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(54) **Title:** NON-REPLICATIVE TRANSDUCTION PARTICLES WITH ONE OR MORE NON-NATIVE TAIL FIBERS AND TRANSDUCTION PARTICLE-BASED REPORTER SYSTEMS

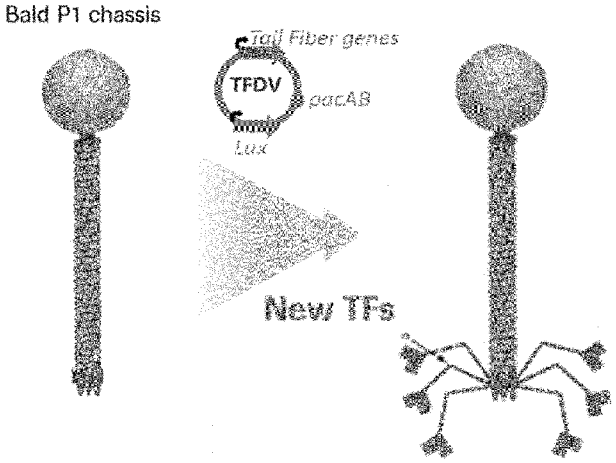

FIG. 1

(57) **Abstract:** The present invention relates to compositions comprising and methods of producing genetically engineered bacteriophages, bacteriophage-like particles and non-replicating transduction particles (NRTPs) that contain non-native tail fibers that display altered host specificity and/or reactivity. The present invention also relates to methods of using these bacteriophages and NRTPs for the development of novel diagnostics, therapeutics and/or research reagents for bacteria-related diseases.

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NON-REPLICATIVE TRANSDUCTION PARTICLES WITH ONE OR MORE NON-NATIVE TAIL FIBERS AND TRANSDUCTION PARTICLE-BASED REPORTER SYSTEMS

BACKGROUND OF THE INVENTION

Field of the invention

The invention relates to methods and compositions for packaging and delivery of non-replicative transduction particles derived from bacteriophages with non-native tail fibers that contain reporter molecules for detecting target organisms.

Description of the Related Art

A transduction particle refers to a virus capable of delivering a non-viral nucleic acid into a cell. Viral-based reporter systems have been used to detect the presence of cells and rely on the lysogenic phase of the virus to allow expression of a reporter molecule from the cell. These viral-based reporter systems use replication-competent transduction particles that express reporter molecules and cause a target cell to emit a detectable signal.

However, the lytic cycle of the virus has been shown to be deleterious to viral-based reporter assays. Carrière, C. *et al.*, *Conditionally replicating luciferase reporter phages: Improved sensitivity for rapid detection and assessment of drug susceptibility of Mycobacterium tuberculosis.* Journal of Clinical Microbiology, 1997. **35**(12): p. 3232-3239. Carrière *et al.* developed *M. tuberculosis*/bacillus Calmette-Guérin (BCG) luciferase reporter phages that have their lytic cycles suppressed at 30°C, but active at 37°C. Using this system, Carrière *et al.* have demonstrated the detection of BCG using phage reporters with a suppressed lytic cycle.

There are disadvantages, however, associated with suppressing but not eliminating the replication functions of the bacteriophage in bacteriophage-based reporter assays. First, controlling replication functions of the bacteriophage imposes limiting assay conditions. For example, the lytic cycle of the reporter phage phAE40 used by Carrière *et al.* was repressed when the phage was used to infect cells at the non-permissive temperature of 30°C. This temperature requirement imposed limiting conditions on the reporter assay in that the optimum temperature for the target bacteria was 37°C. These limiting conditions hinder optimum assay performance.

Moreover, the replication functions of the virus are difficult to control. The replication of the virus should be suppressed during the use of the transduction particles as a reporter system. For example, the lytic activity of the reporter phage phAE40 reported by Carrière *et al.* was reduced

but was not eliminated, resulting in a drop in luciferase signal in the assay. Carrière *et al.* highlighted possible causes for the resulting drop in reporter signal, such as intact phage-expressed genes and temperature limitations of the assay, all stemming from the fact that the lytic cycle of the phage reporter was not eliminated.

Reporter assays relying on the natural lysogenic cycle of phages can be expected to exhibit lytic activity sporadically. In addition, assays that rely on the lysogenic cycle of the phage can be prone to superinfection immunity from target cells already lysogenized with a similar phage, as well as naturally occurring host restriction systems that target incoming virus nucleic acid, thus limiting the host range of these reporter phages.

In other examples, transduction particle production systems are designed to package exogenous nucleic acid molecules, but the transduction particle often contains a combination of exogenous nucleic acid molecules and native progeny virus nucleic acid molecules. The native virus can exhibit lytic activity that is a hindrance to assay performance, and the lytic activity of the virus must be eliminated to purify transduction particles. However, this purification is generally not possible. In U.S. 2009/0155768 A, entitled *Reporter Plasmid Packaging System for Detection of Bacteria*, Scholl *et al.* describes the development of such a transduction particle system. The product of the system is a combination of reporter transduction particles and native bacteriophage (Figure 8 in the reference). Although the authors indicate that the transduction particle and native bacteriophage can be separated by ultracentrifugation, this separation is only possible in a system where the transduction particle and the native virus exhibit different densities that would allow separation by ultracentrifugation. While this characteristic is exhibited by the bacteriophage T7-based packaging system described in the reference, this is not a characteristic that is generally applicable for other virus systems. It is common for viral packaging machinery to exhibit headful packaging that would result in native virus and transduction particles to exhibit indistinguishable densities that cannot be separated by ultracentrifugation. Virus packaging systems also rely on a minimum amount of packaging as a requirement for proper virus structural assembly that results in native virus and transduction particles with indistinguishable densities.

Recently, methods and systems for packaging reporter nucleic acid molecules into non-replicative transduction particles (NRTPs), also referred herein as Smarticles, have been described in U.S Patent No. 9,388,453 and in U.S. Patent Application Publication No. 2017/0166907 in which the production of replication-competent native progeny virus nucleic acid molecules were greatly reduced due to the disruption of the packaging initiation site in the bacteriophage genome

Currently NRTPs are produced from naturally occurring lysogenic bacteriophages found in nature, e.g. φ80α bacteriophage or P1 bacteriophage. It is a time consuming process to find, characterize, and modify new bacteriophages into NRTPs that are able to transduce cells with the desired host range specificity.

Several recent studies (Ando et al., Cell Systems 2015, 1: 187-196; Yosef et al., Molecular Cell 2017, 66: 721-728) have shown that T3 and T7 bacteriophages and bacteriophage/transduction particle mixtures can be re-programmed to recognize desired hosts by swapping tail/tail fibers from T3/T7-like viral sources that would enable the phage to recognize different hosts. To this end, different tail/tail fibers were constructed on the T3/T7 chassis and tested for their ability to inject DNA into different hosts. While these experiments paved the way for producing hybrid particles with novel host ranges, these effects were shown only with phages/tail/tail fibers found in T3-and T7-like bacteriophages. Particularly, these studies do not enable a phage-genome engineering platform that is based on the chassis from other bacteriophage families, such as the *Myroviridae* family which, unlike the T3/T7 bacteriophages, have long and contractile tails and involve completely different tail fiber genes.

One of the most well characterized *Myoviridae* phage is the P1 bacteriophage that infects *Escherichia coli* (*E. coli*) and other bacteria in the Enterobacteriaceae family. Host-range specificity of P1 is controlled within the tail-fiber operon that is approximately 6kb in length (FIG. 2) and includes an invertible C-segment region where either of two alternate 3` ends of the tail fiber gene ($s_v$ or $s_v$') can be fused to a constant 5` end (s_c) located outside of the invertible region. Two alternate genes *u* and *u*' are located outside of the S gene and encode alternate forms of a chaperone protein that is involved in tail fiber assembly. Two other genes in the tail-fiber operon are the *r* gene, whose gene product R is believed to be involved in tail fiber assembly and the gene *cin* which encodes a sequence specific recombinase enzyme which can "flip" the nucleic acid between the Cin recombination sites, known as cix sites, that modifies host specificity, and are described in Guidolin et al., 1989 Gene, 76: 239-243, and in Sandmeier et al., 1992, Journal of Bacteriology, 174(12): 3936-3944.

Accordingly, there is a need in the art for a quick, reliable, and scalable method to generate new NRTPs derived from the tail fibers of many types of phages. Therefore, the present invention relates to a tail fiber replacement platform (or chassis) derived from P1 bacteriophage that can be altered in only the tail fibers in order to change or extend their host specificity, and that this platform can utilize tail fibers from many viral families. Additionally, the present invention relates to methods to improve the yield of NRTPs from an engineered production line and methods of producing multiple natural or engineered tail fibers-endowed NRTPs in a single

fermentation. This approach can be used for other diverse bacteriophages to develop new NRTPs with desired inclusivity and exclusivity profiles with respect to host-range specificities.

SUMMARY OF THE INVENTION

The present invention contemplates a bacteriophage lysogen genetically disrupted to the expression of one or more factors critical to native tail fiber production (examples -genes *s*, *u*, *u*', *r* and *s*'). This could be accomplished through any mutagenesis approach known to one skilled in the art, or through isolation of a tail fiber deficient phage remnant from natural sources. This genetically disrupted phage lysogen that lacks the ability to express functional tail fibers of its own without complementation is referred as a chassis or a "Bald" chassis. In one embodiment, the disrupted lysogen has a mutation or a deletion in one or more of the genes selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes In another embodiment, the disrupted lysogen contains a gene coding for a selectable marker that disrupts the expression of one or more of the genes. In yet another embodiment, the disrupted lysogen comprises a nucleotide sequence of SEQ ID NO: 97.

One further aspect of the invention relates to methods involving the expression of native, new tail fiber genes, or hybrid tail fibers in a lysogen genetically disrupted for native tail fiber expression. This could be accomplished using a packaging plasmid, secondary expression plasmid, genomic expression (phage lysogen or bacterial genome), or any other method known to one skilled in the art.

Therefore, the present invention relates to a bacteriophage tail fiber replacement platform comprising a bacteriophage lysogen from the family Myoviridae (Myoviridae lysogen) that contains a genetic disruption that prevents the expression of one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen, wherein said one or more genes are selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes; and a complementary nucleic acid molecule comprising one or more genes selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a

gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes, wherein the complementary nucleic acid complements the genetic disruption of the Myoviridae lysogen whereby functional tail fibers are produced.

In one embodiment, the Myoviridae lysogen is derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one embodiment, the Myoviridae lysogen is derived from the Punalikevirus genus. In another embodiment, the Myoviridae lysogen is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

In one embodiment, the one or more genes or the combination of genes on the complementary nucleic acid molecule contain regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen such that the functional tail fibers that are produced are not native tail fibers. In such embodiments, the tail fibers that are produced are functional tail fibers that are not native to the initial bacteriophage lysogen. In another embodiment, the one or more genes or the combination of genes contain regions that are not derived from P1 bacteriophage such that the functional tail fibers that are produced are not native P1 bacteriophage tail fibers.

In yet another embodiment, the genetic disruption on the Myoviridae lysogen comprises one or more mutation in any of the one or more genes. In some embodiments, the Myoviridae lysogen may comprise one or more mutation in one of the one or more genes. In other embodiments, the Myoviridae lysogen may comprise one or more mutation in at least two of the one or more genes. In one embodiment, the one or more mutation is in any one of the P1 bacteriophage genes s, s', u, u' and r, in any combination of genes s, s', u, u' and r, or homologs, orthologs, or analogs of genes s, s', u, u' and r.

In one embodiment, the genetic disruption on the Myoviridae lysogen comprises one or more deletion in any one of the one or more genes. In some embodiments, the Myoviridae lysogen may comprise one or more deletion in one of the one or more genes. In other embodiments, the Myoviridae lysogen may comprise one or more deletion in at least two of the one or more genes. In certain embodiments, at least one deletion is a deletion of a complete coding sequence of one of the one or more genes. In other embodiments, any of the one or more deletions is a deletion of a complete coding sequence of one of the one or more genes. In one embodiment, the one or more deletion is in any one of the P1 bacteriophage genes s, s', u, u' and r, in any combination

of genes s, s', u, u' and r, or homologs, orthologs, or analogs of genes s, s', u, u' and r. In another embodiment, the genetic disruption on the Myoviridae lysogen further comprises the insertion of a gene coding for a selectable marker that disrupts the expression of any one of the P1 bacteriophage genes. In one embodiment, the Myoviridae lysogen comprises a nucleic acid sequence of SEQ ID NO: 97.

In one embodiment, the complementary nucleic acid molecule is a plasmid molecule. In another embodiment, the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at the site of the genetic disruption. In yet another embodiment, the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at a site other than the site of the genetic disruption. In yet another embodiment, the complementary nucleic acid molecule is integrated into a genome of a host bacteria cell that contains the Myoviridae lysogen.

In one embodiment, the Myoviridae lysogen contains a further disruption in one or more packaging genes that are involved in packaging the genome of the Myoviridae lysogen. Thereby the one or more packaging genes that are involved in packaging the genome of the Myoviridae lysogen are rendered non-functional. In one embodiment, the one or more packaging genes that complement the disruption in the one or more packaging genes in the Myoviridae lysogen are contained in the plasmid molecule. In another embodiment, the one or more packaging genes that complement the disruption in the one or more packaging genes in the Myoviridae lysogen are contained in a reporter or therapeutic expression plasmid that is separate from the plasmid molecule.

The present invention also relates to a bacterial cell line comprising a P1 bacteriophage lysogen that comprises a nucleic acid sequence of SEQ ID NO: 97.

The present invention further relates to a method for producing bacteriophage particles or non-replicating transduction particles (NRTPs) with non-native tail fibers comprising, providing a bacteria cell containing the bacteriophage tail fiber replacement platform of the present invention, wherein the complementary nucleic acid molecule comprises a gene that encodes a tail fiber structural protein with one or more regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen, and providing conditions to the bacterial cell that induces a lytic phase of the Myoviridae lysogen to produce bacteriophage particles with non-native tail fibers.

In one embodiment, the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacteriophages within the order Caudovirales. In one

embodiment, the tail fiber variable region is obtained from bacteriophages within the family Myoviridae. In another embodiment, the tail fiber variable region is obtained from bacteriophages within a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one embodiment, the genus is Punalikevirus.

In one embodiment, the complementary nucleic acid molecule further comprises a gene that encodes a chaperone protein. In one embodiment, the gene that encodes the chaperone protein is obtained from bacteriophages within the order Caudovirales. In another embodiment, the gene that encodes the chaperone protein is obtained from bacteriophages within the family Myoviridae. In yet another embodiment, the gene that encodes the chaperone protein is obtained from bacteriophages from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one embodiment, the genus is Punalikevirus. In one embodiment, the chaperone protein is obtained from bacterial genomes encoding non-replicative virus derived structures or from nucleic acid delivery particles. In one embodiment, the gene that encodes the chaperone protein comprises an amino acid sequence selected from the group consisting of SEQ ID Nos: 67-96 and 113-117.

In one embodiment, the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacterial genomes encoding phage tail-like particles or structures or from nucleic acid delivery particles. In one embodiment, the gene that encodes the tail fiber structural protein comprises of an amino acid sequence selected from the group consisting of SEQ ID Nos: 35-66, and 108-112. In another embodiment, the gene that encodes the tail fiber structural protein comprises a nucleotide sequence selected from the group consisting of SEQ ID Nos: 1-34, 98, 100, 102, 104 and 106.

The present invention further relates to a method of producing non-replicative transduction particles (NRTPs) with non-native tail fibers comprising: providing a bacteria cell containing the bacteriophage tail fiber replacement platform of the invention, wherein the Myoviridae lysogen comprises a further disruption in one or more packaging genes that are involved in

packaging the Myoviridae lysogen genome, and wherein the complementary nucleic acid comprises a gene encoding the tail fiber structural protein that contains one or more regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen; and wherein the bacteria cell further comprises a reporter plasmid that comprises the one or more packaging genes that are disrupted in the Myoviridae lysogen; and providing conditions to the bacterial cell that induces a lytic phase of the Myoviridae lysogen to produce NRTPs with non-native tail fibers.

In one embodiment, the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacteriophages within the order Caudovirales. In one embodiment, the tail fiber variable region is obtained from bacteriophages within the family Myoviridae. In another embodiment, the tail fiber variable region is obtained from bacteriophages within a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one embodiment, the genus is Punalikevirus.

