superinfection immunity and host restriction mechanisms that target virus nucleic acid molecules and viral functions, all of which can limit the performance of the reporter assay by increasing limits of detection and resulting in false negative results.

[0011] Even where transduction particles have been engineered, methods for using the transduction particles to detect and report the presence of target nucleic acid molecules in cells have limitations. Some methods require disruption of the cell and cumbersome techniques to isolate and detect transcripts in the lysate. Detection methods include using labeled probes such as antibodies, aptamers, or nucleic acid probes. Labeled probes directed to a target gene can result in non-specific binding to unintended targets or generate signals that have a high signal-to-noise ratio. Therefore, there is a need for specific, effective and accurate methods for detection and reporting of endogenous nucleic acid molecules in cells.

[0012] Accordingly, methods and systems are needed for generating non-replicative transduction particles that allow packaging and expression of reporter molecules in cells, while eliminating replication-competent progeny virus. Effective and accurate methods for detecting molecules in cells using the expressed reporter molecules are also needed.

SUMMARY OF THE INVENTION

[0013] Disclosed herein is a bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, said bacterial cell comprising a lysogenized bacteriophage genome lacking a bacteriophage gene encoding a packaging initiation site sequence, wherein deletion of said bacteriophage gene prevents packaging of a bacteriophage nucleic acid molecule into said non-replicative transduction particle; and a reporter nucleic acid molecule comprising a second bacteriophage gene, wherein said second bacteriophage gene encodes a packaging initiation site sequence and facilitates the packaging a replica of said reporter nucleic acid molecule into said non-replicative transduction particle, wherein said second bacteriophage gene is capable of expressing a protein that is encoded by said gene, wherein said replica of said reporter nucleic acid molecule forms a replicon amenable to packaging into said non-replicative transduction particle.

[0013a] In one aspect, there is provided a bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, said bacterial cell comprising:

- a lysogenized bacteriophage genome comprising a first bacteriophage gene comprising a deletion of a packaging initiation site sequence of said first bacteriophage gene that prevents packaging of a bacteriophage nucleic acid molecule into said non-replicative transduction particle; and

- a reporter nucleic acid molecule plasmid comprising a reporter gene and comprising a second bacteriophage gene comprising a second packaging initiation site sequence that facilitates the packaging a replica of said reporter nucleic acid molecule plasmid into said non-replicative transduction particle, wherein said second bacteriophage gene encodes a protein, wherein said replica of said reporter nucleic acid molecule plasmid forms a replicon for packaging into said non-replicative transduction particle.

[0013b] In one aspect, there is provided a bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, said bacterial cell comprising:

- a lysogenized bacteriophage genome lacking a bacteriophage gene encoding a packaging initiation site sequence, wherein deletion of said bacteriophage gene prevents packaging of a bacteriophage nucleic acid molecule into said non-replicative transduction particle; and
- a reporter nucleic acid molecule plasmid comprising a reporter gene and comprising a second bacteriophage gene, wherein said second bacteriophage gene encodes a packaging initiation site sequence and facilitates the packaging a replica of said reporter nucleic acid molecule plasmid into said non-replicative transduction particle, wherein said second bacteriophage gene is capable of expressing a protein that is encoded by said gene, wherein said replica of said reporter nucleic acid molecule plasmid forms a replicon amenable to packaging into said non-replicative transduction particle.

[0013c] In one aspect, there is provided a bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, said bacterial cell comprising:

- a lysogenized bacteriophage genome comprising a first bacteriophage packaging initiation site sequence, wherein said first bacteriophage packaging initiation site sequence comprises a mutation that prevents packaging of a bacteriophage nucleic acid molecule into said non-replicative transduction particle; and
- a reporter nucleic acid molecule plasmid comprising a reporter gene and comprising a second bacteriophage packaging initiation site sequence, wherein said second bacteriophage packaging initiation site sequence lacks said mutation and facilitates the packaging of a replica of said reporter nucleic acid molecule plasmid

into said non-replicative transduction particle, wherein said replica of said reporter nucleic acid molecule plasmid forms a replicon for packaging into said non-replicative transduction particle.

[0013d] In one aspect, there is provided a method for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, comprising:

- providing conditions to said bacterial cell as described herein that induce a lytic phase of said bacteriophage to produce non-replicative transduction particles packaged with said reporter nucleic acid molecule; and
- isolating said non-replicative transduction particle comprising a replica of said reporter nucleic acid molecule plasmid.

[0014] In some embodiments, the reporter nucleic acid molecule is operatively linked to a promoter. In another embodiment, the promoter is selected for contributing to reactivity of a reporter molecule expressed from said reporter nucleic acid molecule in said bacterial cell. In one embodiment, the reporter nucleic acid molecule comprises an origin of replication. In yet another embodiment, the replicon comprises a concatamer amenable to packaging into said non-replicative transduction particle.

