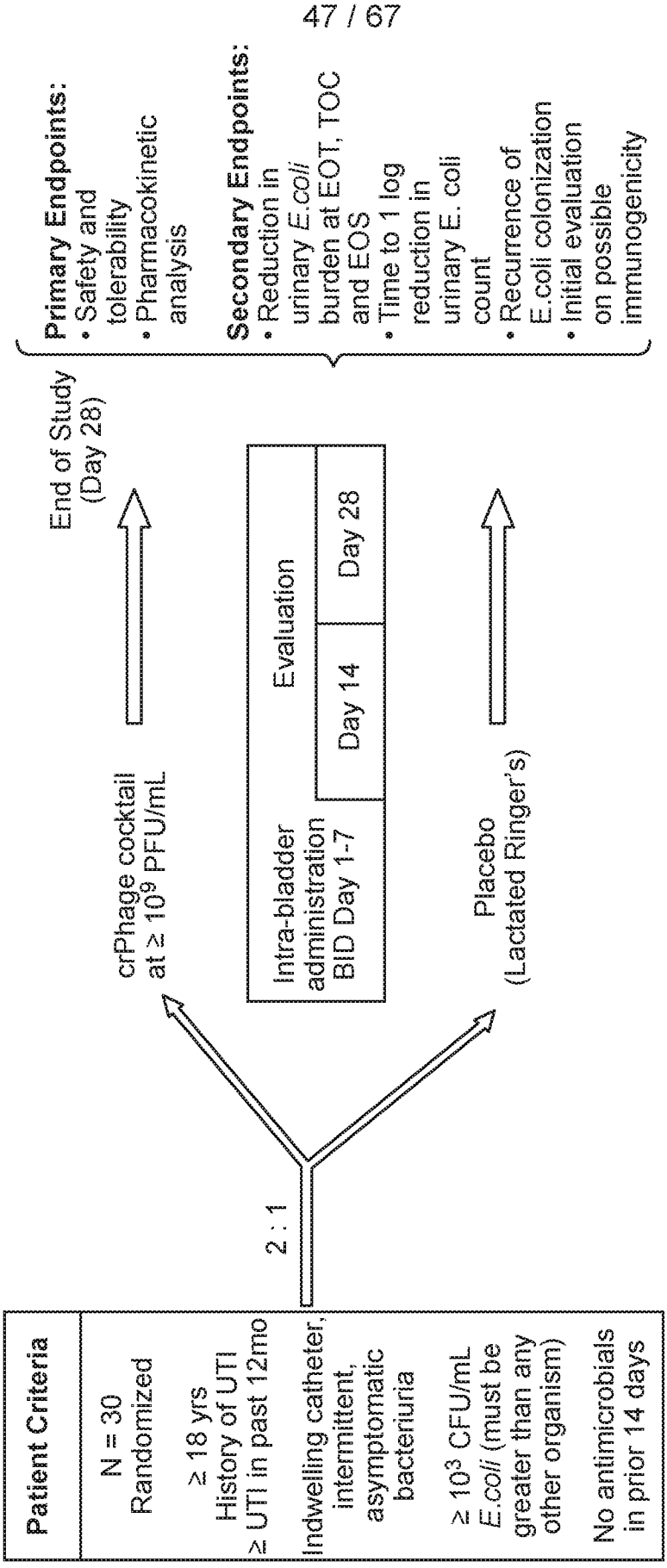

Patient Criteria
N = 30 Randomized
≥ 18 yrs
History of UTI
≥ UTI in past 12mo
Indwelling catheter, intermittent, asymptomatic bacteriuria
≥ 10^3 CFU/mL E.coli (must be greater than any other organism)
No antimicrobials in prior 14 days
2 : 1
crPhage cocktail at ≥ 10^9 PFU/mL
Placebo (Lactated Ringer's)
Intra-bladder administration BID Day 1-7
Evaluation
Day 14
Day 28
End of Study (Day 28)
Primary Endpoints:
• Safety and tolerability
• Pharmacokinetic analysis
Secondary Endpoints:
• Reduction in urinary E.coli burden at EOT, TOC and EOS
• Time to 1 log reduction in urinary E. coli count
• Recurrence of E.coli colonization
• Initial evaluation on possible immunogenicity

FIGURE 34A

Inclusion	Exclusion
• Males or females 18 years of age or older • Indwelling urinary or suprapubic catheters, requiring intermittent catheterization or with asymptomatic bacteriuria and lower urinary tract colonization caused by E. coli (≥ 103 CFU/mL) • Patients with mixed colonization with additional Gram-negative or Gram-positive bacteria isolated will be eligible if the other bacteria are present in lower relative CFU/mL levels than E. coli	• Clinical signs of active UTI or other infection requiring antimicrobial treatment Analgesic use is permitted • Have received Gram-negative bacteria antimicrobials within the past 14 days Patients being treated with Gram-positive antibiotics for non-urinary tract infections are NOT excluded • Presence of a surgically-modified bladder, except for a repaired ruptured bladder