In one embodiment, the complementary nucleic acid molecule further comprises a gene that encodes a chaperone protein. In one embodiment, the gene that encodes the chaperone protein is obtained from bacteriophages within the order Caudovirales. In another embodiment, the gene that encodes the chaperone protein is obtained from bacteriophages within the family Myoviridae. In yet another embodiment, the gene that encodes the chaperone protein is obtained from bacteriophages from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one embodiment, the genus is Punalikevirus. In one embodiment, the chaperone protein is obtained from bacterial genomes encoding non-replicative virus derived structures or from nucleic acid delivery particles. In one embodiment, the gene that encodes the chaperone protein comprises an amino acid sequence selected from the group consisting of SEQ ID Nos: 67-96 and 113-117.

In one embodiment, the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein

(tail fiber variable region) obtained from bacterial genomes encoding phage tail-like particles or structures or from nucleic acid delivery particles. In one embodiment, the gene that encodes the tail fiber structural protein comprises of an amino acid sequence selected from the group consisting of SEQ ID Nos: 35-66, and 108-112. In another embodiment, the gene that encodes the tail fiber structural protein comprises a nucleotide sequence selected from the group consisting of SEQ ID Nos: 1-34, 98, 100, 102, 104 and 106.

In another aspect, the present invention contemplates expressing chimeric tail fiber genes that generate a bacteriophage and/or a NRTP displaying a host-range specificity and/or reactivity that differs from the host-range specificity and/or reactivity of the native bacteriophage chassis. Therefore, the present invention relates to a method of generating an engineered bacteriophage or a non-replicative transduction particle (NRTP) that displays a bacteria host cell specificity that differs from the bacteria host cell specificity displayed by a native bacteriophage comprising, fusing a first nucleotide sequence derived from one tail fiber structural protein encoding gene with a second nucleotide sequence derived from a second tail fiber structural protein encoding gene from a different source to generate a chimeric tail fiber structural protein encoding gene; and expressing the chimeric tail fiber structural protein gene in a bacteria cell that contains the bacteriophage tail fiber replacement platform of the present invention; and providing conditions to the bacteria cell that induces a lytic phase of the Myoviridae lysogen to generate the engineered bacteriophage or NRTP.

In one embodiment, the Myoviridae lysogen is derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one embodiment, the Myoviridae lysogen is derived from the Punalikevirus genus. In another embodiment, the Myoviridae lysogen is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

In one embodiment, the first nucleotide sequence is derived from the Myoviridae lysogen that contains the genetic disruption. In another embodiment, the first nucleotide sequence comprises the s_c region of tail fiber gene s from P1 bacteriophage. In yet another embodiment, the first nucleotide sequence comprises a sequence with at least 90% sequence identity with the nucleotide sequence that encodes amino acid sequence 1-250 of SEQ ID NO: 53. In yet another embodiment, the first nucleotide sequence comprises a sequence with at least 90% sequence identity with the nucleotide sequence that encodes amino acid sequence 1-500 of SEQ ID NO:

53. In one embodiment, the first nucleotide sequences comprises the s_c region derived from plasmid p15B (as described in Ikeda et al., J. Mol. Biol., 1970, 50: 457-470) or from a P1-like type 11 bacteriophage in *Klebsiella pneumoniae* (*Kpn*).

In another aspect, the present invention relates to a method of generating engineered bacteriophages or non-replicative transduction particles (NRTPs) having multiple types of tail fibers from a single bacteria cell line, wherein at least one of the tail fiber type is a non-native tail fiber comprising, providing a bacteria cell containing the bacteriophage tail fiber replacement platform of the present invention, wherein the complementary nucleic acid molecule comprises at least two receptor binding regions from tail fiber structural protein encoding genes that are fused to one or more tail interacting regions from tail fiber structural protein encoding genes, wherein at least one of the receptor binding regions is derived from a different source than the source of one of the tail interacting region; and providing conditions to the bacterial cell that induces a lytic phase of the bacteriophage lysogen to produce bacteriophages or NRTPs having multiple types of tail fibers.

In one embodiment, the Myoviridae lysogen is derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one embodiment, the Myoviridae lysogen is derived from the Punalikevirus genus. In another embodiment, the Myoviridae lysogen is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

In one embodiment, the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at the site of the genetic disruption. In another embodiment, the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at a site other than the site of the genetic disruption. In yet another embodiment, the complementary nucleic acid molecule is integrated into the genome of the bacteria cell. In yet another embodiment, the complementary nucleic acid molecule is integrated into a plasmid molecule.

In one embodiment, fusing the two or more receptor binding regions to the tail interacting region is performed by a cin recombinase and in the presence of multiple cix recombination sites. In one embodiment, fusing the two or more receptor binding regions to the tail interacting region is performed by a cin recombinase or a homolog, ortholog, or paralog of the cin recombinase and in the presence of multiple associated recombination sites. In one

embodiment, the two or more distinct tail fiber structural protein encoding genes are expressed in an operon. In another embodiment, the two or more distinct tail fiber structural protein encoding genes are expressed from independent promoters. In yet another embodiment, the two or more distinct tail fiber structural protein encoding genes are expressed from independent genomic locations.

In another aspect, the present invention relates to a bacterial cell packaging system for packaging a reporter or therapeutic nucleic acid molecule into a non-replicative transduction particle (NRTP) for introduction into a cell, the packaging system comprising, a host bacteria cell; a bacteriophage lysogen that contains a genetic disruption that prevents the expression of one or more genes that are critical for the production of native tail fibers, wherein said one or more genes encode a tail fiber structural protein responsible for binding a receptor, a chaperone needed for folding of one or more regions of a tail fiber structural protein, a protein required for attaching the tail fiber structural protein to the tail structure, or any combination of these genes; and also comprising a first bacteriophage gene that contains a non-functional packaging initiation site sequence, wherein the non-functional packaging initiation site sequence prevents preferential packaging of the bacteriophage genome into the NRTP; one or more complementary nucleic acid molecules comprising one or more genes selected from genes encoding a tail fiber structural protein responsible for binding a receptor, a chaperone needed for folding of one or more regions of a tail fiber structural protein, a protein required for attaching the tail fiber structural protein to the tail structure, or any combination of these genes that complements the genetic disruption of the bacteriophage lysogen whereby functional tail fibers are produced; and a reporter or therapeutic nucleic acid molecule and a second bacteriophage gene that contains a functional packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP, wherein the functional packaging initiation site sequence on the reporter nucleic acid molecule complements the non-functional packaging initiation site sequence in the bacteriophage lysogen.

In one embodiment, the reporter gene is luxAB. In another embodiment, the reporter gene is a fluorescent reporter protein. In some embodiments, the fluorescent reporter protein includes but is not limited to Green Fluorescent Protein (GFP), Yellow Fluorescent Protein (YFP), Cyan Fluorescent Protein (CFP), and Red Fluorescent Protein (RFP). In one embodiment, the first bacteriophage gene and the second bacteriophage gene is a pacA terminase gene. In another embodiment, the first bacteriophage gene and the second bacteriophage gene is a terS terminase gene.

In another aspect, the present invention relates to a method of generating Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs that are able to recognize and transduce bacteria cells for diagnostic or therapeutic use comprising, providing a bacteria cell containing the bacteriophage tail fiber replacement platform of the present invention, wherein the complementary nucleic acid molecule comprises tail fiber genes non-native to the Myovridae lysogen and derived partially or entirely from either Myoviridae, Siphoviridae (as represented in SEQ ID Nos: 5, 10, 32, 33), or Podoviridae tail fiber sequences; and providing conditions to the bacterial cell that induces a lytic phase of the bacteriophage lysogen to produce Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs capable of recognizing and transducing bacteria previously refractory to a native Myoviridae bacteriophage or Myoviridae-family derived NRTP particles for diagnostic or therapeutic use.

In one embodiment, the Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs are derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus. In one embodiment, the Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs are derived from the Punalikevirus genus. In another embodiment, the Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).

BRIEF DESCRIPTION OF THE DRAWINGS

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

FIG. 1 is a diagram showing the tail-fiber swapping platform.

FIG. 2 Diagram of natural P1 tail fiber genomic region. Gene *r* encodes a tail fiber accessory protein putatively involved in attaching tail fibers to the tail structure. The *cin* gene encodes a sequence specific recombinase, which flips the DNA between the Cin recombination sites (called cix sites) enabling expression of two distinct tail fibers from a single piece of DNA, sites are denoted as circles in diagram. Genes *u* and *u'* encode distinct tail fiber accessory proteins, which are thought to act as chaperones to fold the binding domain of the S and S' tail fiber proteins, respectively (binding domains encoded by s_v and s_v' coding sequences, respectively). The conserved region of both S and S' tail fibers proteins are encoded by the s_c

coding sequence which is transcriptionally and translationally fused to either the s_V or s_V' depending on activity of the Cin recombinase.

FIG. 3 Diagram of Tail Fiber Replacement Platform. A tail fiber deficient (bald) packaging line harboring a spectinomycin resistance cassette in place of the *s* and *u* genes is induced to express recombineering machinery (SEQ ID NO: 97) and comprises an operon promoter, the *r* gene, the spectinomycin resistance promoter, the spectinomycin resistance gene, the *u'* gene, the *sv'* coding region, the *cin* gene, and the *cin* promoter. PCR fragments harboring a new tail fiber, hygromycin resistance cassette, and flanking homology arms is then transformed into the induced strain (in this case "Shamrock" and "Shamrock" *u*). The recombineering machinery integrates the new tail fiber operon in place of the spectinomycin resistance cassette-*u'*-s_V' region to generate the new chromosomal packaging line able to produce tail fiber endowed Smarticles.

FIG. 4 Improved Smarticles performance following chromosomal integration of tail fibers. Graphs comparing performance of Smarticles endowed with tail fibers expressed from either plasmids (TFDV) or chromosomally. Reactivity of Smarticles harboring only S' (top left), S (top right), or "Shamrock" (bottom left) tail fibers on various *E. coli* strains is shown. Bottom right, improved Smarticles yield obtained from an S tail fiber packaging line genomically integrated vs. plasmid expressed. V3 Smarticles (native tail fibers region) are supplied as a positive control.

FIG. 5 Shows improved tail fiber activity with expression of native U protein homolog or from bacteriophage genome. A) Performance of "Indian" NRTP produced in packaging line expressing or not expressing plasmid p15B U protein homolog T; B) Performance of "Scarlet" NRTPs produced either from bacteriophage genome or from separate packaging plasmid.

FIG. 6 Production of one, two, or more tail fibers in a single production line A) a single bacterial packaging lines that expresses one, two, three, or more functional tail fibers. S, S', and Shamrock tail fibers were added to a packaging line along with an antibiotic marker. Tail fiber genes can be arranged with cix crossover site in a novel location. Genes can also be arranged for expression not dependent on recombination in large or small operons. B) Lysates were generated from packaging lines expressing single tail fibers (indicated as S, S', Smrk) as well as engineered double and triple tail fiber strains (indicated as V3, Double, Triple). *E. coli* strains that have specific reactivity to a single tail fiber were used to demonstrate multiple functional tail fibers were expressed from a single host.

FIG. 7 Analysis of Bi-functional Smarticles packaging line. Bi-functional P1 derived Smarticles were created endowed with both "Jazzberry" and "Shamrock" tail-fibers. A) Cartoon depiction

of the genetic arrangement of genome integrated "ShamJazz" dual expression system. B) Confirmation of bi-functional activity on strains that are specific for the "Jazzberry", "Shamrock", or "V3" (S and S') tail fibers. C) Predicted and/or observed activity of select individual and combined Smarticles on a collection of *E. coli* strains (EcoAD panel). Percent coverage depicted below, with stem and whisker plot depicting light distribution on the panel. D) Comparison of predicted activity of the bi-functional Smarticles and observed activity (purple) which are indistinguishable. * denotes cumulative predicted coverage vs. actual experimentally observed coverage.

FIG. 8 Establishment of Elements Required for Functional Tail Fibers A) 3 distinct tail fiber Sc regions (see Native P1, "Plum", and "Tangerine") were functionalized on a P1 chassis, expanding the understanding of the determinants required for functional tail fibers. All constructs harbored the P1 *r* gene encoding the R protein. Each tail fiber variable region (S_v) was most functional when expressed with its cognate U accessory protein. B) Protein alignments of the 3 functional S_c regions demonstrate that the N-terminal 250 amino acids are important.

FIG. 9 Displays the various arrangements for expression of tail fiber genes from fermentation strain. A) shows expression of tail fibers and viral chassis from phage genome. B) Shows viral chassis expression from phage genome and tail fibers genes from packaging plasmid. C) Shows viral chassis from phage genome and tail fibers from independent plasmid. D) Shows viral chassis from phage genome and tail fibers from bacterial genome. E) Shows viral chassis and one set of tail fibers from phage genome. A second, unique set of tail fibers is expressed from an independent plasmid. F) Shows viral chassis and tail fibers from phage genome. A second, unique set of tail fibers is expressed from the packaging plasmid. G) Shows viral chassis and two, unique sets of tail fibers from phage genome.

DETAILED DESCRIPTION OF THE INVENTION

Definitions

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

As used herein, "reporter nucleic acid molecule" refers to a nucleotide sequence comprising a DNA or RNA molecule. The reporter nucleic acid molecule can be naturally occurring or an artificial or synthetic molecule. In some embodiments, the reporter nucleic acid molecule is exogenous to a host cell and can be introduced into a host cell as part of an exogenous nucleic acid molecule, such as a plasmid or vector. In certain embodiments, the reporter nucleic acid

molecule can be complementary to a target gene in a cell. In other embodiments, the reporter nucleic acid molecule comprises a reporter gene encoding a reporter molecule (*e.g.*, reporter enzyme, protein). In some embodiments, the reporter nucleic acid molecule is referred to as a "reporter construct" or "nucleic acid reporter construct."

As used herein, "therapeutic nucleic acid molecule" refers to a nucleotide sequence that has a therapeutic effect to treat diseases, either by sequence-specific recognition of target nucleic acid that relates to the disease (e.g. antisense nucleic acids) or by encoding a gene product that possesses therapeutic activity (e.g. antimicrobial proteins). In some embodiments, the therapeutic nucleic molecule can be introduced into a host cell as part of an exogenous nucleic acid molecule, such as a plasmid. As used herein, a "therapeutic expression plasmid" refers to a plasmid that contains a therapeutic nucleic acid molecule that encodes a gene product having therapeutic activity.

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

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

nucleic acid molecule forms a replicon capable of being packaged as concatameric DNA into a progeny virus during virus replication.

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

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

A transcript derived from a reporter nucleic acid molecule can be referred to as a "reporter transcript." The reporter transcript can include a reporter sequence and a cis-repressing sequence. The reporter transcript can have sequences that form regions of complementarity, such that the transcript includes two regions that form a duplex (*e.g.*, an intermolecular duplex region). One region can be referred to as a "cis-repressing sequence" and has complementarity to a portion or all of a target transcript and/or a reporter sequence. A second region of the transcript is called a "reporter sequence" and can have complementarity to the cis-repressing sequence. Complementarity can be full complementarity or substantial complementarity. The presence and/or binding of the cis-repressing sequence with the reporter sequence can form a conformation in the reporter transcript, which can block further expression of the reporter molecule. The reporter transcript can form secondary structures, such as a hairpin structure, such that regions within the reporter transcript that are complementary to each other can hybridize to each other.

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

tissue site or administered systemically. *In vitro* introduction into a cell includes methods known in the art, such as electroporation and lipofection. Further approaches are described herein or known in the art.

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

A "phage tail-like particle" or "phage tail-like structures" refers to a bacterially produced high-molecular-weight phage tail-like protein complexe that resemble bacteriophage tails in form, however, do not encode or deliver replicative viral genomes and therefore are not viruses. Non-limiting examples of phage tail-like particles or structures include Type 6 Secretion Systems (T6SS) and R-type tailocins (also known as pyocins) which are found in diverse bacteria types. Further descriptions of these particles are found in Williams et al., Applied and Environmental Microbiology, 2008, 74:12, 3868-3876, and in Scholl et al., Antimicrobial Agents and Chemotherapy, 2009, 53:7, 3074-3080. While these example structures do not encode viral capsid heads, they are able to mediate binding and tail injection into target cells resulting in either killing target cells or delivering protein effectors. Phage tail-like particles or structures can harbor and utilize specificity determining elements such as tail fibers and therefore their genetic sequences can be mined to re-target cell binding specificity. One example of a pyocin-derived tail fiber is shown in the nucleotide sequence of SEQ ID NO: 11.