CLAIMS

1. A bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, said bacterial cell comprising:
   - a lysogenized bacteriophage genome comprising a first bacteriophage gene comprising a deletion of a packaging initiation site sequence of said first bacteriophage gene that prevents packaging of a bacteriophage nucleic acid molecule into said non-replicative transduction particle; and
   - a reporter nucleic acid molecule plasmid comprising a reporter gene and comprising a second bacteriophage gene comprising a second packaging initiation site sequence that facilitates the packaging a replica of said reporter nucleic acid molecule plasmid into said non-replicative transduction particle, wherein said second bacteriophage gene encodes a protein, wherein said replica of said reporter nucleic acid molecule plasmid forms a replicon for packaging into said non-replicative transduction particle.
2. A bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, said bacterial cell comprising:
   - a lysogenized bacteriophage genome lacking a bacteriophage gene encoding a packaging initiation site sequence, wherein deletion of said bacteriophage gene prevents packaging of a bacteriophage nucleic acid molecule into said non-replicative transduction particle; and
   - a reporter nucleic acid molecule plasmid comprising a reporter gene and comprising a second bacteriophage gene, wherein said second bacteriophage gene encodes a packaging initiation site sequence and facilitates the packaging a replica of said reporter nucleic acid molecule plasmid into said non-replicative transduction particle, wherein said second bacteriophage gene is capable of expressing a protein that is encoded by said gene, wherein said replica of said reporter nucleic acid molecule plasmid forms a replicon amenable to packaging into said non-replicative transduction particle.
3. A bacterial cell packaging system for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, said bacterial cell comprising:
   - a lysogenized bacteriophage genome comprising a first bacteriophage packaging initiation site sequence, wherein said first bacteriophage packaging initiation site sequence comprises a mutation that prevents packaging of a

bacteriophage nucleic acid molecule into said non-replicative transduction particle; and

- a reporter nucleic acid molecule plasmid comprising a reporter gene and comprising a second bacteriophage packaging initiation site sequence, wherein said second bacteriophage packaging initiation site sequence lacks said mutation and facilitates the packaging of a replica of said reporter nucleic acid molecule plasmid into said non-replicative transduction particle, wherein said replica of said reporter nucleic acid molecule plasmid forms a replicon for packaging into said non-replicative transduction particle.

4. The bacterial cell packaging system of any of claims 1 to 3, wherein said reporter nucleic acid molecule is operatively linked to a promoter.

5. The bacterial cell packaging system of any of claims 1 to 3, wherein said reporter nucleic acid molecule plasmid comprises an origin of replication.

6. The bacterial cell packaging system of any of claims 1 to 3, wherein said replicon comprises a concatamer amenable to packaging into said non-replicative transduction particle.

7. The bacterial cell packaging system of claim 1 or claim 2, wherein said second bacteriophage gene comprises the sequence of SEQ ID NO:9.

8. The bacterial cell packaging system of claim 1, wherein said first bacteriophage gene comprises the sequence of SEQ ID NO:8.

9. The bacterial cell packaging system of claim 1 or claim 2, wherein said first and said second bacteriophage genes each comprises a small terminase (terS) gene comprising said packaging initiation site sequence, optionally wherein said terS gene is a *S. aureus* bacteriophage φ11 or φ80α terS gene.

10. The bacterial cell packaging system of claim 3, wherein said first and said second bacteriophage packaging initiation site sequences each comprise a packaging initiation site sequence from a small terminase (terS) gene, or wherein said first and said second bacteriophage packaging initiation site sequences each comprise a pac-site sequence from a small terminase (terS) gene of an *S. aureus* bacteriophage φ11 or φ80α.

11. The bacterial cell packaging system of any of claims 1 to 3, wherein said replicon is derived from a *S. aureus* pT181 plasmid origin of replication.

12. The bacterial cell packaging system of any of claims 1 to 3, wherein said replicon comprises the sequence of SEQ ID NO:5.

13. The bacterial cell packaging system of any of claims 1 to 3, wherein said packaging initiation site sequence of said second bacteriophage gene or said packaging gene comprises a pac-site.

14. The bacterial cell packaging system of claim 1 or 2, wherein said second bacteriophage gene is operatively linked to a promoter, optionally wherein said promoter is an inducible promoter or a constitutive promoter.

15. The bacterial cell packaging system of any of claims 1 to 3, wherein said bacteriophage comprises a *S. aureus* bacteriophage φ80α or a bacteriophage φ11.

16. The bacterial cell packaging system of any of claims 1 to 3, wherein said bacterial cell comprises:

an *E. coli* cell;

a Gram-negative cell; or

a Gram-positive cell.

17. The bacterial cell packaging system of any of claims 1 to 3, wherein said bacterial cell comprises an *S. aureus* cell.

18. The bacterial cell packaging system of any of claims 1 to 3, wherein said reporter gene encodes a detectable and/or a selectable marker,

or optionally wherein said reporter gene is selected from the group consisting of genes encoding enzymes mediating luminescence reactions (luxA, luxB, luxAB, luc, ruc, nluc), genes encoding enzymes mediating colorimetric reactions (lacZ, HRP), genes encoding fluorescent proteins (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent proteins), nucleic acid molecules encoding affinity peptides (His-tag, 3X-FLAG), and genes encoding selectable markers (ampC, tet(M), CAT, erm).

19. A method for packaging a reporter nucleic acid molecule into a non-replicative transduction particle, comprising:

- providing conditions to said bacterial cell of any of the preceding claims that induce a lytic phase of said bacteriophage to produce non-replicative transduction particles packaged with said reporter nucleic acid molecule; and
- isolating said non-replicative transduction particle comprising a replica of said reporter nucleic acid molecule plasmid.

20. The method of claim 19, wherein said non-replicative transduction particle does not contain a replicated bacteriophage genome.

21. A composition comprising said non-replicative transduction particle comprising a replica of said reporter nucleic acid molecule plasmid produced from said method of any of claims 19 to 20.