FIGURE 34B

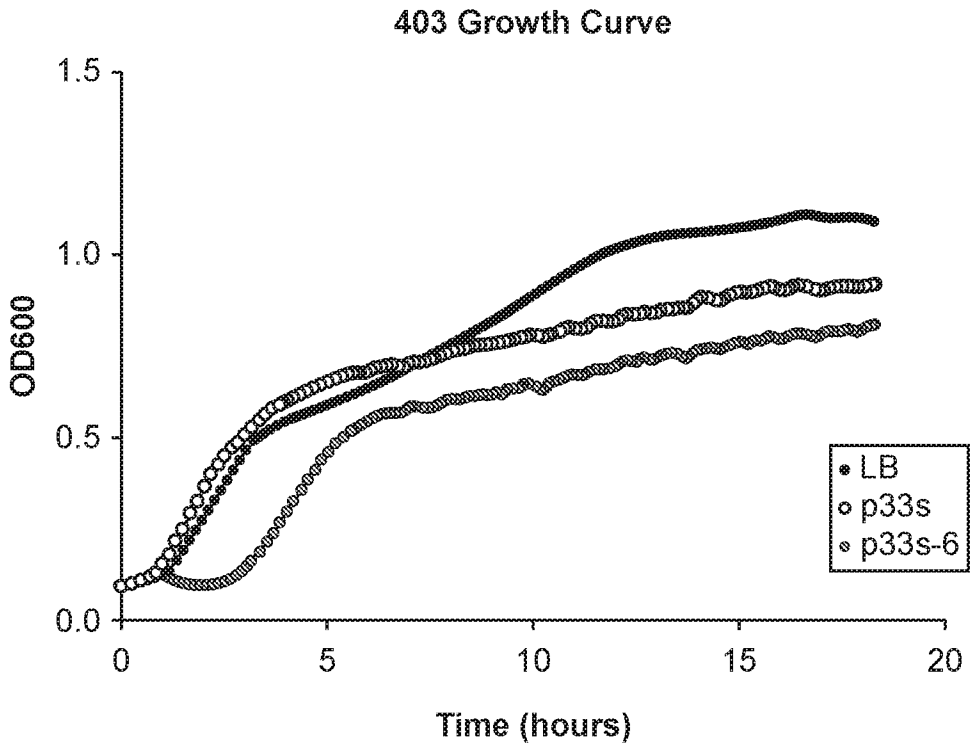

403 Growth Curve
OD600
1.5
1.0
0.5
0.0
0
5
10
15
20
Time (hours)
LB
p33s
p33s-6

FIGURE 35A

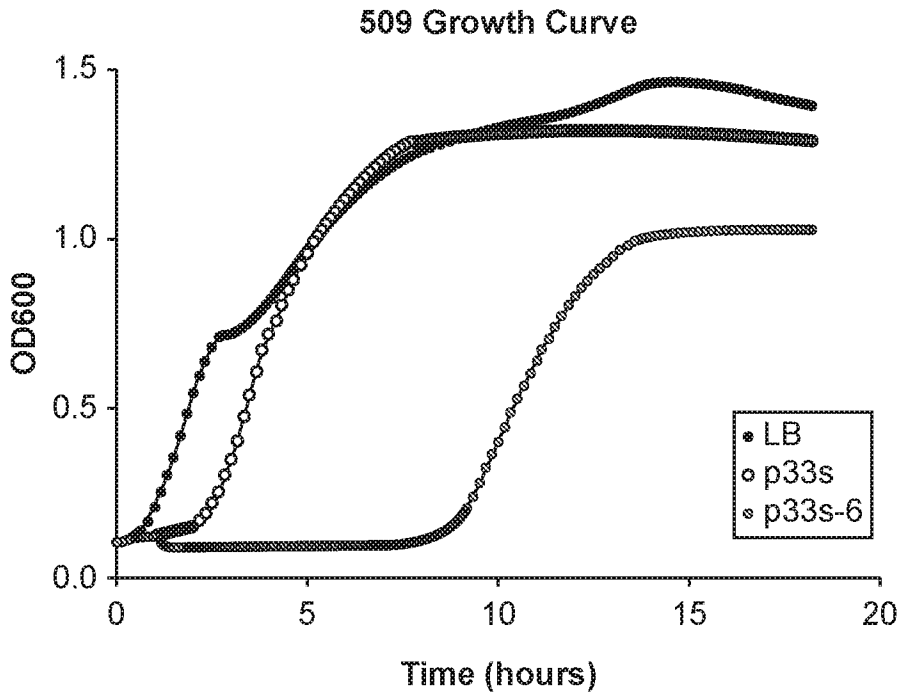

509 Growth Curve
OD600
1.5
1.0
0.5
0.0
0
5
10
15
20
Time (hours)
LB
p33s
p33s-6

FIGURE 35B

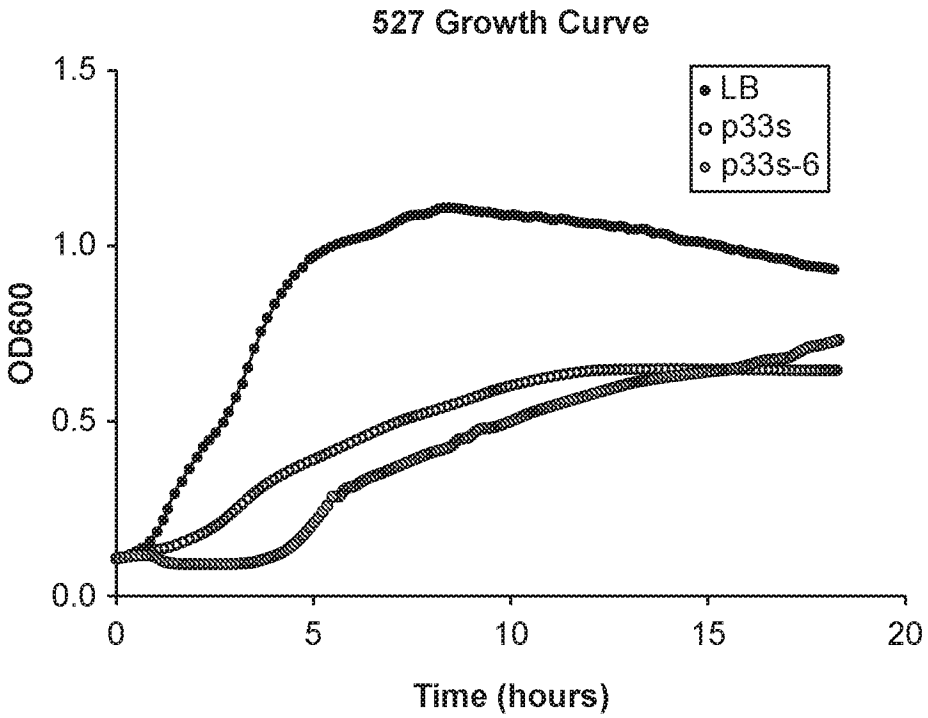

527 Growth Curve
LB
p33s
p33s-6
OD600
1.5
1.0
0.5
0.0
0
5
10
15
20
Time (hours)

FIGURE 35C

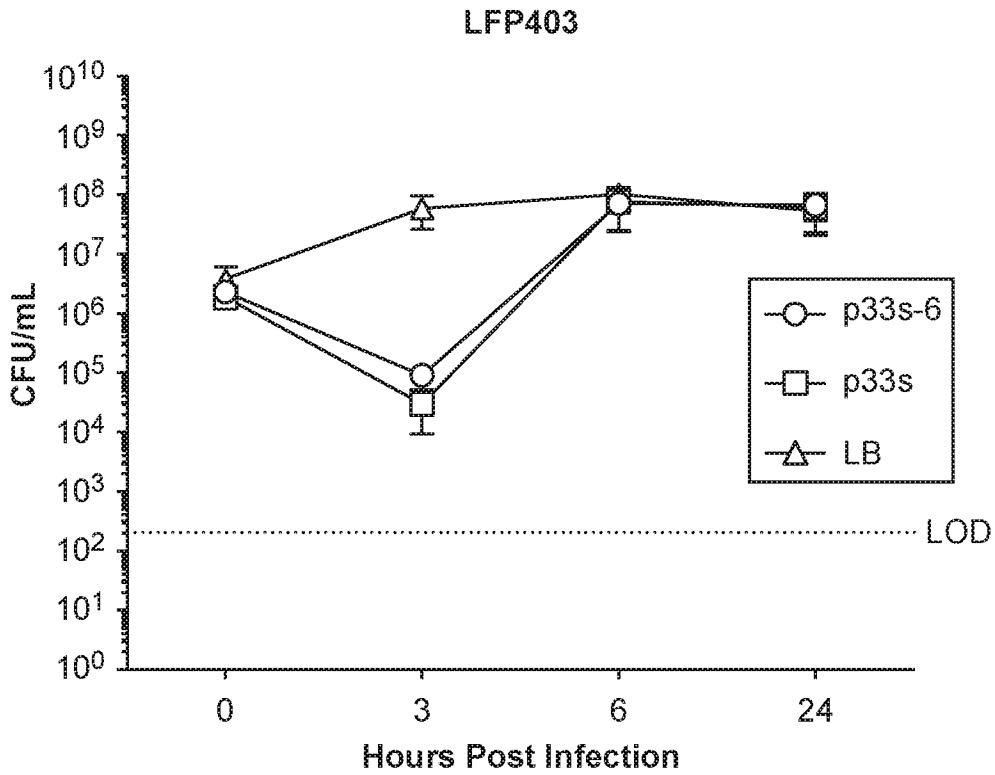

LFP403
CFU/mL
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
0
3
6
24
Hours Post Infection
p33s-6
p33s
LB
LOD