A "non-replicative transduction particle" or "NRTP" refers to a virus capable of delivering a non-viral nucleic acid molecule into a cell, but is incapable of packaging its own replicated viral genome into the transduction particle. The virus can be a bacteriophage, adenovirus, *etc.*

A "plasmid" is a small DNA molecule that is physically separate from, and can replicate independently of, chromosomal DNA within a cell. Most commonly found as small circular, double-stranded DNA molecules in bacteria, plasmids are sometimes present in archaea and eukaryotic organisms. Plasmids are considered replicons, capable of replicating autonomously within a suitable host. As used herein, the term "packaging plasmid" refers to a plasmid encoding both the elements required for being packaged into a given viral capsid (i.e. packaging genes) and an expressible reporter gene. As used herein, the term "tail fiber display vector" and abbreviated as "TFDV" refers to a plasmid encoding the elements required for being packaged into a given viral capsid, an expressible reporter gene, and a tail fiber encoded sequence that produces a binding protein able to attach to the given capsid. As used herein, the term "secondary tail fiber expression plasmid" refers to a plasmid which encodes a functional tail fiber that can be attached to a given capsid, but does not necessarily harbor a reporter molecule or packaging site/machinery.

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

A "virus" is a small infectious agent that replicates only inside the living cells of other organisms. Virus particles (known as virions) include two or three parts: i) the genetic material made from either DNA or RNA molecules that carry genetic information; ii) a protein coat that protects these genes; and in some cases, iii) an envelope of lipids that 9388

As used herein, the term "complement" refers to a non-disrupted sequence that is in the presence of an identical sequence that has been disrupted, or to the relationship of the non-disrupted sequence to the disrupted sequence. In one embodiment, the complement comprises a gene encoded on a polynucleotide in a cell that is functional and capable of expression, and expresses a protein with the same function as a disrupted gene on a bacteriophage prior to disruption. In some embodiments, the complement gene has greater than 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% sequence identity to the disrupted bacteriophage gene prior to disruption, i.e., the native bacteriophage gene. In some embodiments, the complement gene is identical to the disrupted bacteriophage gene prior to disruption, i.e., the native bacteriophage gene. In some embodiments, the complement gene comprises a polynucleotide sequence that has been deleted from the bacteriophage. In some embodiments, the complement gene refers to a gene encoding packaging machinery of a bacteriophage on a plasmid, where the same gene has been disrupted in a bacteriophage. Thus, the plasmid is required to be in the presence of a bacteriophage with a mutated packaging machinery gene to provide the necessary packaging machinery necessary for packaging a polynucleotide into a transduction particle.

As used herein, the term "packaging-related enzymatic activity" refers to one or more polypeptides crucial for the interaction with a packaging initiation site sequence to package a polynucleotide into a transduction particle. In some embodiments, a pair of terminase genes is required for such an interaction, wherein each terminase encodes a packaging-related enzymatic activity. In some embodiments, the enzymatic activity is encoded by a *terS* and/or *terL* gene from a *S. aureus* bacteriophage φ11 or φ80α, a *terA* and *terB* gene from an *E. faecalis* bacteriophage φEf11, or a *pacA* and *pacB* gene of *Enterobacteriaceae* bacteriophage P1. In these embodiments, each of the pair of terminase genes express a packaging-related enzymatic activity, and a functional version of both are required for packaging of a polynucleotide with the packaging initiation site. In some embodiments, disruption of one of the genes of a plurality of genes associated with a packaging-related enzymatic activity eliminates the packaging-related enzymatic activity. In some embodiments, both of a pair of terminase genes are

disrupted on the bacteriophage, thus disrupting the entire set of packaging-related enzymatic activity encoding genes on the bacteriophage.

The term "in situ" refers to processes that occur in a living cell growing separate from a living organism, *e.g.*, growing in tissue culture.

The term "in vivo" refers to processes that occur in a living organism.

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

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

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

For example, a first nucleotide sequence can be described as complementary to a second nucleotide sequence when the two sequences hybridize (*e.g.*, anneal) under stringent hybridization conditions. Hybridization conditions include temperature, ionic strength, pH, and organic solvent concentration for the annealing and/or washing steps. The term "stringent hybridization conditions" refers to conditions under which a first nucleotide sequence will hybridize preferentially to its target sequence, *e.g.*, a second nucleotide sequence, and to a lesser extent to, or not at all to, other sequences. Stringent hybridization conditions are sequence

dependent, and are different under different environmental parameters. Generally, stringent hybridization conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the nucleotide sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the first nucleotide sequences hybridize to a perfectly matched target sequence. An extensive guide to the hybridization of nucleic acids is found in, *e.g.*, Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology--Hybridization with Nucleic Acid Probes part I, chap. 2, "Overview of principles of hybridization and the strategy of nucleic acid probe assays," Elsevier, N.Y. ("Tijssen"). Other conditions, such as physiologically relevant conditions as may be encountered inside an organism, can apply. The skilled person will be able to determine the set of conditions most appropriate for a test of complementarity of two sequences in accordance with the ultimate application of the hybridized nucleotides.

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

"Complementary" sequences, as used herein, may also include, or be formed entirely from, non-Watson-Crick base pairs and/or base pairs formed from non-natural and modified nucleotides, provided the above requirements with respect to their ability to hybridize are fulfilled. Such non-Watson-Crick base pairs includes, but not limited to, G:U Wobble or Hoogstein base pairing.

The terms "complementary," "fully complementary" and "substantially complementary" herein may be used with respect to the base matching between two strands of a dsRNA, or between

the antisense strand of a dsRNA and a target sequence, between complementary strands of a single stranded RNA sequence or a single stranded DNA sequence, as will be understood from the context of their use.

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

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

The term "percent identity," in the context of two or more nucleic acid or polypeptide sequences, refer to two or more sequences or subsequences that have a specified percentage of nucleotides or amino acid residues that are the same, when compared and aligned for maximum correspondence, as measured using one of the sequence comparison algorithms described below (*e.g.*, BLASTP and BLASTN or other algorithms available to persons of skill) or by visual

inspection. Depending on the application, the percent "identity" can exist over a region of the sequence being compared, *e.g.*, over a functional domain, or, alternatively, exist over the full length of the two sequences to be compared.

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

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

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

The term "sufficient amount" means an amount sufficient to produce a desired effect, *e.g.*, an amount sufficient to produce a detectable signal from a cell.

The term "therapeutically effective amount" is an amount that is effective to ameliorate a symptom of a disease. A therapeutically effective amount can be a "prophylactically effective amount" as prophylaxis can be considered therapy.

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

Lysogenic and Lytic Cycle of Viruses

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

In addition, virus-based reporter assays, such as phage-based reporters, can suffer from limited reactivity (*i.e.*, analytical inclusivity) due to limits in the phage host range caused by host-based and prophage-derived phage resistance mechanisms. These resistance mechanisms target native phage nucleic acid that can result in the degradation or otherwise inhibition of the phage DNA and functions. Such resistance mechanisms include restriction systems that cleave phage DNA and CRISPR systems that inhibit phage-derived transcripts.

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

III. Methods for Producing Non-Replicative Transduction Particles (NRTP)

Disruption/Complementation-Based Methods For Producing Non-Replicative Transduction Particles .

Disclosed herein are non-replicative transduction particle packaging systems based on disruption of a component of the genome of a virus that is recognized by the viral packaging machinery as the element from which genomic packaging is initiated during viral production. In an embodiment, this disruption disrupts a packaging initiation site from a bacteriophage, and also disrupts a terminase function. Examples of the disrupted elements include the pac-site sequence of pac-type bacteriophages and the cos-site sequence of cos-type bacteriophages. When the packaging initiation site sequence within the phage is disrupted, the phage cannot produce functional terminases. In an example, the pac-site is encoded within a pacA gene sequence, and terminase functions require both a functional PacA and PacB. Plasmid DNA is packaged into a phage capsid by complementing said disrupted terminases and including a recognizable packaging initiation site on the plasmid DNA. The bacteriophage can be any bacteriophage, such as an *Enterobacteriaceae* bacteriophage P1 or φEF11, or an *S. aureus* bacteriophage φ80α or a bacteriophage φ11.

Packaging initiation sites are often found within coding regions of genes that are essential to virus production. A region of the bacteriophage genome can be disrupted by an insertion,

replacement, deletion, or mutation that disrupts the packaging initiation site. Examples of disruptions that accomplish this include, but are not limited to, an allelic exchange event that replaces a sequence on the bacteriophage genome that contains the packaging initiation site sequence with another sequence such as that of an antibiotic resistance gene, or the complete deletion of the small and large terminase genes. In an example employing the terminase genes pacA and pacB, pacA can be disrupted in a manner that causes polar effects that also disrupt pacB expression and/or overall terminase function mediated by PacA and PacB. Other examples can include terminase genes can also include terS and terL genes from *S. aureus* bacteriophage φ11 or φ80α, or the terS and terL genes from *E. faecalis* bacteriophage φEf11 In one example, a cell's genome is lysogenized with a viral genome where the packaging initiation site has been disrupted. In some embodiments, the cell can be an *E. coli* cell, an *S. aureus* cell, or an *E. faecalis* cell. The cell can be Gram-negative or Gram-positive. A complementing plasmid (or reporter nucleic acid molecule) is introduced into the cell, and the plasmid DNA includes at least the gene that has been disrupted in the bacteriophage, as well as the packaging initiation site sequence, and optionally additional bacteriophage genes and a reporter gene, which can encode a detectable and/or a selectable marker. The plasmid can be constructed using methods found in U.S. Patent No. 9,388,453, and in U.S Patent Application Publication No. 2017/0166907. One or more genes of the plasmid can be operatively linked to a promoter, such as an inducible promoter (which can be induced when packaging is initiated by inducing the bacteriophage). In some embodiments, the promoter can be a native promoter of a small terminase gene or a large terminase gene. The native promoter can be controlled by the bacteriophage, and thus effectively acts as a conditional promoter induced during packaging.

In some examples, it is preferable that the disruption/complementation is designed such that there is no homology between the mutated virus DNA and the complementing exogenous DNA. This is because lack of homology between the mutated virus DNA and the complementing exogenous DNA avoids the possibility of homologous recombination between the two DNA molecules that can result in re-introduction of a packaging sequence into the virus genome. To accomplish a lack of homology, one strategy is to delete the entire gene (or genes) that contains the packaging initiation site sequence from the virus genome and then complement this gene with an exogenous DNA molecule that preferably contains no more than exactly the DNA sequence that was deleted from virus. In this strategy, the complementing DNA molecule is designed to express the gene that was deleted from the virus. Another example of such a system is provided using the bacteriophage φ80α, a pac-type phage. The phage genome is lysogenized

in a host bacterial cell, and the phage genome includes a small terminase gene where the pac-site of a pac-type prophage φ80α has been deleted. A plasmid including a complementary small terminase gene with a native pac-site is transformed into the cell. When the lytic cycle of the lysogenized prophage is induced, the bacteriophage packaging system packages plasmid DNA into progeny bacteriophage structural components, rather than packaging the native bacteriophage DNA. The packaging system thus produces non-replicative transduction particles carrying plasmid DNA.

The reporter gene encodes a detectable marker or a selectable marker. In an example, the reporter gene is selected from the group consisting of enzymes mediating luminescence reactions (*luxA*, *luxB*, *luxAB*, *luc*, *ruc*, *nluc*), enzymes mediating colorimetric reactions (lacZ, HRP), fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent proteins), affinity peptides (His-tag, 3X-FLAG), and selectable markers (ampC, tet(M), CAT, erm). In an embodiment, the reporter gene is *luxA*. In some embodiments, the resistance marker comprises an antibiotic resistance gene. In some embodiments, the resistance marker is a kanamycin resistance gene (*kan*). In some embodiments, the constitutive promoter comprises Pblast. In some embodiments, the bacteriophage genome disruption is accomplished by an allelic exchange event that replaces or disrupts a sequence on the bacteriophage genome that contains the packaging initiation site sequence.

In an example, a pair of terminase genes on a bacteriophage genome, *e.g.*, *pacA* and *pacB*, *terA* and *terB*, or *terS* and *terL*, can be disrupted in a manner that causes polar effects that also disrupt expression of one of the terminase genes and/or overall terminase function mediated by the terminase genes. The disrupted bacteriophage can be complemented with a plasmid comprising terminase genes, *e.g.*, pacA and pacB, terA and terB, or terS and terL, of the bacteriophage genome. When the mutated virus is undergoing a lytic cycle, the viral packaging proteins, produced either from the bacteriophage genome or (if disrupted) the complementing plasmid, package a replicon of the plasmid DNA into the packaging unit because it contains a packaging initiation site, and non-replicative transduction particles are produced carrying the replicated plasmid DNA.

Reporters

In some embodiments, the NRTPs and constructs of the invention comprise a reporter nucleic acid molecule including a reporter gene. The reporter gene can encode a reporter molecule, and the reporter molecule can be a detectable or selectable marker. In certain embodiments, the reporter gene encodes a reporter molecule that produces a detectable signal when expressed in a cell.

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

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

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

Reporters and reporter assays are described further in Section V herein.

V. NRTPs and Reporter Assays

Inducer Reporter Assay

In some embodiments, the invention comprises methods for the use of NRTPs as reporter molecules for use with endogenous or native inducers that target gene promoters within viable cells. The NRTPs of the invention can be engineered using the methods described in Section III and below in Examples 1-2.

In some embodiments, the method comprises employing a NRTP as a reporter, wherein the NRTP comprises a reporter gene that is operably linked to an inducible promoter that controls the expression of a target gene within a target cell. When the NRTP that includes the reporter gene is introduced into the target cell, expression of the reporter gene is possible via induction of the target gene promoter in the reporter nucleic acid molecule.

Transcripts

As described above, a transcript is a length of nucleotide sequence (DNA or RNA) transcribed from a DNA or RNA template sequence or gene. The transcript can be a cDNA sequence transcribed from an RNA template or an mRNA sequence transcribed from a DNA template. The transcript can be transcribed from an engineered nucleic acid construct. The transcript can have regions of complementarity within itself, such that the transcript includes two regions that

can form an intra-molecular duplex. One region can be referred to as a "cis-repressing sequence" that binds to and blocks translation of a reporter sequence. A second region of the transcript is called a "reporter sequence" that encodes a reporter molecule, such as a detectable or selectable marker.

The transcripts of the invention can be a transcript sequence that can be 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 nucleotides in length. In other embodiments, the transcript can be at least 25, 30, 40, 50, 60, 70, 80, 90, 100, 500, 1000, 1500, 2000, 3000, 4000, 5000 or more nucleotides in length. The cis-repressing sequence and the reporter sequence can be the same length or of different lengths.

In some embodiments, the cis-repressing sequence is separated from the reporter sequence by 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, 35, 40, 45, 50, 55, 60, or more spacer nucleotides.

Vectors

In another aspect, the transcripts (including antisense and sense sequences) of the invention are expressed from transcription units inserted into DNA or RNA vectors (*see*, *e.g.*, Couture, A, *et al.*, *TIG.* (1996), 12:5-10; Skillern, A., *et al.*, International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, U.S. Pat. No. 6,054,299). These sequences can be introduced as a linear construct, a circular plasmid, or a viral vector, including bacteriophage-based vectors, which can be incorporated and inherited as a transgene integrated into the host genome. The transcript can also be constructed to permit it to be inherited as an extrachromosomal plasmid (Gassmann, *et al.*, *Proc. Natl. Acad. Sci. USA* (1995) **92**:1292).

The transcript sequences can be transcribed by a promoter located on the expression plasmid. In one embodiment, the cis-repressing and reporter sequences are expressed as an inverted repeat joined by a linker polynucleotide sequence such that the transcript has a stem and loop structure.

Recombinant expression vectors can be used to express the transcripts of the invention. Recombinant expression vectors are generally DNA plasmids or viral vectors. Viral vectors expressing the transcripts can be constructed based on, but not limited to, adeno-associated virus (for a review, see Muzyczka, *et al.*, *Curr. Topics Micro. Immunol.* (1992) 158:97-129)); adenovirus (see, for example, Berkner, *et al.*, BioTechniques (1998) 6:616), Rosenfeld *et al.* (1991, Science 252:431-434), and Rosenfeld *et al.* (1992), *Cell* 68:143-155)); or alphavirus as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types, including epithelial cells, *in vitro* and/or *in vivo* (see, *e.g.*, Eglitis, *et*

al., *Science* (1985) 230:1395-1398; Danos and Mulligan, Proc. Natl. Acad. Sci. *USA* (1998) 85:6460-6464; Wilson *et al.*, 1988, Proc. Natl. Acad. Sci. USA 85:3014-3018; Armentano *et al.*, 1990, Proc. Natl. Acad. Sci. USA 87:61416145; Huber *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8039-8043; Ferry *et al.*, 1991, Proc. Natl. Acad. Sci. USA 88:8377-8381; Chowdhury *et al.*, 1991, Science 254:1802-1805; van Beusechem. *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:7640-19 ; Kay *et al.*, 1992, Human Gene Therapy 3:641-647; Dai *et al.*, 1992, Proc. Natl. Acad. Sci. USA 89:10892-10895; Hwu *et al.*, 1993, J. Immunol. 150:4104-4115; U.S. Patent No. 4,868,116; U.S. Patent No. 4,980,286; PCT Application WO 89/07136; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). Recombinant retroviral vectors capable of transducing and expressing genes inserted into the genome of a cell can be produced by transfecting the recombinant retroviral genome into suitable packaging cell lines such as PA317 and Psi-CRIP (Comette *et al.*, 1991, Human Gene Therapy 2:5-10; Cone *et al.*, 1984, Proc. Natl. Acad. Sci. USA 81:6349). Recombinant adenoviral vectors can be used to infect a wide variety of cells and tissues in susceptible hosts (*e.g.*, rat, hamster, dog, and chimpanzee) (Hsu *et al.*, 1992, J. Infectious Disease, 166:769), and also have the advantage of not requiring mitotically active cells for infection.

Any viral vector capable of accepting the coding sequences for the transcript(s) to be expressed can be used, for example, vectors derived from adenovirus (AV); adeno-associated virus (AAV); retroviruses (*e.g.*, lentiviruses (LV), Rhabdoviruses, murine leukemia virus); herpes virus, and the like. The tropism of viral vectors can be modified by pseudotyping the vectors with envelope proteins or other surface antigens from other viruses, or by substituting different viral capsid proteins, as appropriate.

For example, lentiviral vectors featured in the invention can be pseudotyped with surface proteins from vesicular stomatitis virus (VSV), rabies, Ebola, Mokola, and the like. AAV vectors featured in the invention can be made to target different cells by engineering the vectors to express different capsid protein serotypes. Techniques for constructing AAV vectors which express different capsid protein serotypes are within the skill in the art; see, *e.g.*, Rabinowitz J E *et al.* (2002), J Virol 76:791-801.

Selection of recombinant viral vectors suitable for use in the invention, methods for inserting nucleic acid sequences for expressing the transcripts into the vector, and methods of delivering the viral vector to the cells of interest are within the skill in the art. See, for example, Dornburg R (1995), Gene Therap. 2: 301-310; Eglitis M A (1988), Biotechniques 6: 608-614; Miller A D (1990), Hum Gene Therap. 1: 5-14; Anderson W F (1998), Nature 392: 25-30; and Rubinson D A *et al.*, Nat. Genet. 33: 401-406.

Viral vectors can be derived from AV and AAV. A suitable AV vector for expressing the transcripts featured in the invention, a method for constructing the recombinant AV vector, and a method for delivering the vector into target cells, are described in Xia H *et al.* (2002), *Nat. Biotech.* 20: 1006-1010. Suitable AAV vectors for expressing the transcripts featured in the invention, methods for constructing the recombinant AV vector, and methods for delivering the vectors into target cells are described in Samulski R *et al.* (1987), J. Virol. 61: 3096-3101; Fisher K J *et al.* (1996), J. Virol, 70: 520-532; Samulski R *et al.* (1989), J. Virol. 63: 3822-3826; U.S. Pat. No. 5,252,479; U.S. Pat. No. 5,139,941; International Patent Application No. WO 94/13788; and International Patent Application No. WO 93/24641.

The promoter driving transcript expression in either a DNA plasmid or viral vector featured in the invention may be a eukaryotic RNA polymerase I (*e.g.,* ribosomal RNA promoter), RNA polymerase II (*e.g.,* CMV early promoter or actin promoter or U1 snRNA promoter) or generally RNA polymerase III promoter (*e.g.,* U6 snRNA or 7SK RNA promoter) or a prokaryotic promoter, for example the T7 promoter, provided the expression plasmid also encodes T7 RNA polymerase required for transcription from a T7 promoter. The promoter can also direct transgene expression to the pancreas (see, *e.g.*, the insulin regulatory sequence for pancreas (Bucchini *et al.*, 1986, Proc. Natl. Acad. Sci. USA 83:2511-2515)).

In addition, expression of the transcript can be precisely regulated, for example, by using an inducible regulatory sequence and expression systems such as a regulatory sequence that is sensitive to certain physiological regulators, *e.g.*, circulating glucose levels, or hormones (Docherty *et al.*, 1994, FASEB J. 8:20-24). Such inducible expression systems, suitable for the control of transgene expression in cells or in mammals include regulation by ecdysone, by estrogen, progesterone, tetracycline, chemical inducers of dimerization, and isopropyl-beta-D-1-thiogalactopyranoside (IPTG). A person skilled in the art would be able to choose the appropriate regulatory/promoter sequence based on the intended use of the dsRNA transgene.

Generally, recombinant vectors capable of expressing transcript molecules are delivered as described below, and persist in target cells. Alternatively, viral vectors can be used that provide for transient expression of transcript molecules. Such vectors can be repeatedly administered as necessary. Once expressed, the transcript binds to target RNA and modulates its function or expression. Delivery of transcript expressing vectors can be systemic, such as by intravenous or intramuscular administration, by administration to target cells ex-planted from the patient followed by reintroduction into the patient, or by any other means that allows for introduction into a desired target cell.

Transcript expression DNA plasmids are typically transfected into target cells as a complex with cationic lipid carriers (*e.g.,* Oligofectamine) or non-cationic lipid-based carriers (*e.g.,* Transit-TKO™). Multiple lipid transfections for dsRNA-mediated knockdowns targeting different regions of a single PROC gene or multiple PROC genes over a period of a week or more are also contemplated by the invention. Successful introduction of vectors into host cells can be monitored using various known methods. For example, transient transfection can be signaled with a reporter, such as a fluorescent marker, such as Green Fluorescent Protein (GFP). Stable transfection of cells *ex vivo* can be ensured using markers that provide the transfected cell with resistance to specific environmental factors (*e.g.*, antibiotics and drugs), such as hygromycin B resistance.

The delivery of the vector containing the recombinant DNA can by performed by abiologic or biologic systems. Including but not limited to liposomes, virus-like particles, transduction particles derived from phage or viruses, and conjugation.

Reporters for Transcript Assay

In some embodiments, the nucleic acid construct comprises a reporter sequence (*e.g.*, a reporter gene sequence). The reporter gene encodes a reporter molecule that produces a signal when expressed in a cell. In some embodiments, the reporter molecule can be a detectable or selectable marker. In certain embodiments, the reporter molecule can be a fluorescent reporter molecule, such as a green fluorescent protein (GFP), yellow fluorescent protein (YFP), cyan fluorescent protein (CFP), blue fluorescent protein (BFP), or red fluorescent protein (RFP). In other embodiments, the reporter molecule can be a chemiluminescent protein.

Reporter molecules can be a bacterial luciferase, an eukaryotic luciferase, a fluorescent protein, an enzyme suitable for colorimetric detection, a protein suitable for immunodetection, a peptide suitable for immunodetection or a nucleic acid that function as an aptamer or that exhibits enzymatic activity.

Selectable markers can also be used as a reporter. The selectable marker can be an antibiotic resistance gene, for example.

EXAMPLES

Example 1: Disruption/Complementation-Based Methods For Producing Non-Replicative Transduction Particles with Non-Native Tail Fibers.

A diagram of the tail-fiber replacement platform is shown in FIG 1. The attachment of NTRPs to their target bacteria cell is mediated by bacteriophage tail fibers. By removing the native P1 NRTP tail fiber genes, a "bald" chassis was created to test the functionality of heterologous tail fibers. New tail fibers were then expressed in the "bald" background using a Tail Fiber Display Vector (TFDV). The P1 based Smarticles genome was modified using the λ-RED Recombineering method from Murphy et al. (1998) to create a deletion in the genes encoding the S structural tail fiber protein and the U tail fiber assembly chaperone protein. This disruption created a platform to express any desired tail fibers in trans. Bacterial strains were grown on Luria-Bertani (LB) broth and LB agar with the following antibiotic concentrations Gentamicin 15 μg/mL, Cabinicillin 100 μg/mL, Spectinomycin 50 μg/mL, Zeocin 100 μg/mL and Hygromycin 150 μg/mL. New England Biolabs (NEB) cloning strain NEB5α was used to propagate and archive plasmids. Assembly of DNA for plasmids as well as linear DNA products for Recombineering were produced using NEB Gibson Assembly Master Mix. EMD Millipore KOD Xtreme Hot Start Polymerase was used for amplification of DNA in all PCR reactions.

Example 2: Construction of a "Bald" P1 Chassis

The λ-RED encoding Recombineering plasmid pKM208 was first transformed into the bacterial strain BAA-1001 (with the *pacAB* genes replaced with a hygromycin cassette) to facilitate the Recombineering reaction. The strain was made electrocompetent and induced with 1mM IPTG for expression of recombination proteins. Linear DNA containing the antibiotic resistance marker spectinomycin was then transformed into the strain, this DNA contains homology to the surrounding regions of the *s* and *u* genes of P1. After recovery at 30°C, transformants were plated onto LB agar containing spectinomycin, then patched. Colony PCR for the removal of the *s* and *u* genes followed by Sanger sequencing were used to ensure the desired modification was made. During construction the *u'* and s_v' regions were left intact, but because the s_v' does not code for the full structural protein, the S' tail fiber cannot be produced. This bacterial lysogen represented the "Bald" P1 chassis that lacks the ability to express functional tail fibers of its own without complementation. The resulting bacterial strain containing the Bald P1 chassis was designated as SEG_170 cell line. Functional tests of the chassis consisted of packaging plasmids with and without coding sequences for the native S and U proteins. Loss of Smarticles functionality was seen from lysate derived from the packaging line with no tail

fibers on the packaging plasmid. Activity was recovered from lysate derived from the packaging line with tail fibers on the packaging plasmid, achieving successful complementation in trans.

Example 3: Construction of Tail Fiber Display Vectors (TFDV)

Tail Fiber Display Vectors (TFDV) were utilized to express native and/or novel tail fibers in the bald bacteriophage P1 chassis. These display vectors utilized either Gentamicin or Zeocin resistance cassettes, pUC/RO1600 origins of replication, and tail fiber genes under the control of the pTac IPTG inducible promoter. All vectors also contained the *pacAB* genes/packaging site together with the P23-luxAB-TT (luciferase) cassette for transduction and light assays. Tail fiber candidates were *de novo* synthesized at IDT or PCR amplified from bacterial gDNA, Gibson Assembled into PCR amplified vectors, transformed into NEBa cells, patched, colony PCR verified, and sequence verified. Sequence verified plasmids were transformed into the well characterized SEG_170 bald packaging line (competent cells purchased from Lucigen). Transformants were patched onto selective media and screened in small volume lysates to determine the best Smarticles producing clones. Top clones were handed off for large volume lysate production, QC, and reactivity analysis on bacterial panels.

Example 4: Construction of genomic expression of tail fibers

To facilitate optimal Smarticles production with native and/or novel tail fibers, tail fiber and chaperone genes were integrated into the P1 Smarticles genome at the *s* and *u* genes native locations. This expression from the genome instead of *in trans* ensured the proper expression and timing with the rest of the P1 based genome, reducing the burden from *in trans* expression. Tail fibers were first amplified by PCR with primers containing homology to the hygromycin cassette on the C-terminal portion of the tail fiber structural gene (*s* gene) or tail fiber chaperone gene (*u* gene). PCR was also used to amplify the hygromycin cassette. These two fragments were assembled with Gibson Assembly and further amplified with primers that contain homology to the P1 genome where *s* was previously found and homology in the recombinase *cin* which lies just downstream of the native *s* and *u* genes (see FIG. 2). This Recombineering event performed the dual functions of inserting the tail fiber on the P1 genome as well as disrupting the cin recombinase responsible for switching the variable regions of the *s* tail fiber gene. This linear DNA product of tail fiber and hygromycin cassette was transformed into BAA-1001 containing the spectinomycin resistance cassette. Recovery at 30°C and plating followed transformation. Cells were plated onto LB agar containing hygromycin and patched for spectinomycin sensitivity. Promising colonies were checked with colony PCR and Sanger sequencing.

Positive colonies containing the non-native tail fiber and hygromycin resistance were then transformed with a packaging plasmid. Cells were grown overnight in media containing antibiotic. Lysates were generated using a 90 minute 42°C heat induction to inactivate the P1 master repressor and facilitate Smarticles production. Lysates were then spun down to pellet the bacterial cell debris and then filter sterilized through a 2 μm filter. Lysates were checked for proper Smarticles functionality on the new hosts similar to the process for the TFDV packaging lines described above.

To construct a packaging line that expresses two or more tail fibers from a single Smarticles genome, a similar Recombineering approach was used as described above. The first tail fiber was similarly placed on the P1 genome, but instead of the tail fiber being linked to hygromycin resistance, it was attached to an alternative antibiotic, such as kanamycin or gentamicin. The cin recombinase was also left intact to facilitate switching of the multiple tail fibers. A second recombineering reaction was performed to place the second variable portion of the tail fiber attached to the hygromycin casstte to replacement of the kanamycin (or gentamicin) cassette already on the genome. This could be repeated to add three, four, five, etc. tail fibers to the genome (see FIG. 6).

Example 5: NRTP assay conditions

The assay involved an initial 2.5 hour pre-treatment of bacterial cells at a concentration of 5.0E+05 CFU/mL in assay media (10g/L Tryptone + 5g/L Yeast Extract + 5% PEG8000). Following the pre-treatment step, both NRTPs and transduction salts (1M MgCl2 + 0.5M CaCl2) were added to the reaction and incubated for 2 hours – which allowed for transduction of the reporter molecule that contained the luciferase gene, luxAB. Light production from bacteria was measured by Relative Luminometer Units (RLU) output using a luminometer.

Example 6: Performance of NRTPs with engineered tail fibers.

The improved reactivity of engineered NRTPs against various species and strains can be seen on Table 1. These data clearly show 1) the diversity of tail fibers analyzed, 2) the robust alterations reactivity observed by different tail fiber protein binding domains, 3) that some tail fibers are highly specific for certain species, and 4) some tail fibers are highly reactive against many diverse species of bacteria. Both nucleotide and amino acid sequences of the tail fibers from these engineered NRTPs (e.g. “Sunset”, “Indian”, “Tangerine” etc.) are listed in the SEQUENCE LISTING.