FIGURE 35D

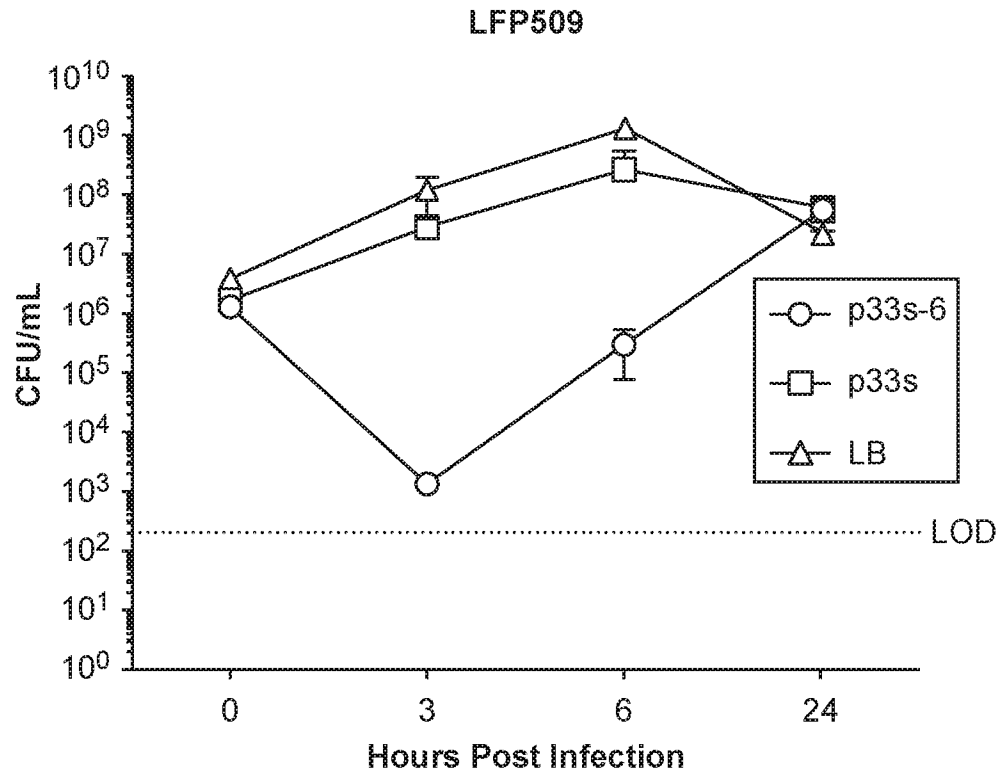

LFP509
CFU/mL
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
LOD
p33s-6
p33s
LB
0
3
6
24
Hours Post Infection

FIGURE 35E

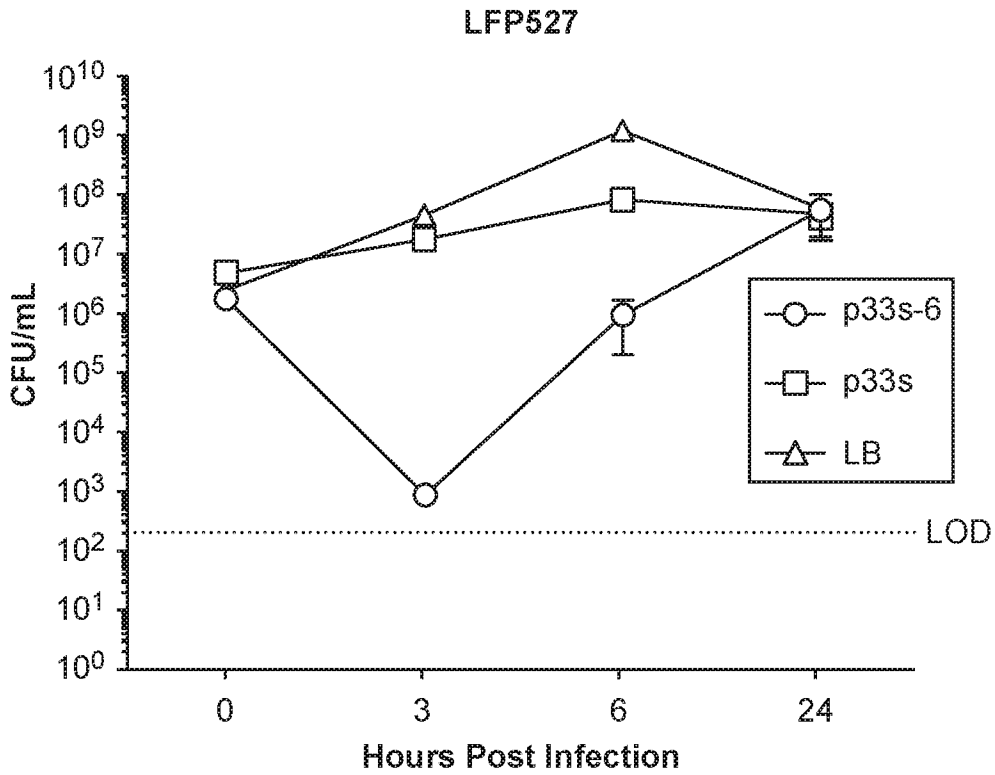

LFP527
CFU/mL
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
10^{4}
10^{3}
10^{2}
10^{1}
10^{0}
LOD
p33s-6
p33s
LB
0
3
6
24
Hours Post Infection

FIGURE 35F

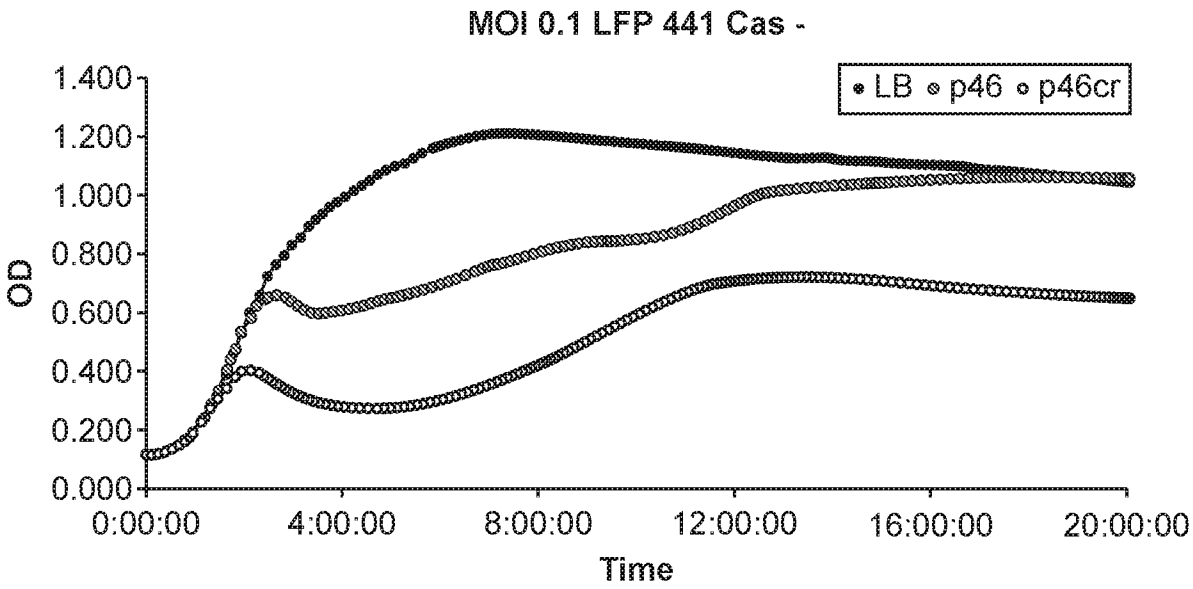

MOI 0.1 LFP 441 Cas -
LB
p46
p46cr
OD
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
0:00:00
4:00:00
8:00:00
12:00:00
16:00:00
20:00:00
Time