Table 1

Lysate*	Test Panels										
	Abi (n=73)	Cfi (N=88)	Cko (N=88)	Eae (N=88)	Ecl (N=68)	Eco (N=87)	Kox (N=88)	Kpn (N=89)	Pae (N=87)	Pms (N=88)	Sms (N=88)
5X V3	45.9%***	56.8%*	63.6%*	61.4%*	95.6%*	88.5%*	94.3%*	82%*	23%***	17%*	89.8%*
S	**40.3%****	NA	NA	NA	**67.6%****	**71.3%****	NA	**75.3%****	**10.3%****	**20.5%****	NA
S'	**41.6%****	NA	NA	NA	**89.7%****	**60.9%****	NA	**73.0%****	**3.4%****	**29.5%****	NA
Sunset TFDV	**0%****	5.7%*	0%*	2.3%*	**7.4%****	**12.6%****	1.1%*	**1.1%****	**0%****	0%*	1.1%*
Indian TFDV	**1.3%****	10.2%*	0%*	4.5%*	**14.7%****	**2.3%****	40.9%*	**14.6%****	**1.1%****	5.7%*	6.8%*
Tangerine Genomic	*1.4%****	NA	NA	NA	*1.5%**	*13.8%**	NA	*0%**	*9.2%****	NA	NA
Tangerine TFDV	NA	3.4%*	0%*	0%*	NA	NA	0%*	NA	NA	0%*	6.8%*
Maroon TFDV	**0%****	0%*	0%*	0%*	**4.4%****	**14.9%****	0%*	**0%****	**0%****	0%*	4.5%*
Scarlett Genomic	**0%****	6.8%*	6.8%*	45.5%*	**8.8%****	**14.9%****	47.7%*	**11.2%****	**0%****	6.8%*	11.4%*
Bittersweet Genomic	*1.4%****	NA	NA	NA	*0%**	*4.6%**	NA	*0%**	*0%****	NA	NA
Bittersweet TFDV	**2.7%*****	4.5%*	0%*	0%*	**0%***	**3.4%***	0%*	**0%***	**0%*****	1.1%*	0%*
Indigo Genomic	*0%****	NA	NA	NA	*16.2%**	*1.1%**	NA	*0%**	*0%****	NA	NA
5x Indigo TFDV	1.4%***	NA	NA	NA	14.7%*	1.1%*	NA	0%*	0%***	NA	NA
DenimTFDV	4.1%***	NA	NA	NA	4.4%*	12.6%*	NA	22.5%*	0%***	NA	NA
Cobalt TFDV	5.5%***	NA	NA	NA	5.9%*	16.1%*	NA	29.2%*	0%***	NA	NA
Pine TFDV	**35.1%**	23.9%*	63.6%*	47.7%*	**83.8%**	**79.3%****	78.4%*	**80.9%****	**9.2%****	6.8%*	38.6%*
Jungle TFDV	**50.6%****	42%*	12.5%*	48.9%*	**88.2%****	**65.5%****	90.9%*	**79.8%****	**1.1%****	26.1%*	97.7%*
Shamrock	**6.8%*****	34.1%*	1.1%*	1.1%*	**22.1%***	**73.6%***	0%*	**0%***	**8%*****	9.3%*	17.1%*
Violet TFDV	**56.8%*****	23.9%*	1.1%*	35.2%*	**80.9%***	**66.7%***	71.6%*	**65.2%***	**20.7%*****	2.3%*	79.6%*
Plum TFDV	**2.7%*****	0%*	0%*	0%*	**1.5%***	**43.7%***	0%*	**0%***	**8%*****	0%*	0%*
Salmon TFDV	**40.5%*****	28.4%*	76.1%*	34.1%*	**33.8%***	**55.2%***	77.3%*	**46.1%***	**20.7%*****	9.1%*	73.6%*
Jazzberry TFDV	**2.7%*****	1.1%*	0%*	0%*	**1.5%***	**42.5%***	0%*	**0%***	**8%*****	0%*	0%*
Razzmatazz TFDV	52.1%***	NA	NA	NA	85.3%*	62.1%*	NA	60.7%*	16.1%***	NA	NA
ShamJazz genomic	*4.1%****	NA	NA	NA	*14.7%**	*72.4%**	NA	*0%**	*0%****	NA	NA
5x Fuzzy+Chaperone TFDV	0%***	NA	NA	NA	0%*	2.3%*	NA	20.2%*	0%***	NA	NA
Banana TFDV	*0%****	NA	NA	*1.1%**	*82.4%**	*0%**	NA	*1.1%**	*0%****	NA	NA
Mango TFDV	*13.7%****	NA	NA	NA	*97.1%**	*26.4%**	NA	*47.2%**	*2.3%****	NA	NA
10x Queen TFDV (Eco0229)	*6.8%****	NA	NA	NA	*25%**	*80.5%**	NA	*0%**	*0.0%****	NA	NA
Tropical TFDV	*0%****	NA	NA	NA	*2.9%**	*0%**	NA	*0%**	*0%****	NA	NA
Gold TFDV	*2.7%***	*5.7%***	NA	*37.5%***	*22.1%***	*32.2%***	*87.5%***	*62.9%***	*0%***	*22.7%***	*68.2%***
Bluetiful TFDV	*0%***	*1.1%***	NA	*22.7%***	NA	*0.0%***	*30.7%***	NA	*0%***	*4.5%***	*76.1%***
Rio TFDV	*0%***	*3.4%***	NA	*3.4%***	*92%***	*0%***	*84%***	*53.9%***	*0%***	*86.4%***	*80.7%**
Orchid TFDV	*8.2%***	*17.0%***	NA	*22.7%***	*42.6%***	*79.3%***	NA	*55.1%***	*0%***	*5.7%***	*63.6%***
Limon TFDV	*1.4%***	*56.8%***	*34.1%***	NA	NA	*0%***	NA	NA	*0%***	NA	NA

* Detection observed at 1E4 CFU/mL	**Bold denotes lysate concentration of 1x utilized in diagnostic assay**
** Detection observed at 5E5 CFU/mL	Normal font denotes concentration of 5x utilized in diagnostic assay
*** Detection observed at 1E7 CFU/mL	*Italics denote lysate concentration of 10x utilized in diagnostic assay*

A. baumannii	**Abi**
C. freundii	**Cfi**
C. koseri	**Cko**
E. aerogenes	**Eae**
E. cloacae	**Ecl**
E. coli	**Eco**
K. oxytoca	**Kox**
K. pneumoniae	**Kpn**
P. aeruginosa	**Pae**
P. mirabilis	**Pms**
S. marcescens	**Sms**

FIG. 3 graphically depicts how a bald (tail fiber deficient) P1 lysogen can be altered to express a new tail fiber from the genome. FIG. 4 depicts how genomic integration of a given tail fiber can improve performance compared to trans expression from a TFDV. Light activity is shown for 3 distinct tail fibers on 3 indicator strains for both the TFDV and genomically expressed versions. Additionally, transduction efficiency of the TFDV and genomic S tail fiber NRTPs were also compared to the WT P1 NRTP control (V3).

FIG. 5 A depicts a graph that show improved tail fiber activity of the engineered "Indian" NRTP as light production (RLU) comparing a packaging line that expresses the plasmid p15B U protein "t chaperon" compared to packaging line not expressing this protein. Figure 5B depicts a graph that shows improved performance of "Scarlet" NRTPs that are produced from a bacteriophage genome compared to "Scarlet" NRTPs produced from a separate packaging plasmid (TFDV).

Figure 6A displays the genomic arrangement of production lines harboring 0, 1, 2, or 3 distinct tail fiber genes and demonstrates how lysogens can be engineered to produce multiple NRTPs

from a single fermentation. Tail fiber genes can be arranged with cix crossover site in a novel location. Genes can also be arranged for expression not dependent on recombination in large or small operons. Figure 6B depicts indicator strains specifically detected by given NRTPs endowed individual tail fibers (left). While the right panel shows that packaging lines expressing multiple tail fibers can detect multiple indicator stains, demonstrating that multiple functional NRTPs with varying specificities can be produced at once.

FIG. 7 compares the performance of NRTPs derived from a bi-functional (two tail fiber) expression strain relative to individual NRTPs produced in separate production runs. Top image shows genomic arrangement of bi-functional tail fiber region from the Jazzberry and Shamrock tail fibers. Left middle, compares individually produced and bi-functional NRTPs on indicator strains specific for the different tail fibers. Right middle, shows how detection and light production were improved on a large panel of *E. coli* strains by making bi-functional production lines in addition to mixing into a cocktail with other NRTPs (such as the native P1 NRTP, called V3). The bottom part of FIG. 7 directly compares the performance of bi-functionally produced NRTPs to the cumulative performance of the individual tail fibers produced separately.

FIG. 8 shows the results of experiments performed that identified the elements that are required for functionalizing new tail fibers on a viral chassis. Panel A illustrates that the P1 *r* gene is required and that the tail fiber variable region (S_V) should be pared with its cognate *u* homolog accessory protein for maximal activity. Additionally, panel A shows that 3 distinct tail fiber conserved regions could allow functionalization of non-native tail fibers on a new chassis. Panel B shows the alignment of the amino acid sequence of the 3 S_C regions from P1 bacteriophage, type 11 Kpn bacteriophage, and p15B plasmid (related to P1), highlighting a highly conserved N-terminal 150 amino acid sequence motif.

FIG. 9 Displays the various arrangements for expression of tail fiber genes from fermentation strain. A) shows expression of tail fibers and viral chassis from phage genome. B) Shows viral chassis expression from phage genome and tail fibers genes from packaging plasmid. C) Shows viral chassis from phage genome and tail fibers from independent plasmid. D) Shows viral chassis from phage genome and tail fibers from bacterial genome. E) Shows viral chassis and one set of tail fibers from phage genome. A second, unique set of tail fibers is expressed from an independent plasmid. F) Shows viral chassis and tail fibers from phage genome. A second, unique set of tail fibers is expressed from the packaging plasmid. G) Shows viral chassis and two, unique sets of tail fibers from phage genome.

While the disclosure has been particularly shown and described with reference to a preferred embodiment and various alternate embodiments, it will be understood by persons skilled in the relevant art that various changes in form and details can be made, whereas the true scope of the invention is set forth in the appended claims.

Description of Sequences Listed in Sequence Listing:

Nucleotide sequences of tail fibers functionally expressed on a P1 chassis.

Asparagus Tail Fiber Nucleotide Sequence	SEQ ID NO: 1
Banana Tail Fiber Nucleotide Sequence	SEQ ID NO: 2
Bittersweet Tail Fiber Nucleotide Sequence	SEQ ID NO: 3
Cobalt Tail Fiber Nucleotide Sequence	SEQ ID NO: 4
Cornflower Tail Fiber Nucleotide Sequence	SEQ ID NO: 5
Denim Tail Fiber Nucleotide Sequence	SEQ ID NO: 6
Eggplant Tail Fiber Nucleotide Sequence	SEQ ID NO: 7
Fern Tail Fiber Nucleotide Sequence	SEQ ID NO: 8
Fuchsia Tail Fiber Nucleotide Sequence	SEQ ID NO: 9
Fuzzy Tail Fiber Nucleotide Sequence	SEQ ID NO: 10
Inchworm Tail Fiber Nucleotide Sequence	SEQ ID NO: 11
Indian Tail Fiber Nucleotide Sequence	SEQ ID NO: 12
Indigo Tail Fiber Nucleotide Sequence	SEQ ID NO: 13
Jazzberry Tail Fiber Nucleotide Sequence	SEQ ID NO: 14
Jazzberry-ShamrocK Dual Tail Fiber Nucleotide Seq.	SEQ ID NO: 15
Jungle Tail Fiber Nucleotide Sequence	SEQ ID NO: 16
Mango Tail Fiber Nucleotide Sequence	SEQ ID NO: 17
Maroon Tail Fiber Nucleotide Sequence	SEQ ID NO: 18
Mulberry Tail Fiber Nucleotide Sequence	SEQ ID NO: 19
P1_S'gene Nucleotide Sequence	SEQ ID NO: 20
P1S-P1S'-Shamrock Dual Tail Fiber Nucleotide Seq.	SEQ ID NO: 21
Pine Tail Fiber Nucleotide Sequence	SEQ ID NO: 22
Plum Tail Fiber Nucleotide Sequence	SEQ ID NO: 23
Queen Tail Fiber Nucleotide Sequence	SEQ ID NO: 24
Razzmatazz Tail Fiber Nucleotide Sequence	SEQ ID NO: 25
Salmon Tail Fiber Nucleotide Sequence	SEQ ID NO: 26
Scarlet Tail Fiber Nucleotide Sequence	SEQ ID NO: 27
Shamrock Tail Fiber Nucleotide Sequence	SEQ ID NO: 28
Sunset Tail Fiber Nucleotide Sequence	SEQ ID NO: 29
Tangerine Tail Fiber Nucleotide Sequence	SEQ ID NO: 30
Thistle Tail Fiber Nucleotide Sequence	SEQ ID NO: 31
Tropical_pNS88 Tail Fiber Nucleotide Sequence	SEQ ID NO: 32
Tropical_pNS92 Tail Fiber Nucleotide Sequence	SEQ ID NO: 33
Violet Tail Fiber Nucleotide Sequence	SEQ ID NO: 34

Amino Acid sequences of tail fibers functionally expressed on a P1 chassis.

Asparagus_-_P1Sc-(P1Sv-MuS_hybrid)_translation	SEQ ID NO: 35
Banana_-_P1Sc(aa1-114)-Ecl112(aa218-end)_translation	SEQ ID NO: 36
Bittersweet_-_p15b_Sc-p15b_SvQ_translation	SEQ ID NO: 37
Cobalt_-_p15bSc-Kpn_SCKP_TF4_translation	SEQ ID NO: 38
Cornflower_-_P1Sc(aa1-208)-Kpn478_tail_fiber(aa131-499)_translation	SEQ ID NO: 39
Denim_-_p15bSc-Kpn_SCKP_TF1_translation	SEQ ID NO: 40
Eggplant_-_D6Sc-D6Sv'_translation	SEQ ID NO: 41
Fern_-_P1Sc-(P1Sv-D108S_hybrid)_translation	SEQ ID NO: 42
Fuchsia_-_P1Sc-D6Sv_translation	SEQ ID NO: 43
Fuzzy_-_P1Sc(aa1-208)-Kpn051_TF(aa40-382)_translation	SEQ ID NO: 44
Inchworm_-_P1Sc-R2_pyocin_translation	SEQ ID NO: 45
Indian_-_p15bSc-p15bSvN_translation	SEQ ID NO: 46
Indigo_-_p15bSc-Kpn_SCKP_TF3_translation	SEQ ID NO: 47
Jazzberry_-_P1Sc-KpnSv_translation	SEQ ID NO: 48
Jungle_-_P1Sc-(P1Sv'-MuS'_hybrid)_translation	SEQ ID NO: 49
Mango_-_P1Sc(aa1-214)-Ecl117B(aa214-764)_translation	SEQ ID NO: 50
Maroon_-_p15bSc-p15bSvP_translation	SEQ ID NO: 51
Mulberry_-_D6Sc-D6Sv_translation	SEQ ID NO: 52
P1_S'_-_P1Sc-P1Sv'_translation	SEQ ID NO: 53
Pine_-_P1Sc(nt1-987)-P2H_translation	SEQ ID NO: 54
Plum_-_KpnSc-KpnSv_translation	SEQ ID NO: 55
Queen_-_S-Queen_Sv_translation	SEQ ID NO: 56
Razzmatazz_-_P1Sc-P7Sv_translation	SEQ ID NO: 57
Salmon_-_P1Sc-P7Sv'_translation	SEQ ID NO: 58
Scarlet_-_p15bSc-p15bSvI_translation	SEQ ID NO: 59
Shamrock_-_P1Sc-(P1S-SfMuS'_hybrid)_translation	SEQ ID NO: 60
Sunset_-_p15bSc-p15bSvR_translation	SEQ ID NO: 61
Tangerine_-_p15bSc-p15bSv0_translation	SEQ ID NO: 62
Thistle_-_P1Sc-KpnSv_translation	SEQ ID NO: 63
Tropical_pNS88_-_P1Sc(aa1-214)-Ecl117A(aa91-437)_translation	SEQ ID NO: 64
Tropical_pNS92_-_P1Sc(aa1-214)-Ecl117A(aa96-437)_translation	SEQ ID NO: 65
Violet_-_RCS47Sc-RCS47Sv_translation	SEQ ID NO: 66

Amino Acid sequences of chaperone proteins

Asparagus_-_MuU_translation	SEQ ID NO: 67
Banana_-_Ecl112_U_chaperone_translation	SEQ ID NO: 68
Bittersweet_-_p15b_T_translation	SEQ ID NO: 69
Cobalt_-_p15b_T_translation	SEQ ID NO: 70
Denim_-_p15b_T_translation	SEQ ID NO: 71
Eggplant_-_P1_U_translation	SEQ ID NO: 72
Fern_-_D108_U_translation	SEQ ID NO: 73
Fuchsia_-_P1_U'_translation	SEQ ID NO: 74
Fuzzy_-_PROKKA_00043_(Kpn478_chaperone)_translation	SEQ ID NO: 75
Inchworm_-_PA0621_translation	SEQ ID NO: 76

Indian_-_p15b_T_translation	SEQ ID NO: 77
Indigo_-_p15b_T_translation	SEQ ID NO: 78
Jazzberry_-_Kpn_U_translation	SEQ ID NO: 79
Jungle_-_Mu_U'_translation	SEQ ID NO: 80
Mango_-_Ecl117B_U_chaperone_translation	SEQ ID NO: 81
Maroon_-_p15_T_translation	SEQ ID NO: 82
Mulberry_-_P1_U'_translation	SEQ ID NO: 83
P1_S'_-_P1_U'_translation	SEQ ID NO: 84
Pine_-_P2_G_translation	SEQ ID NO: 85
Plum_-_Kpn_U_translation	SEQ ID NO: 86
Queen_-_Queen_U_chaperone_translation	SEQ ID NO: 87
Razzmatazz_-_P7_Ua_translation	SEQ ID NO: 88
Razzmatazz_-_P7_Ub_translation	SEQ ID NO: 89
Salmon_-_P7_U'_translation	SEQ ID NO: 90
Scarlet_-_p15b_T_translation	SEQ ID NO: 91
Shamrock_-_SfMuU'_translation	SEQ ID NO: 92
Sunset_-_p15b_T_translation	SEQ ID NO: 93
Tangerine_-_p15_T_translation	SEQ ID NO: 94
Thistle_-_KpnU_translation	SEQ ID NO: 95
Violet_-_RCS47_U_translation	SEQ ID NO: 96

Other Sequences

Bald_Chassis_tail_fiber_region_P1_s-u::aadA	SEQ ID NO: 97
Rio Tail Fiber_s_hybrid_nucleotide sequence	SEQ ID NO: 98
Rio Chaperone_u_nucleotide_sequence	SEQ ID NO: 99
Limon Tail Fiber_s_hybrid_nucleotide sequence	SEQ ID NO: 100
Limon Chaperone_u_nucleotide_sequence	SEQ ID NO: 101
Orchid Tail Fiber_s_hybrid_nucleotide sequence	SEQ ID NO: 102
Orchid Chaperone_u_nucleotide sequence	SEQ ID NO: 103
Gold Tail Fiber_s_hybrid_nucleotide sequence	SEQ ID NO: 104
Gold Chaperone_u_nucleotide sequence	SEQ ID NO: 105
Bluetiful Tail Fiber_s_hybrid_nucleotide sequence	SEQ ID NO: 106
Bluetiful Chaperone_u_nucleotide sequence	SEQ ID NO: 107
Rio Tail Fiber_S_hybrid_amino acid sequence	SEQ ID NO: 108
Limon Tail Fiber_S_hybrid_amino acid sequence	SEQ ID NO: 109
Orchid Tail Fiber_S_hybrid_amino acid sequence	SEQ ID NO: 110
Gold Tail Fiber_S_hybrid_amino acid sequence	SEQ ID NO: 111
Bluetiful Tail Fiber_S_hybrid_amino acid sequence	SEQ ID NO: 112
Rio Chaperone_U_amino acid sequence	SEQ ID NO: 113
Limon Chaperone_U_amino acid sequence	SEQ ID NO: 114
Orchid Chaperone_U_amino acid sequence	SEQ ID NO: 115
Gold Chaperone_U_amino acid sequence	SEQ ID NO: 116
Bluetiful Chaperone_U_amino acid sequence	SEQ ID NO: 117

CLAIMS

1. A bacteriophage tail fiber replacement platform comprising:
   - a bacteriophage lysogen from the family Myoviridae (Myoviridae lysogen) that contains a genetic disruption that prevents the expression of one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen, wherein said one or more genes are selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes; and
   - a complementary nucleic acid molecule comprising one or more genes selected from a gene that encodes a tail fiber structural protein responsible for binding a bacteria cell receptor, a gene that encodes a chaperone protein needed for folding of one or more regions of a tail fiber structural protein, a gene that encodes a protein required for attaching the tail fiber structural protein to a tail, or any combination of these genes, wherein the complementary nucleic acid complements the genetic disruption of the Myoviridae lysogen whereby functional tail fibers are produced.
2. The platform of claim 1, wherein the Myoviridae lysogen is derived from a genus selected from: Bcep781likevirus, Bcepmulikevirus, FelixO1likevirus, Hapunalikevirus, I3likevirus, Mulikevirus, Punalikevirus, Pbunalikevirus, PhiCD119likevirus, Phihlikevirus, Phikzlikevirus, Viunalikevirus, Eucampyvirinae, Cp220likevirus, Cp8unalikevirus, Peduovirinae, Hpunalikevirus, P2likevirus, Spounavirinae, Spounalikevirus, Twortlikevirus, Tevenvirinae, Schizot4likevirus, or T4likevirus.
3. The platform of claim 2, wherein the Myoviridae lysogen is derived from the Punalikevirus genus.
4. The platform of claim 3, wherein the Myoviridae lysogen is Enterobacteria bacteriophage phage P1 (P1 bacteriophage).
5. The platform of any one of claims 1 to 4, wherein the one or more genes or the combination of genes on the complementary nucleic acid molecule contain regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen such that the functional tail fibers that are produced are not native tail fibers.

6. The platform of claim 5, wherein the one or more genes or the combination of genes contain regions that are not derived from P1 bacteriophage such that the functional tail fibers that are produced are not native P1 bacteriophage tail fibers.

7. The platform of any one of claims 1 to 6, wherein the complementary nucleic acid molecule is a plasmid molecule; or

wherein the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at the site of the genetic disruption; or

wherein the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at a site other than the site of the genetic disruption; or

wherein the complementary nucleic acid molecule is integrated into a genome of a host bacteria cell that contains the Myoviridae lysogen.

8. The platform of any one of claims 1 to 7, wherein the Myoviridae lysogen contains a further disruption in one or more packaging genes that are involved in packaging the genome of the Myoviridae lysogen.

9. The platform of claim 8, wherein the complementary nucleic acid molecule is a plasmid molecule and wherein one or more packaging genes that complement the disruption in the one or more packaging genes in the Myoviridae lysogen are contained in said plasmid molecule; or

wherein the complementary nucleic acid molecule is a plasmid molecule and wherein one or more packaging genes that complement the disruption in the one or more packaging genes in the Myoviridae lysogen are contained in a reporter or therapeutic expression plasmid that is separate from said plasmid molecule.

10. A bacterial cell line comprising a P1 bacteriophage lysogen that comprises a nucleic acid sequence of SEQ ID NO: 97.

11. A method for producing bacteriophage particles with non-native tail fibers comprising:

- providing a bacteria cell containing the bacteriophage tail fiber replacement platform of any one of claims 1 to 9, wherein the complementary nucleic acid molecule comprises a gene that encodes a tail fiber structural protein with one or more regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen; and
- providing conditions to the bacterial cell that induces a lytic phase of the Myoviridae lysogen to produce bacteriophage particles with non-native tail fibers.

12. The method of claim 11, wherein the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the

tail fiber structural protein (tail fiber variable region) obtained from bacteriophages within the order Caudovirales.

13. The method of claim 12, wherein the tail fiber variable region is obtained from bacteriophages within the family Myoviridae.

14. The method of any one of claims 11 to 13, wherein the complementary nucleic acid molecule further comprises a gene that encodes a chaperone protein.

15. The method of claim 14, wherein the gene that encodes the chaperone protein is obtained from bacteriophages within the order Caudovirales.

16. The method of claim 15, wherein the gene that encodes the chaperone protein is obtained from bacteriophages within the family Myoviridae.

17. The method of claim 14, wherein the chaperone protein is obtained from bacterial genomes encoding non-replicative virus derived structures or from nucleic acid delivery particles.

18. The method of claim 11, wherein the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacterial genomes encoding phage tail-like particles or structures or from nucleic acid delivery particles.

19. A method of producing non-replicative transduction particles (NRTPs) with non-native tail fibers comprising:

- providing a bacteria cell containing the bacteriophage tail fiber replacement platform of any one of claims 1 to 9, wherein the Myoviridae lysogen comprises a further disruption in one or more packaging genes that are involved in packaging the Myoviridae lysogen genome, and wherein the complementary nucleic acid comprises a gene encoding the tail fiber structural protein that contains one or more regions that are not derived from the one or more genes that are critical for the production of tail fibers native to the Myoviridae lysogen; and wherein the bacteria cell further comprises a reporter plasmid that comprises the one or more packaging genes that are disrupted in the Myoviridae lysogen; and
- providing conditions to the bacterial cell that induces a lytic phase of the Myoviridae lysogen to produce NRTPs with non-native tail fibers.

20. The method of claim 19, wherein the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacteriophages within the order Caudovirales.

21. The method of claim 20, wherein the tail fiber variable region is obtained from bacteriophages within the family Myoviridae.

22. The method of any one of claims 19 to 21, wherein the complementary nucleic acid molecule further comprises a gene that encodes a chaperone protein.

23. The method of claim 22, wherein the gene that encodes the chaperone protein is obtained from bacteriophages within the order Caudovirales.

24. The method of claim 23, wherein the gene that encodes the chaperone protein is obtained from bacteriophages within the family Myoviridae.

25. The method of claim 22, wherein the chaperone protein is obtained from bacterial genomes encoding phage tail-like particles or structures or from nucleic acid delivery particles.

26. The method of claim 19, wherein the one or more regions that are not derived from the one or more genes native to the Myoviridae lysogen comprises a variable region of the tail fiber structural protein (tail fiber variable region) obtained from bacterial genomes encoding non-replicative virus derived structures or from nucleic acid delivery particles.

27. A method of generating an engineered bacteriophage or a non-replicative transduction particle (NRTP) that displays a bacteria host cell specificity that differs from the bacteria host cell specificity displayed by a native bacteriophage comprising:

- fusing a first nucleotide sequence from one tail fiber structural protein encoding gene with a second nucleotide sequence from a second tail fiber structural protein encoding gene from a different source to generate a chimeric tail fiber structural protein encoding gene; and
- expressing the chimeric tail fiber structural protein gene in a bacteria cell that contains the bacteriophage tail fiber replacement platform of any one of claims 1 to 9; and
- providing conditions to the bacteria cell that induces a lytic phase of the Myoviridae lysogen to generate the engineered bacteriophage or NRTP.

28. The method of claim 27, wherein the first nucleotide sequence is derived from the Myoviridae lysogen that contains the genetic disruption.

29. A method of generating engineered bacteriophages or non-replicative transduction particles (NRTPs) having multiple types of tail fibers from a single bacteria cell line, wherein at least one of the tail fiber type is a non-native tail fiber comprising:

- providing a bacteria cell containing the bacteriophage tail fiber replacement platform of any one of claims 1 to 9, wherein the complementary nucleic acid molecule

comprises at least two receptor binding regions from tail fiber structural protein encoding genes that are fused to one or more tail interacting regions from tail fiber structural protein encoding genes, wherein at least one of the receptor binding regions is derived from a different source than the source of one of the tail interacting region; and

- providing conditions to the bacterial cell that induces a lytic phase of the bacteriophage lysogen to produce bacteriophages or NRTPs having multiple types of tail fibers.

30. The method of claim 29, wherein the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at the site of the genetic disruption; or wherein the complementary nucleic acid molecule is integrated into the Myoviridae lysogen at a site other than the site of the genetic disruption; or wherein the complementary nucleic acid molecule is integrated into the genome of the bacteria cell; or wherein the complementary nucleic acid molecule is integrated into a plasmid molecule.

31. The method of any one of claims 29 to 30, wherein fusing the two or more receptor binding regions to the tail interacting region is performed by a cin recombinase and in the presence of multiple cix recombination sites; or
wherein fusing the two or more receptor binding regions to the tail interacting region is performed by a cin recombinase or a homolog, ortholog, or paralog of the cin recombinase and in the presence of multiple associated recombination sites.

32. The method of any one of claims 29 to 31, wherein the two or more distinct tail fiber structural protein encoding genes are expressed in an operon.

33. The method of any one of claims 29 to 31, wherein the two or more distinct tail fiber structural protein encoding genes are expressed from independent promoters.

34. The method of any one of claims 29 to 31, wherein the two or more distinct tail fiber structural protein encoding genes are expressed from independent genomic locations.

35. A bacterial cell packaging system for packaging a reporter or therapeutic nucleic acid molecule into a non-replicative transduction particle (NRTP) for introduction into a cell, the packaging system comprising:

- a host bacteria cell;
- a bacteriophage lysogen that contains a genetic disruption that prevents the expression of one or more genes that are critical for the production of native tail fibers, wherein said one or more genes encode a tail fiber structural protein responsible for binding a receptor, a chaperone needed for folding of one or more regions of a tail fiber structural protein, a protein required for attaching the tail fiber structural protein to the tail structure, or any combination of these genes; and also comprising a first bacteriophage

gene that contains a non-functional packaging initiation site sequence, wherein the non-functional packaging initiation site sequence prevents preferential packaging of the bacteriophage genome into the NRTP;

- one or more complementary nucleic acid molecules comprising one or more genes selected from genes encoding a tail fiber structural protein responsible for binding a receptor, a chaperone needed for folding of one or more regions of a tail fiber structural protein, a protein required for attaching the tail fiber structural protein to the tail structure, or any combination of these genes that complements the genetic disruption of the bacteriophage lysogen whereby functional tail fibers are produced; and
- a reporter or therapeutic nucleic acid molecule and a second bacteriophage gene that contains a functional packaging initiation site sequence for facilitating packaging of a replicon of the reporter nucleic acid molecule into the NRTP,

wherein the functional packaging initiation site sequence on the reporter nucleic acid molecule complements the non-functional packaging initiation site sequence in the bacteriophage lysogen.

36. The bacterial cell packaging system of claim 35, wherein the reporter gene is luxAB or wherein the reporter gene is a fluorescent reporter protein.

37. The bacterial cell packaging system of any one of claims 35 to 36, wherein the first bacteriophage gene and the second bacteriophage gene is a pacA terminase gene.

38. The bacterial cell packaging system of any one of claims 35 to 36, wherein the first bacteriophage gene and the second bacteriophage gene is a terS terminase gene.

39. A method of generating Myoviridae-family bacteriophages or Myoviridae-family derived non-replicative transduction particles (NRTPs) that are able to recognize and transduce bacteria cells for diagnostic or therapeutic use comprising:

- providing a bacteria cell containing the bacteriophage tail fiber replacement platform of any one of claims 1 to 9, wherein the complementary nucleic acid molecule comprises tail fiber genes non-native to the Myovridae lysogen and derived partially or entirely from either Myoviridae, Siphoviridae, or Podoviridae tail fiber sequences; and
- providing conditions to the bacterial cell that induces a lytic phase of the bacteriophage lysogen to produce Myoviridae-family bacteriophages or Myoviridae-family derived NRTPs capable of recognizing and transducing bacteria previously refractory to a native Myoviridae bacteriophage or Myoviridae-family derived NRTP particles for diagnostic or therapeutic use.