FIGURE 36A

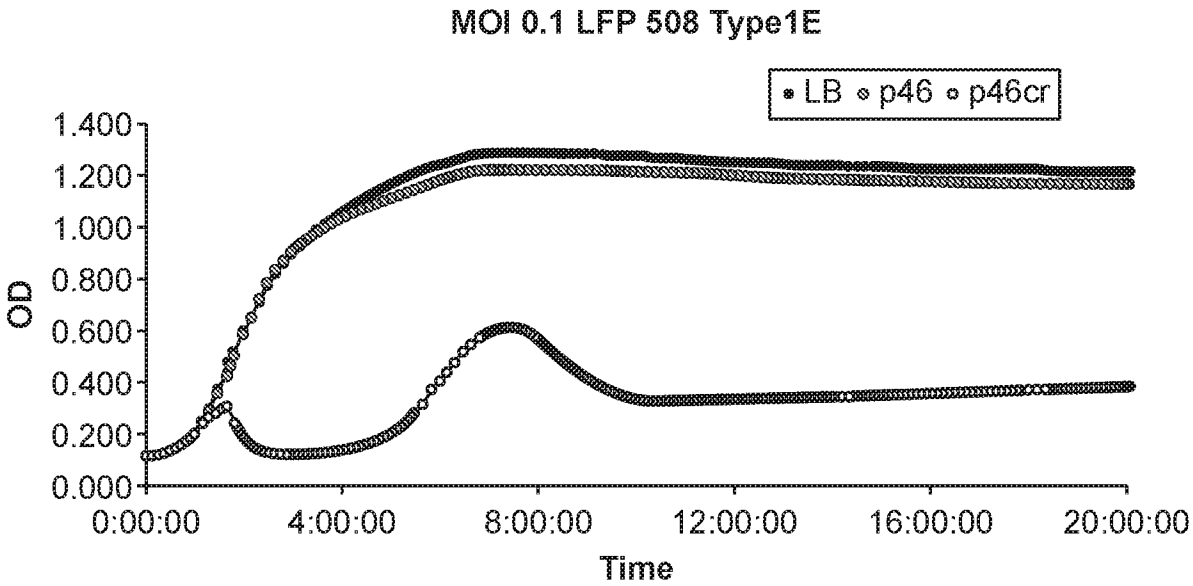

MOI 0.1 LFP 508 Type1E
LB
p46
p46cr
1.400
1.200
1.000
0.800
0.600
0.400
0.200
0.000
OD
0:00:00
4:00:00
8:00:00
12:00:00
16:00:00
20:00:00
Time

FIGURE 36B

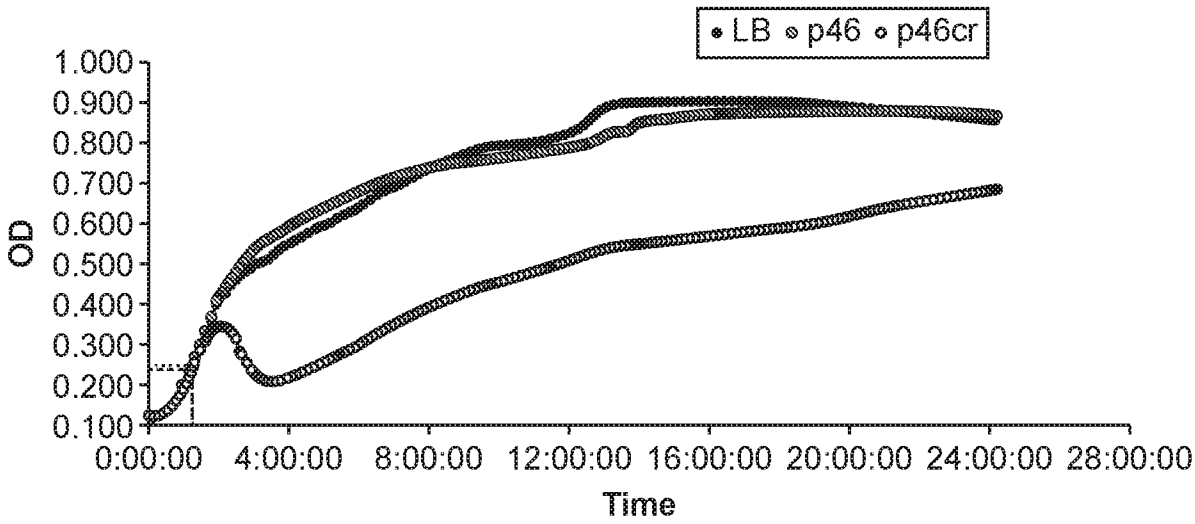

LB
p46
p46cr
OD
1.000
0.900
0.800
0.700
0.600
0.500
0.400
0.300
0.200
0.100
0:00:00
4:00:00
8:00:00
12:00:00
16:00:00
20:00:00
24:00:00
28:00:00
Time

FIGURE 36C

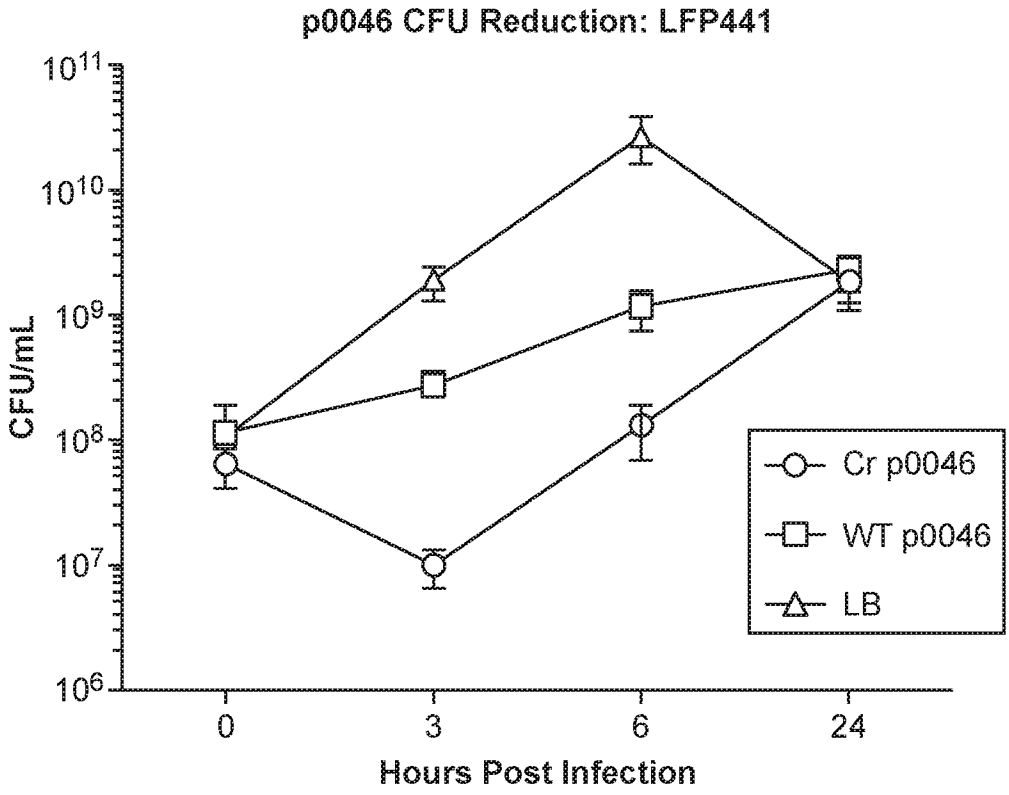

p0046 CFU Reduction: LFP441
CFU/mL
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
0
3
6
24
Hours Post Infection
Cr p0046
WT p0046
LB