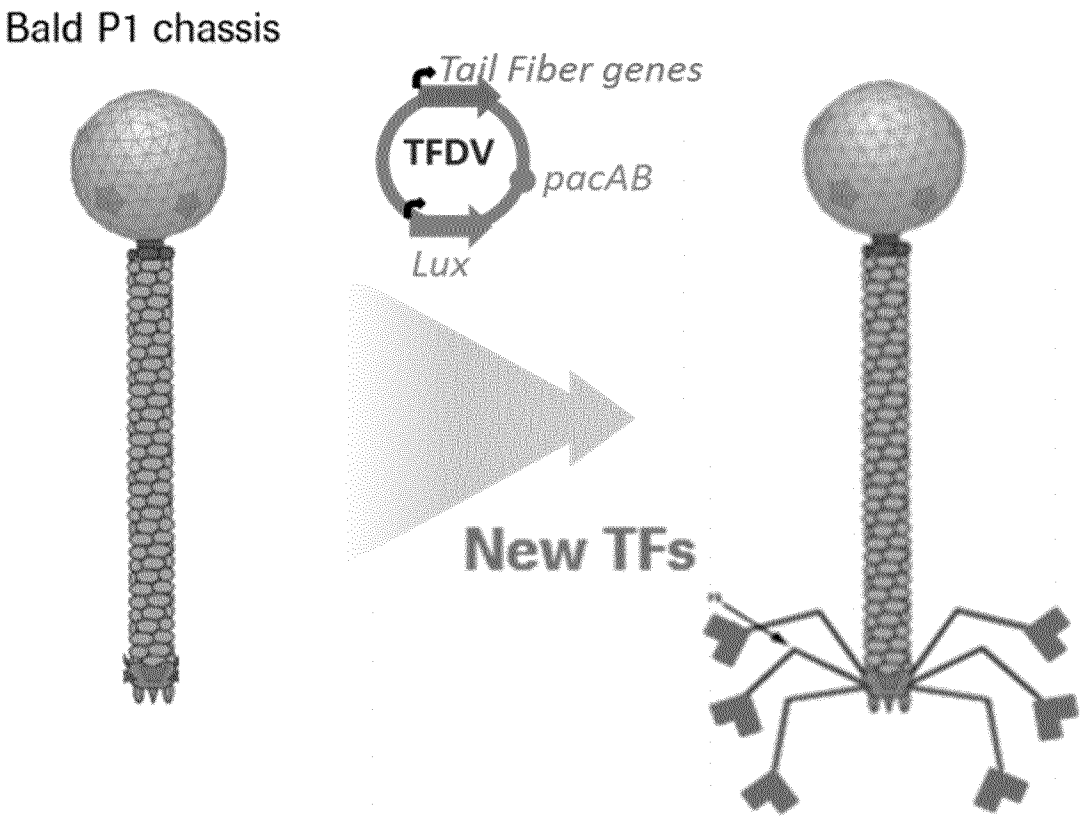


FIG. 1

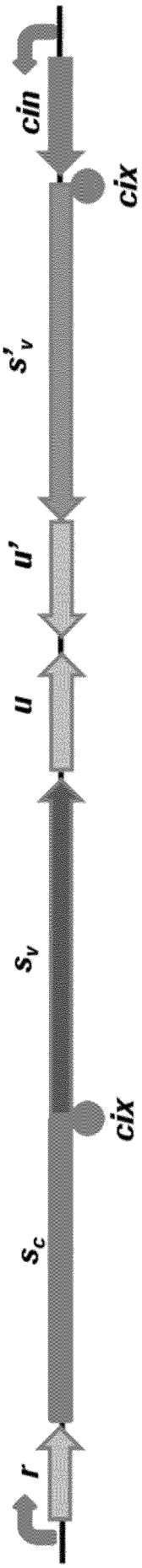


FIG. 2

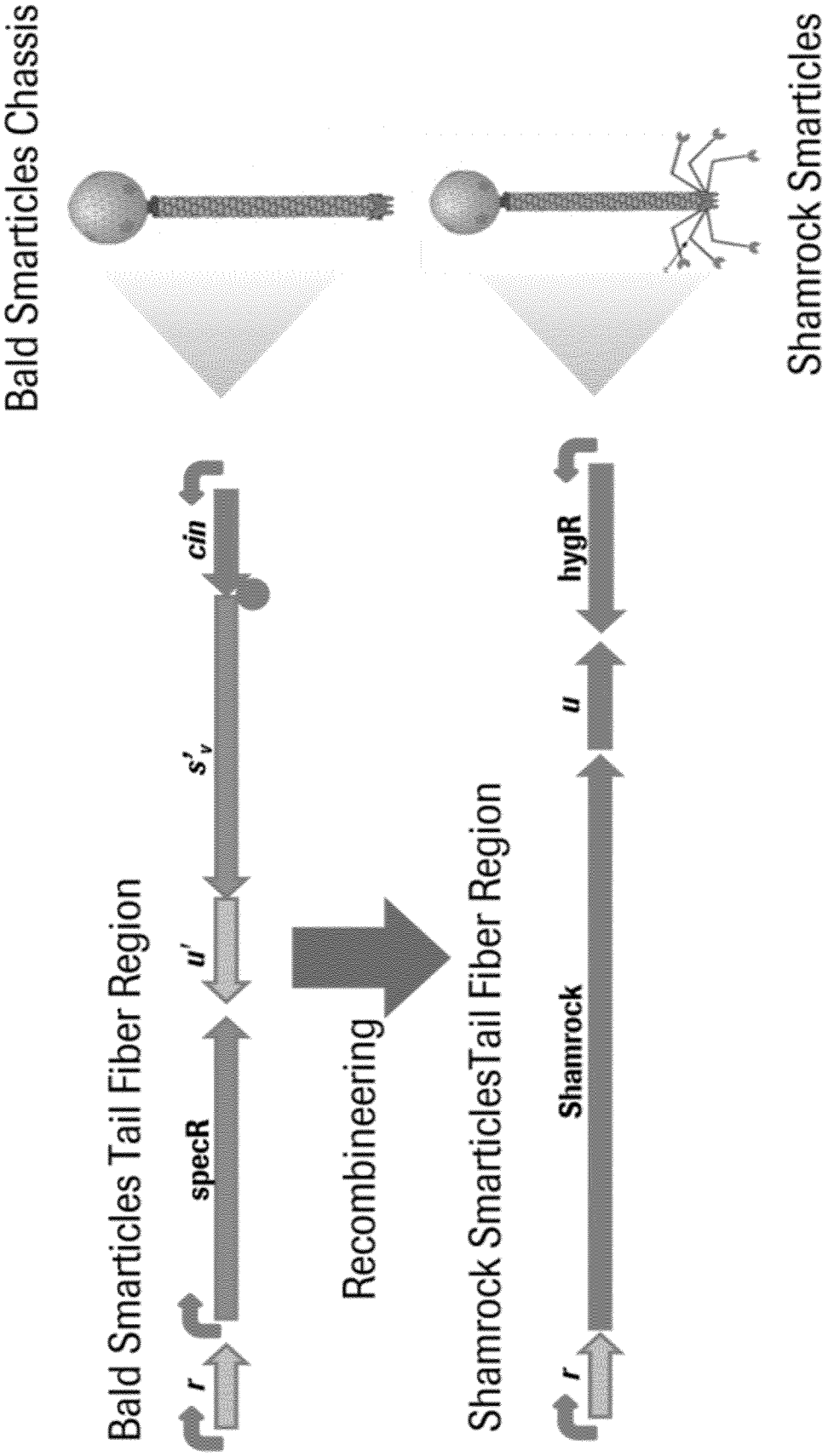

Bald Smarticles Chassis
Shamrock Smarticles
Bald Smarticles Tail Fiber Region
r
specR
u'
s'v
cin
Recombineering
Shamrock SmarticlesTail Fiber Region
r
Shamrock
u
hygR

FIG. 3

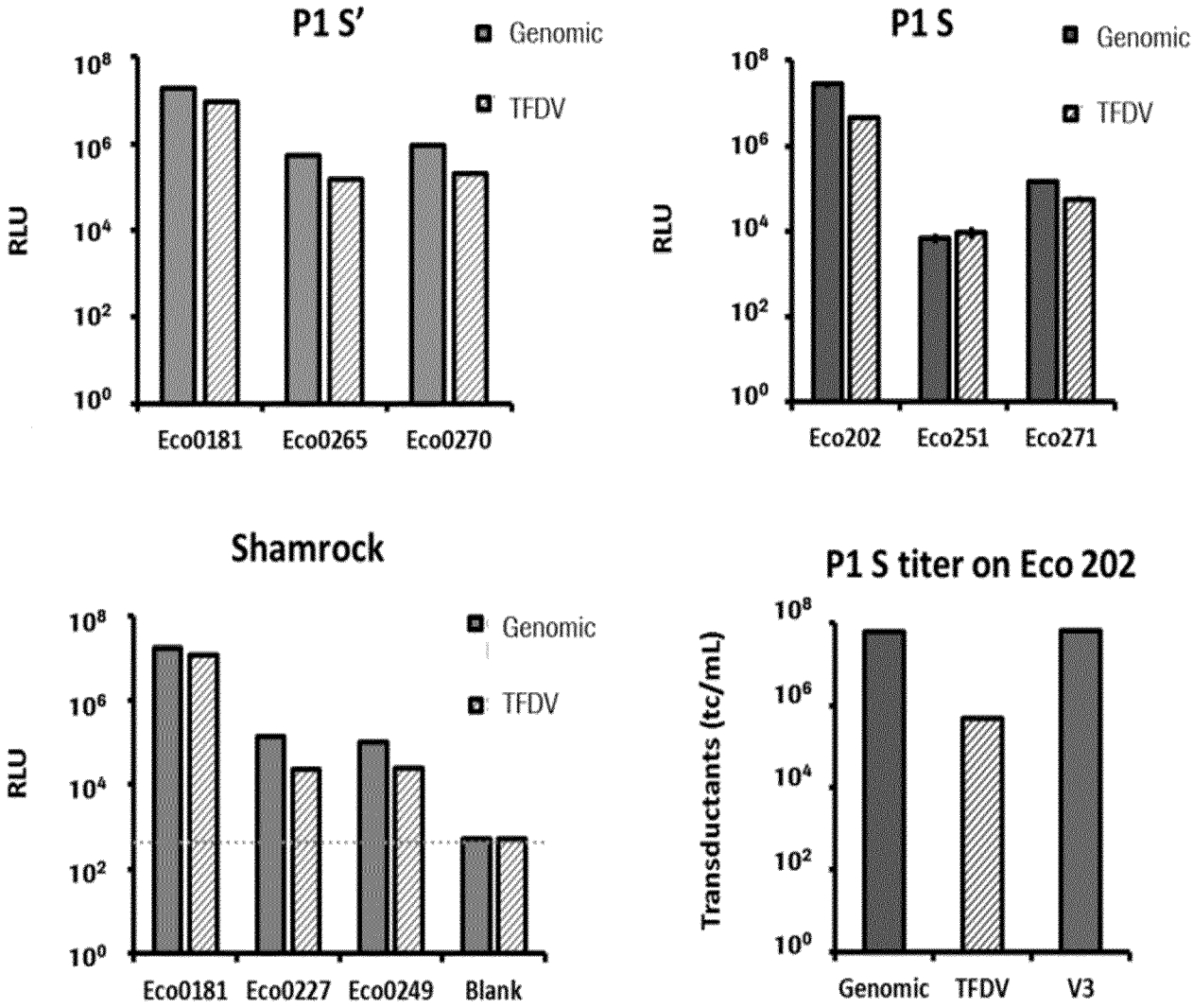

P1 S'
Genomic
TFDV
RLU
10^8
10^6
10^4
10^2
10^0
Eco0181
Eco0265
Eco0270
P1 S
Genomic
TFDV
RLU
10^8
10^6
10^4
10^2
10^0
Eco202
Eco251
Eco271
Shamrock
Genomic
TFDV
RLU
10^8
10^6
10^4
10^2
10^0
Eco0181
Eco0227
Eco0249
Blank
P1 S titer on Eco 202
Transductants (tc/mL)
10^8
10^6
10^4
10^2
10^0
Genomic
TFDV
V3

FIG. 4

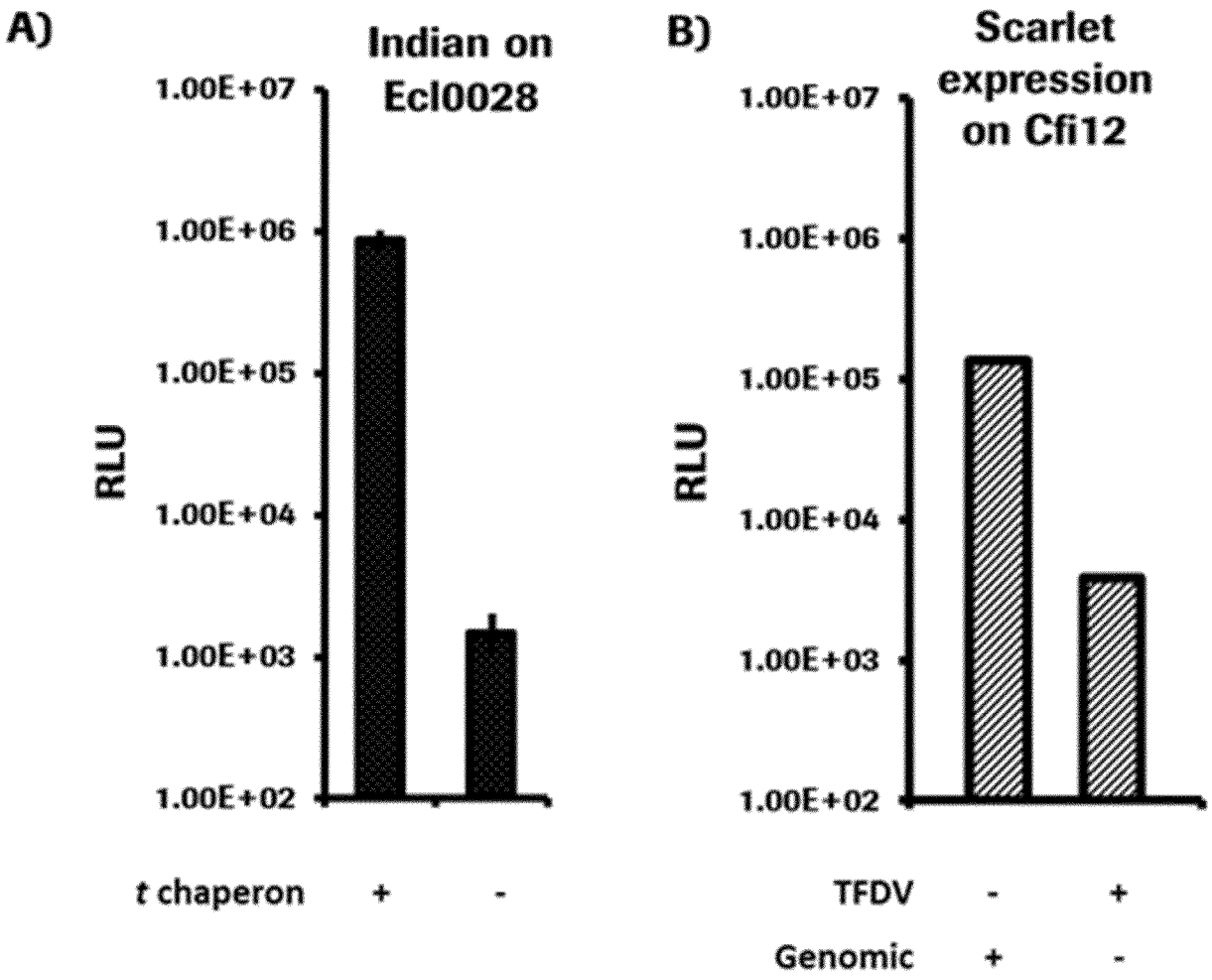
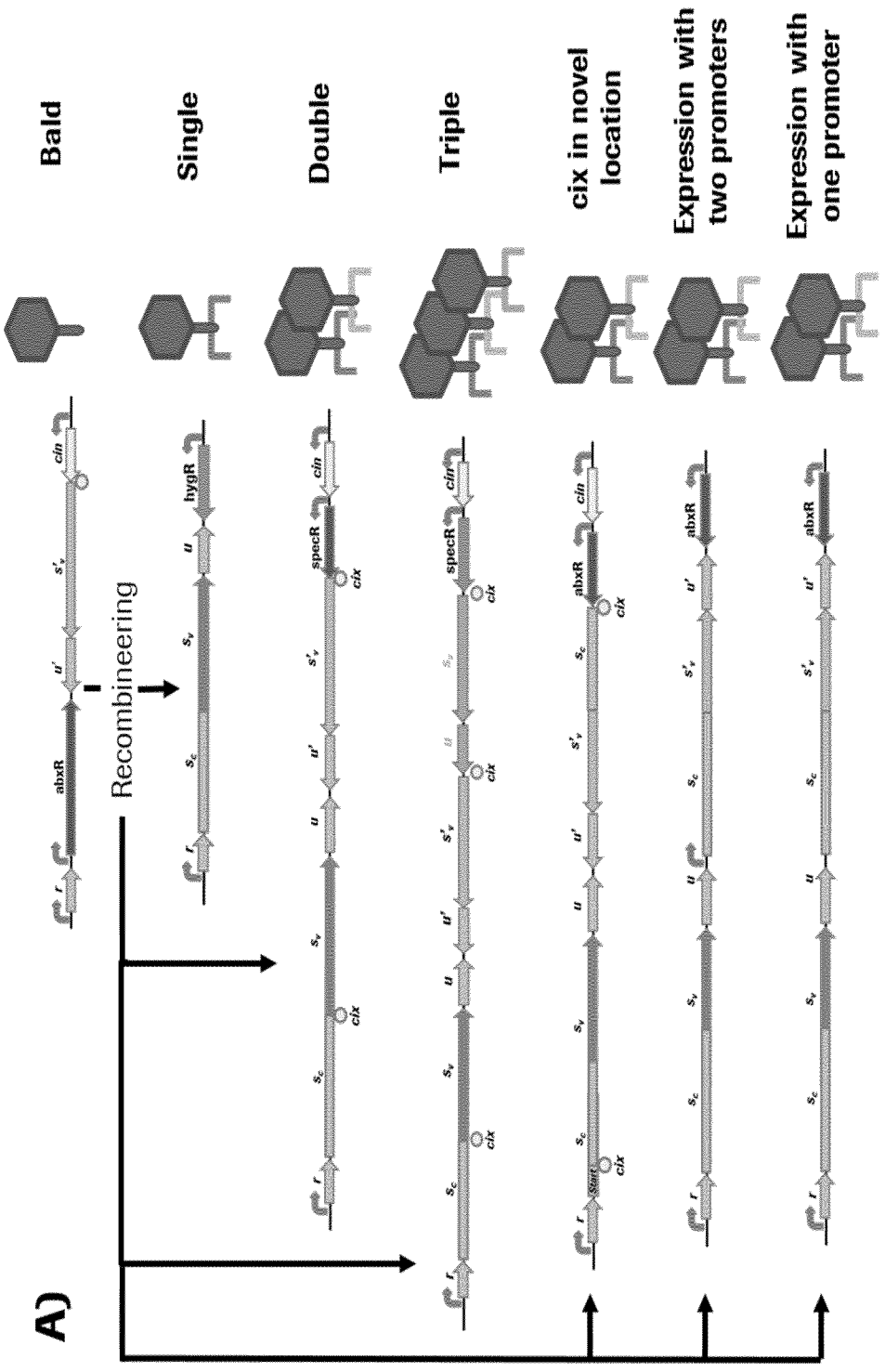