FIGURE 36D

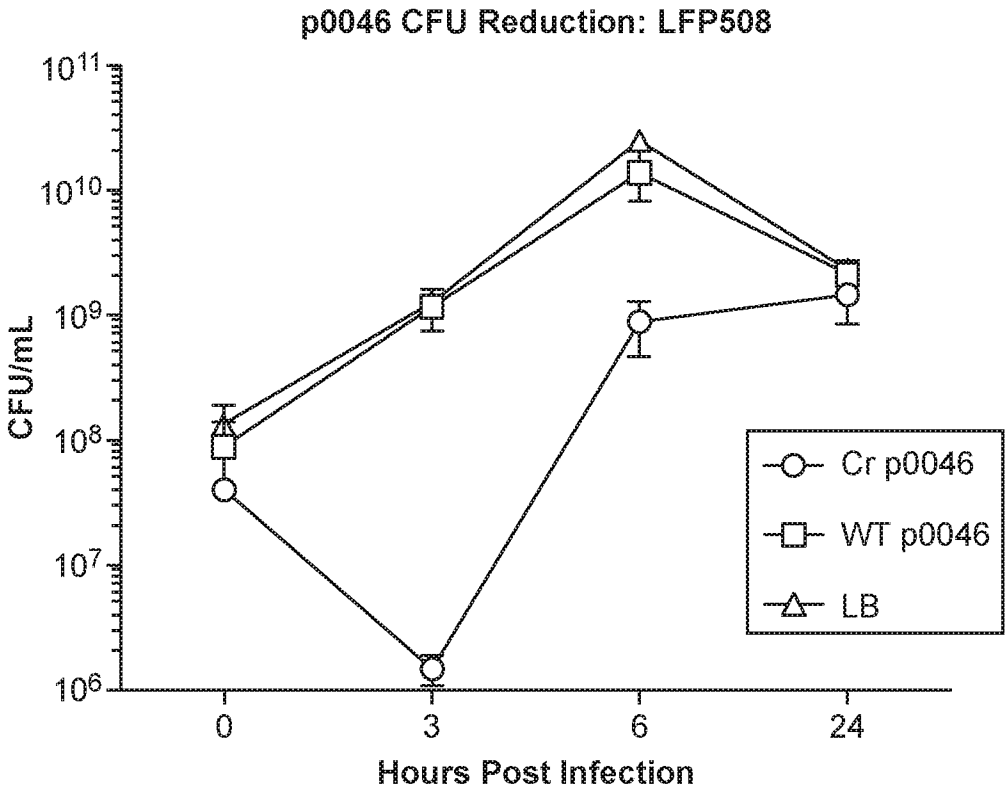

p0046 CFU Reduction: LFP508
CFU/mL
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
0
3
6
24
Hours Post Infection
Cr p0046
WT p0046
LB

FIGURE 36E

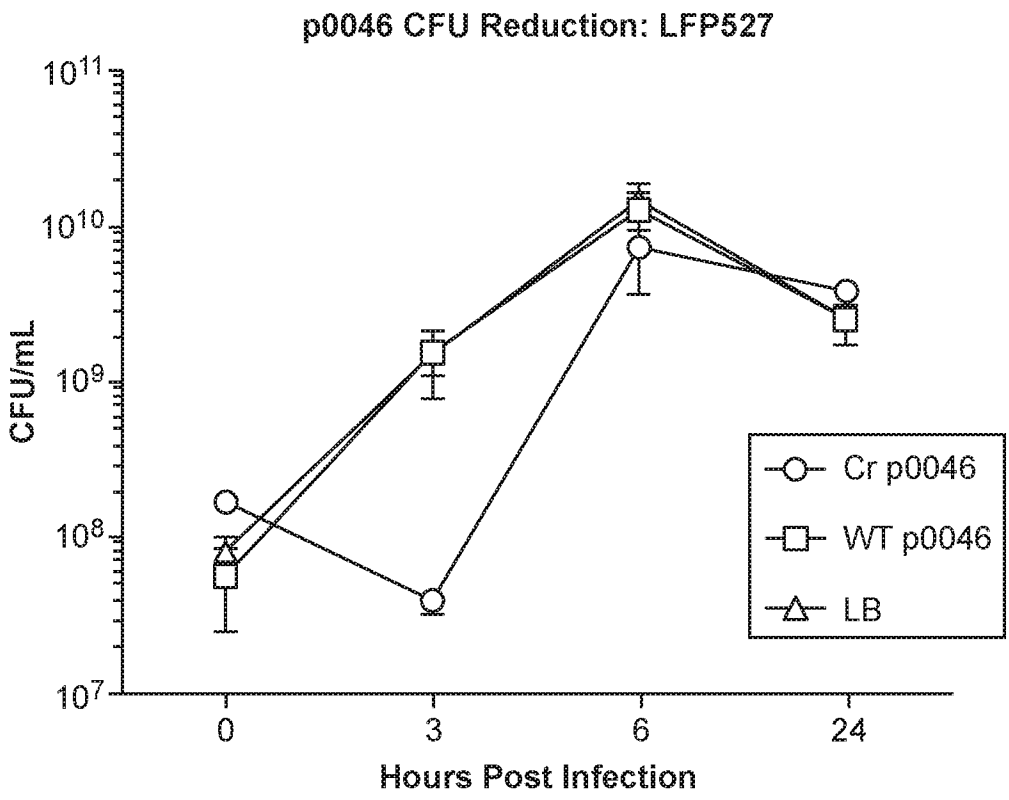

p0046 CFU Reduction: LFP527
CFU/mL
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
0
3
6
24
Hours Post Infection
Cr p0046
WT p0046
LB

FIGURE 36F

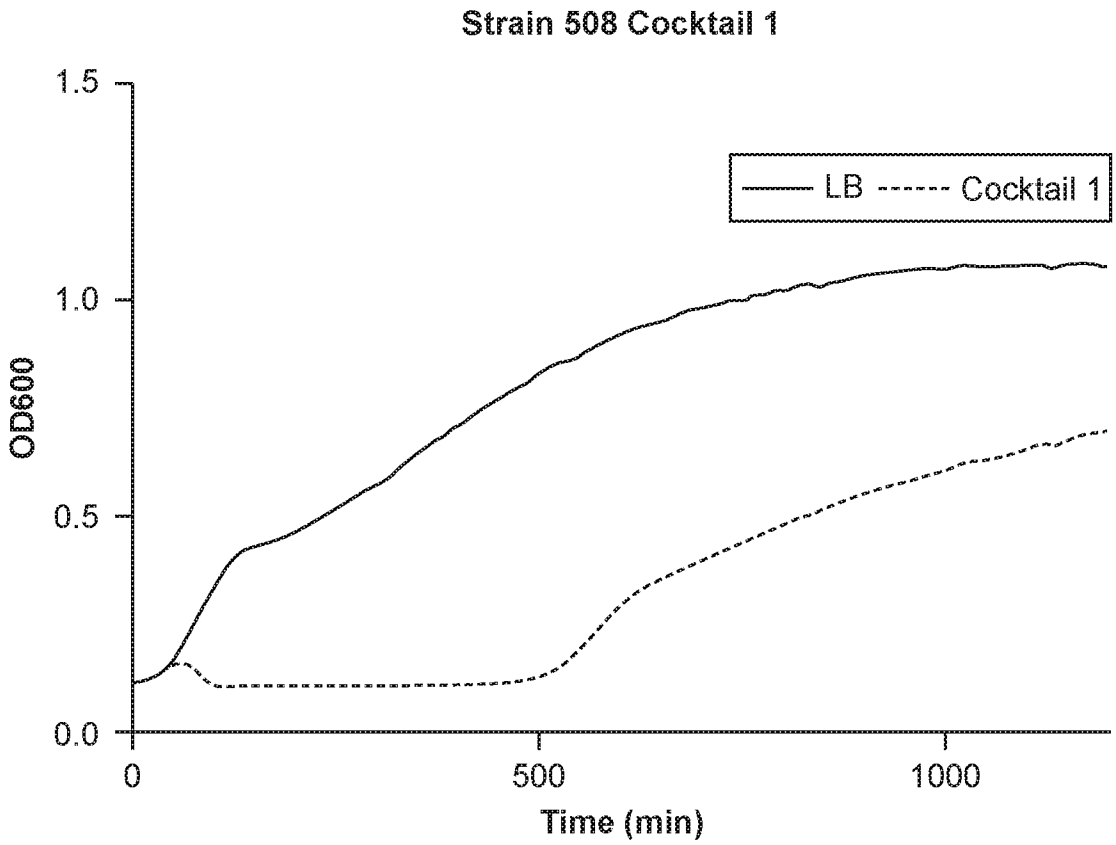

Strain 508 Cocktail 1
1.5
1.0
0.5
0.0
OD600
LB
Cocktail 1
0
500
1000
Time (min)

FIGURE 37A

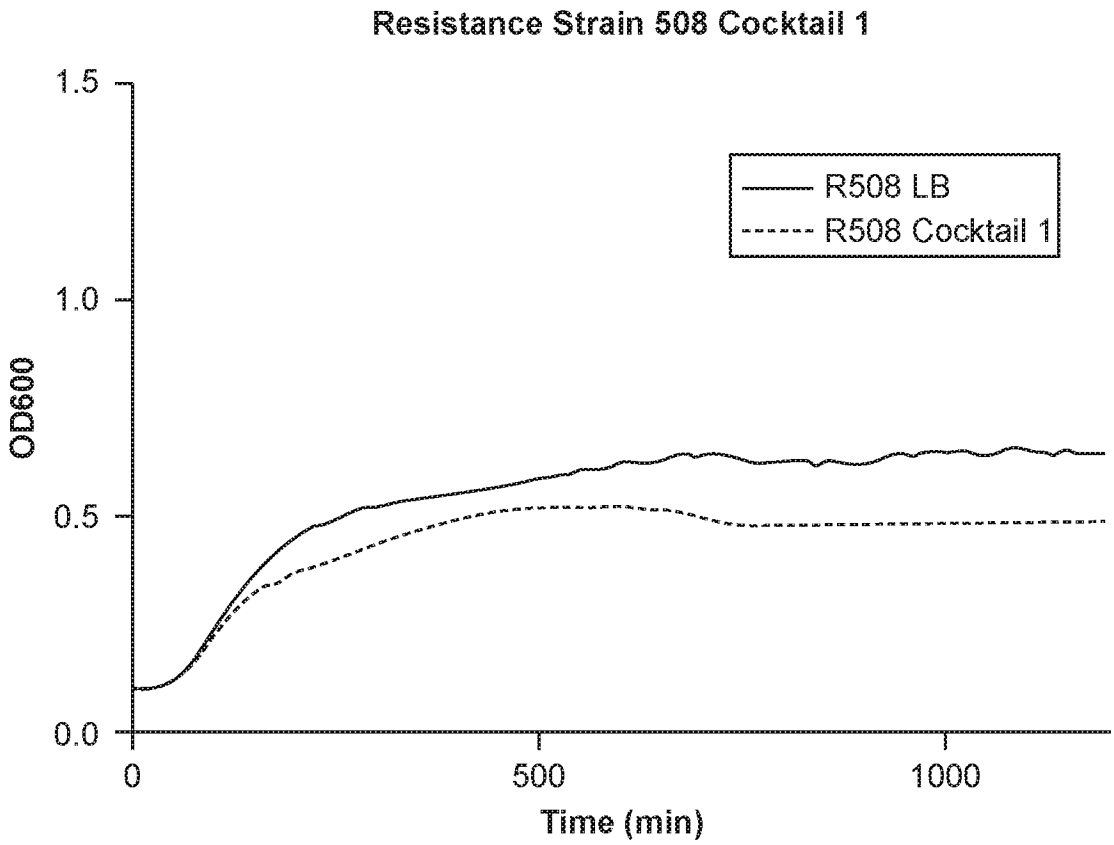

Resistance Strain 508 Cocktail 1
1.5
1.0
0.5
0.0
OD600
R508 LB
R508 Cocktail 1
0
500
1000
Time (min)