A)
Indian on Ecl0028
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
RLU
t chaperon + -
B)
Scarlet expression on Cfi12
1.00E+07
1.00E+06
1.00E+05
1.00E+04
1.00E+03
1.00E+02
RLU
TFDV - +
Genomic + -

FIG. 5



B)

Strain	Lysate						
	S	S'	Smrk	V3	Double	Triple
Eco202	1	0	0	1	1	1
Eco109	0	1	0	1	1	1
Eco228	0	0	1	0	0	1

FIG. 6

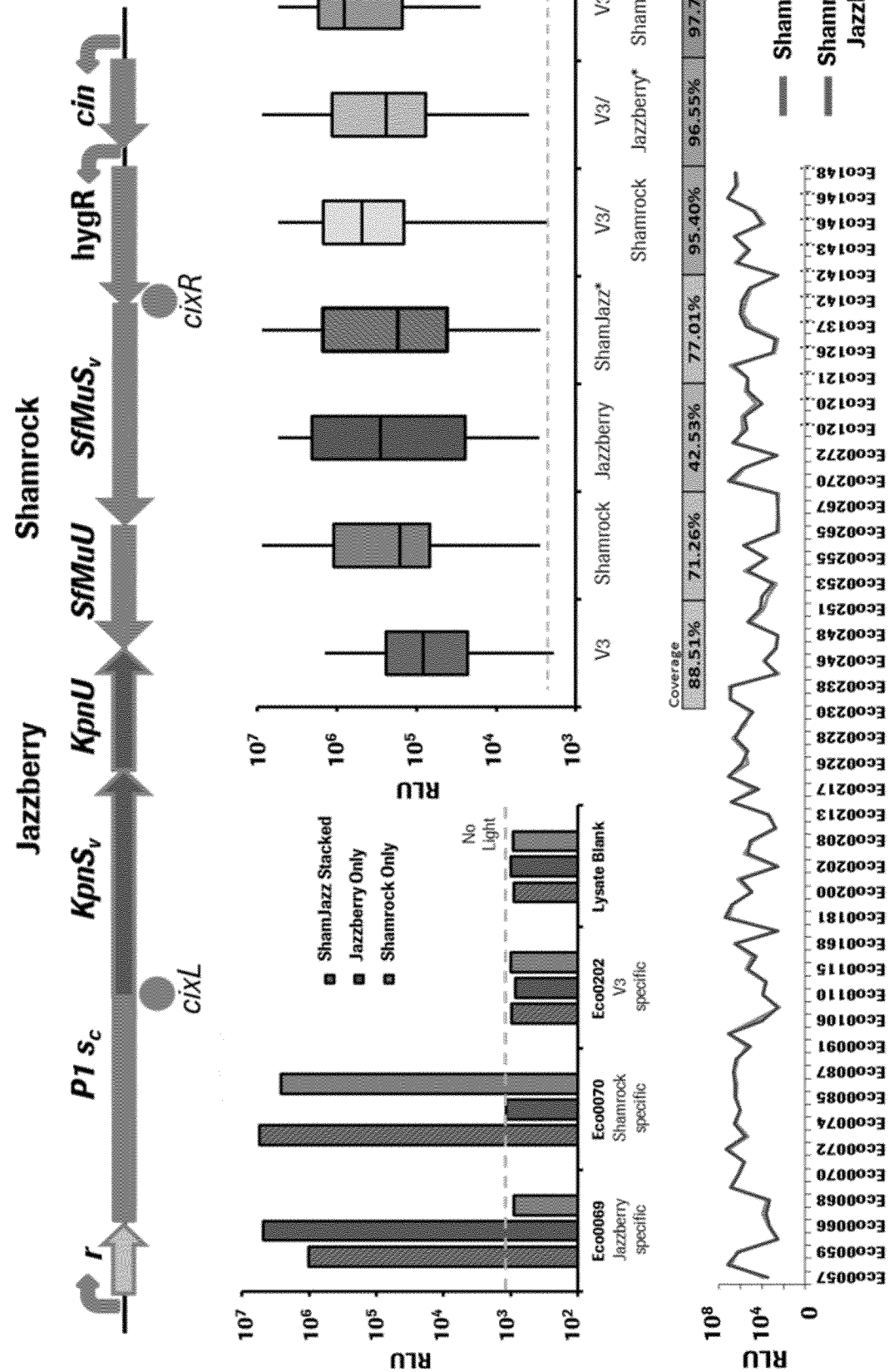
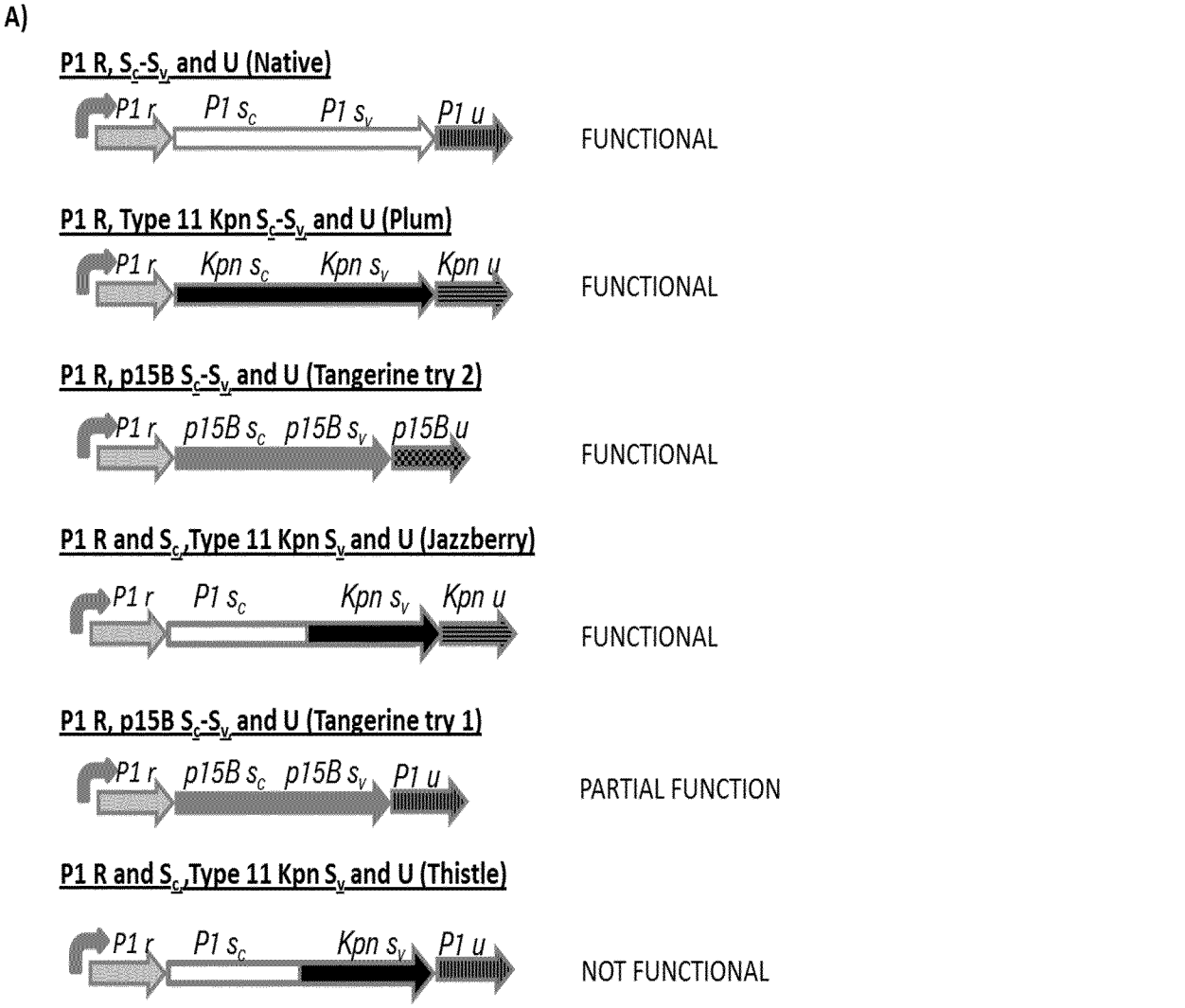

Jazzberry
Shamrock
P1 sc
KpnSv
KpnU
SfMuU
SfMuSv
hygR
cin
r
cixL
cixR
ShamJazz Stacked
Jazzberry Only
Shamrock Only
No Light
RLU
Eco0069 Jazzberry specific
Eco0070 Shamrock specific
Eco0202 V3 specific
Lysate Blank
V3
Shamrock
Jazzberry
ShamJazz*
V3/ Shamrock
V3/ Jazzberry*
V3/ ShamJazz*
Coverage
88.51%
71.26%
42.53%
77.01%
95.40%
96.55%
97.70%
ShamJazz
Shamrock or Jazzberry

FIG. 7

A)


P1 R, Sc-Sv and U (Native)
P1 r
P1 sc
P1 sv
P1 u
FUNCTIONAL
P1 R, Type 11 Kpn Sc-Sv and U (Plum)
P1 r
Kpn sc
Kpn sv
Kpn u
FUNCTIONAL
P1 R, p15B Sc-Sv and U (Tangerine try 2)
P1 r
p15B sc
p15B sv
p15B u
FUNCTIONAL
P1 R and Sc,Type 11 Kpn Sv and U (Jazzberry)
P1 r
P1 sc
Kpn sv
Kpn u
FUNCTIONAL
P1 R, p15B Sc-Sv and U (Tangerine try 1)
P1 r
p15B sc
p15B sv
P1 u
PARTIAL FUNCTION
P1 R and Sc,Type 11 Kpn Sv and U (Thistle)
P1 r
P1 sc
Kpn sv
P1 u
NOT FUNCTIONAL

B)

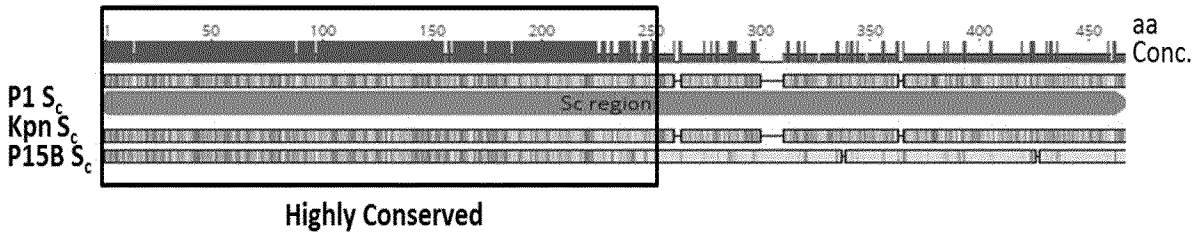

1
50
100
150
200
250
300
350
400
450
aa
Conc.
P1 Sc
Kpn Sc
P15B Sc
Sc region
Highly Conserved

FIG. 8

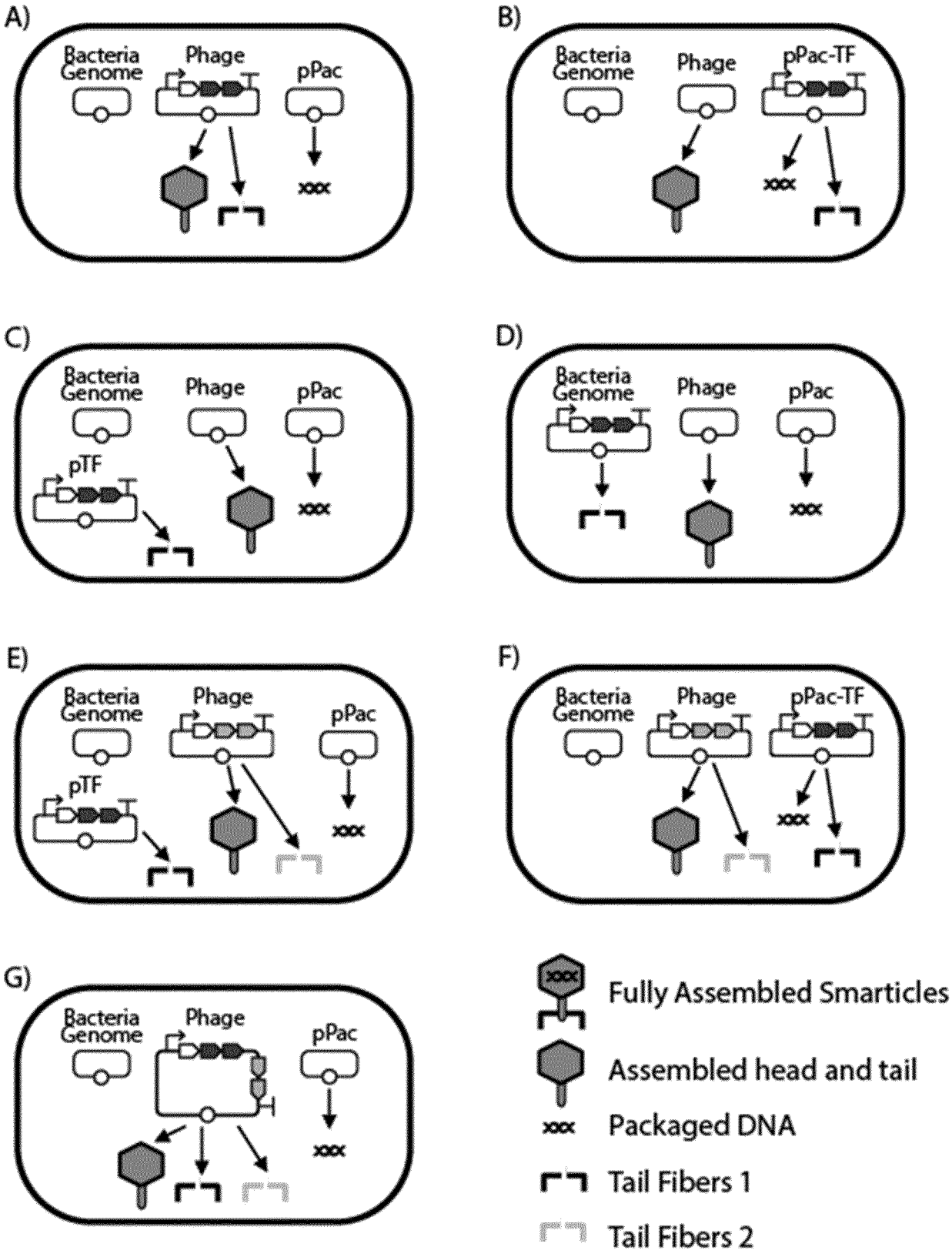

A)
Bacteria Genome
Phage
pPac
xxx
B)
Bacteria Genome
Phage
pPac-TF
xxx
C)
Bacteria Genome
Phage
pPac
pTF
xxx
D)
Bacteria Genome
Phage
pPac
xxx
E)
Bacteria Genome
Phage
pPac
pTF
xxx
F)
Bacteria Genome
Phage
pPac-TF
xxx
G)
Bacteria Genome
Phage
pPac
xxx
Fully Assembled Smarticles
Assembled head and tail
Packaged DNA
Tail Fibers 1
Tail Fibers 2

FIG. 9