FIGURE 37B

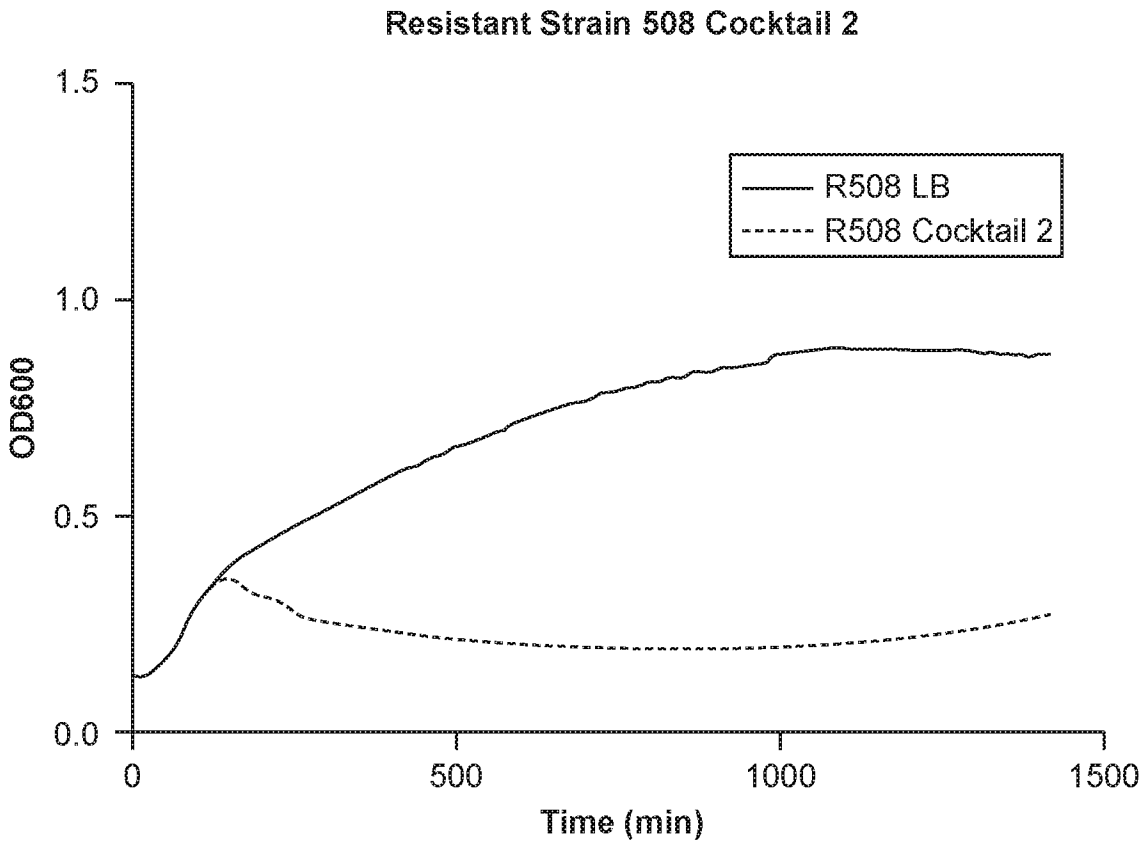

Resistant Strain 508 Cocktail 2
1.5
1.0
0.5
0.0
OD600
R508 LB
R508 Cocktail 2
0
500
1000
1500
Time (min)

FIGURE 37C

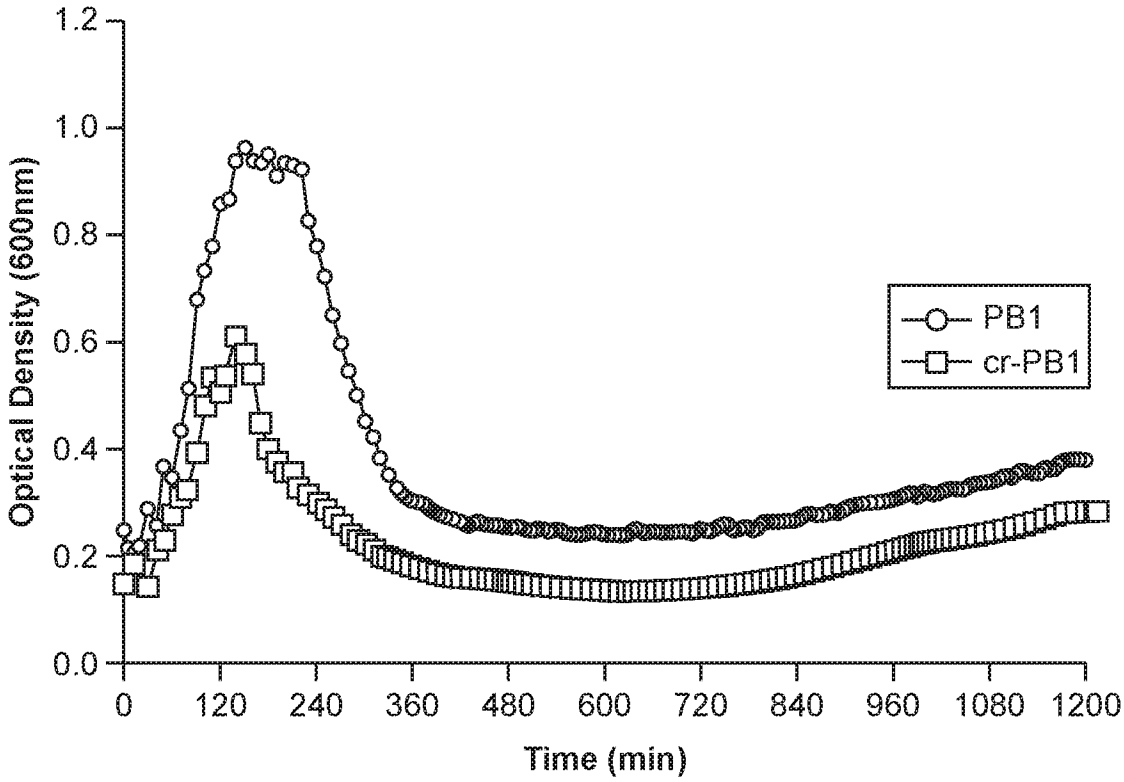

Optical Density (600nm)
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0
120
240
360
480
600
720
840
960
1080
1200
Time (min)
PB1
cr-PB1

FIGURE 38A

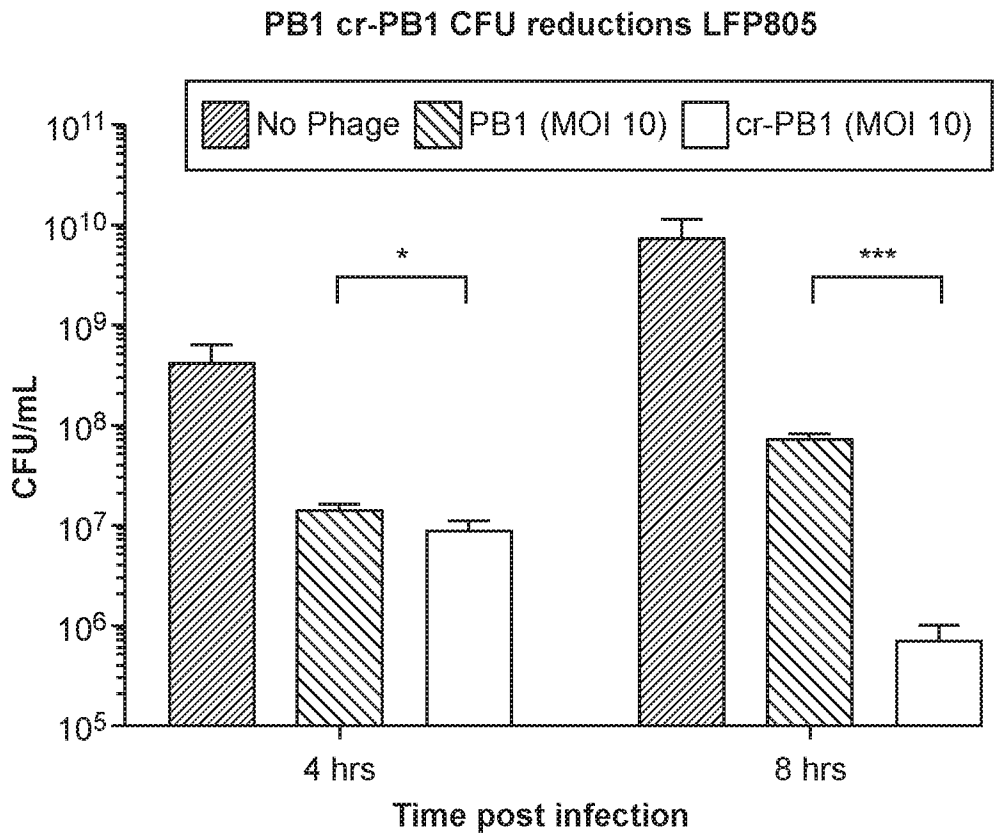

PB1 cr-PB1 CFU reductions LFP805
No Phage
PB1 (MOI 10)
cr-PB1 (MOI 10)
CFU/mL
10^{11}
10^{10}
10^{9}
10^{8}
10^{7}
10^{6}
10^{5}
*
***
4 hrs
8 hrs
Time post infection

FIGURE 38B

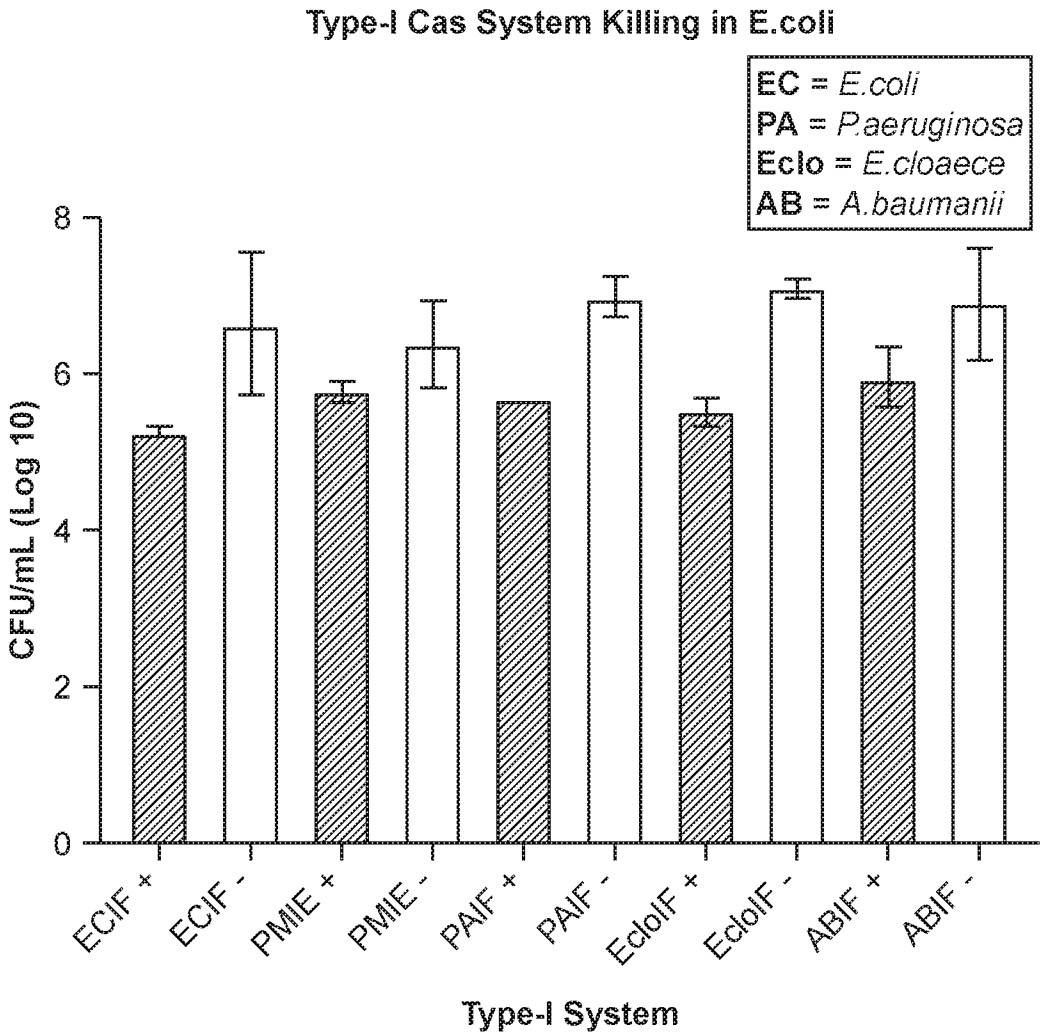

Type-I Cas System Killing in E.coli
EC = E.coli
PA = P.aeruginosa
Eclo = E.cloaece
AB = A.baumanii
CFU/mL (Log 10)
0
2
4
6
8
ECIF +
ECIF -
PMIE +
PMIE -
PAIF +
PAIF -
EcloIF +
EcloIF -
ABIF +
ABIF -
Type-I System

FIGURE 39A

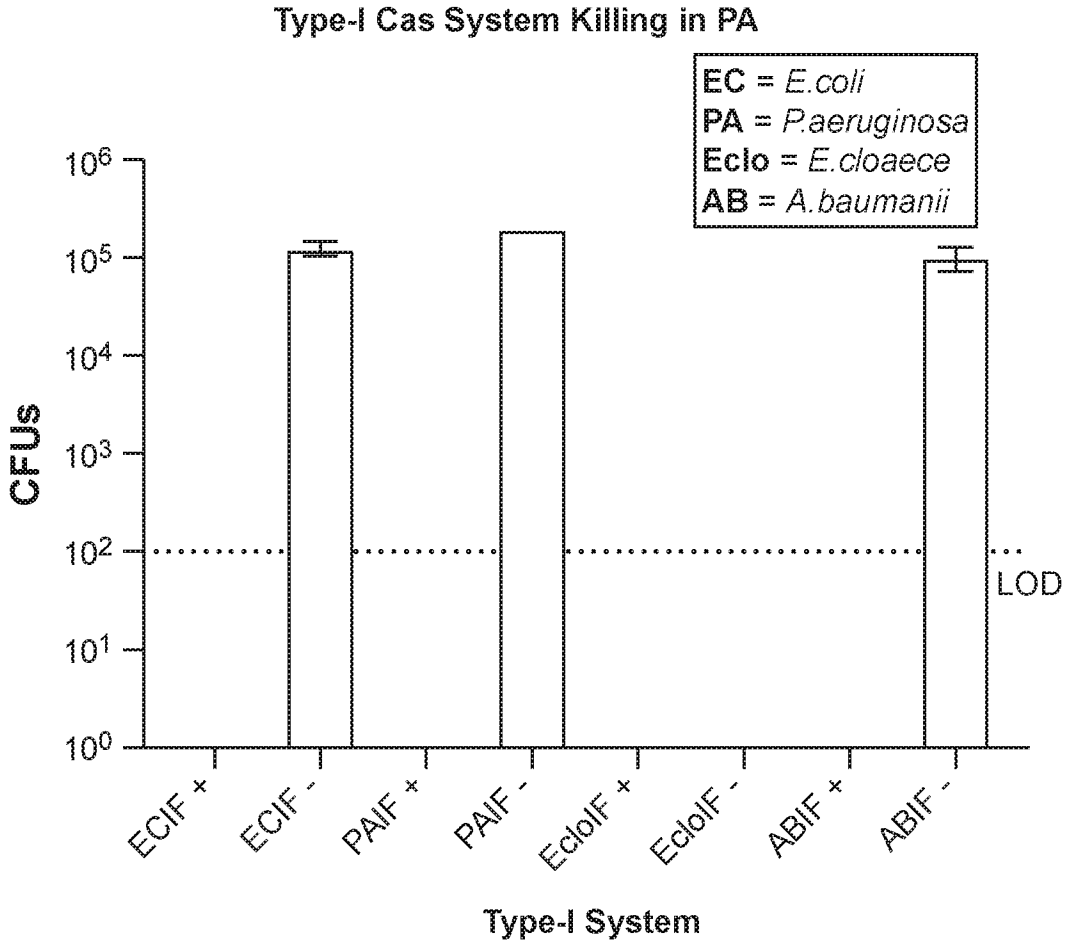

Type-I Cas System Killing in PA
EC = E.coli
PA = P.aeruginosa
Eclo = E.cloaece
AB = A.baumanii
CFUs
10^6
10^5
10^4
10^3
10^2
10^1
10^0
LOD
ECIF +
ECIF -
PAIF +
PAIF -
EcloIF +
EcloIF -
ABIF +
ABIF -
Type-I System

FIGURE 39B