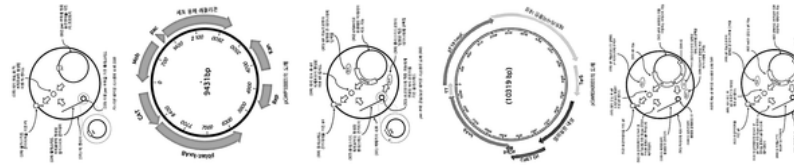
Patents

Non-replicative transduction particles and transduction particle-based reporter systems

Abstract

Methods and systems are provided for packaging reporter nucleic acid molecules in non-replicating transduced particles for use as reporter molecules. Non-replicating transduction particles may be composed of viruses, or viral transduction and replication systems may be used. The reporter nucleic acid molecule includes a reporter gene, such as a reporter molecule or a selectable marker, for detecting a target gene or cell. Methods and systems are provided for the detection of cells and target nucleic acid molecules using non-replicating transduced particles as reporter molecules.

Images (35)



Classifications

C12Q1/6897 Measuring or testing processes involving enzymes, nucleic acids or microorganisms; Compositions therefor; Processes of preparing such compositions involving nucleic acids involving reporter genes operably linked to promoters

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Claims (240)

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1. A bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle, said bacterial cell comprising
A lytic bacteriophage genome lacking a bacteriophage gene encoding a packaging initiation site sequence; And
A reporter nucleic acid molecule comprising a second bacteriophage gene
/ RTI >
Wherein the deletion of the bacteriophage gene blocks packaging of the bacteriophage nucleic acid molecule into the non-replicating transduction particle, wherein the second bacteriophage gene encodes the packaging initiation site sequence and binds the reporter nucleic acid molecule Wherein the second bacteriophage gene is capable of expressing a protein encoded by the gene, and the replicas of the reporter nucleic acid molecule are capable of being packaged with the replicon, which can be packaged with the non- Lt; RTI ID = 0.0 > cell < / RTI > packaging system.

2. 2. The bacterial cell packaging system of claim 1, wherein said reporter nucleic acid molecule is operably linked to a promoter.

3. 3. The bacterial cell packaging system of claim 2, wherein the promoter is selected to contribute to the reactivity of the reporter molecule expressed from the reporter nucleic acid molecule in the bacterial cell.

4. The bacterial cell packaging system of claim 1, wherein the reporter nucleic acid molecule comprises a replication origin.

5. 2. The bacterial cell packaging system of claim 1, wherein the replicon comprises a conchamer that can be packaged in the non-replicating transduced particles.

6. The bacterial cell packaging system according to claim 1, wherein the first and second bacteriophage genes each comprise the pacA gene of Enterobacteriaceae bacteriophage P1 and the packaging initiation site sequence.

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

8. The bacterial cell packaging system according to claim 1, wherein the replicon is a bacteriophage P1 cell lysate replicon in the Enterobacteriaceae.

9. The bacterial cell packaging system of claim 1, wherein said replicon comprises in-frame deletion of a C1 inhibitor-regulated P53 promoter, a promoter P53 antisense, a repL gene, and a kilA gene.

10. 2. The bacterial cell packaging system of claim 1, wherein said replicon comprises the sequence of SEQ ID NO: 3.

11. 2. The bacterial cell packaging system of claim 1, wherein the first and second bacteriophage genes each comprise a small teratinase (terS) gene comprising the packaging initiation site sequence.

12. 2. The method according to claim 1, wherein the terS gene is selected from the group consisting of S. A bacterial cell packaging system characterized in that it is a S. aureus bacteriophage? 11 or? 80? TerS gene.

13. The method according to claim 1, A bacterial cell packaging system characterized in that it originates from the Aureus pT181 plasmid replication origin.

14. The bacterial cell packaging system of claim 1, wherein the replicon comprises the sequence of SEQ ID NO: 5.

15. 2. The bacterial cell packaging system of claim 1, wherein the packaging initiation site sequence of the second bacteriophage gene comprises a pac-site.

16. The bacterial cell packaging system of claim 1, wherein the pac-region of the second bacteriophage gene comprises the sequence of SEQ ID NO: 7.

17. 2. The bacterial cell packaging system of claim 1, wherein the packaging initiation site sequence of the second bacteriophage gene comprises a cos-site.

18. 2. The bacterial cell packaging system of claim 1, wherein the packaging initiation site sequence of the second bacteriophage gene comprises a concatamer junction.

19. The bacterial cell packaging system of claim 1, further comprising a plasmid comprising the reporter nucleic acid molecule.

20. The bacterial cell packaging system of claim 1, wherein the second bacteriophage gene is operably linked to a promoter.

21. 2. The bacterial cell packaging system according to claim 1, wherein the promoter is an inducible promoter or a constitutive promoter.

22. 2. The bacterial cell packaging system of claim 1, wherein the bacteriophage comprises bacteriophage P1 in the Enterobacteriaceae.

23. 23. The method of claim 22, wherein the bacteriophage comprises: Aberrant bacteriophage? 80? Or bacteriophage? 11.

24. 2. The method of claim 1, wherein the bacterial cell is selected from the group consisting of: Lt; RTI ID = 0.0 > E. coli < / RTI > cells.

25. 2. The method of claim 1, wherein the bacterial cell is selected from the group consisting of S. Aureus cells. ≪ / RTI >

26. The bacterial cell packaging system of claim 1, wherein the bacterial cell comprises gram negative cells.

27. The bacterial cell packaging system of claim 1, wherein the bacterial cell comprises Gram-positive cells.

28. The bacterial cell packaging system according to claim 1, wherein the reporter nucleic acid molecule comprises a reporter gene.

29. 29. The bacterial cell packaging system of claim 28, wherein the reporter gene encodes a detectable marker and / or a selectable marker.

30. 29. The method according to claim 28, wherein the reporter gene is selected from the group consisting of enzymes (luxA, luxB, luxAB, luc, ruc, nluc) mediating the luminescence reaction, enzyme (lacZ, HRP) (RFP, CFP, BFP, mCherry, near infrared fluorescent protein), affinity peptide (His-tag, 3X-FLAG) and selectable markers ampC, tet (M), CAT, erm A bacterial cell packaging system.

31. The bacterial cell packaging system of claim 1, wherein the reporter nucleic acid molecule comprises an aptamer.

32. 29. The bacterial cell packaging system of claim 28, wherein the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to a second sequence of the reporter nucleic acid molecule.

33. 33. The bacterial cell packaging system of claim 32, wherein the nucleic acid transcript sequence is complementary to a cell transcript.

34. 33. The bacterial cell packaging system of claim 32, wherein the nucleic acid transcript sequence comprises a cis-suppression sequence.

35. 35. A method according to any one of claims 1 to 34, wherein said replicas of said reporter nucleic acid molecules comprise a nucleic acid transcript sequence complementary to a second sequence of said replica of said reporter nucleic acid molecule. system.

36. 36. The bacterial cell packaging system of claim 35, wherein the nucleic acid transcript sequence is complementary to a cell transcript.

37. 37. The bacterial cell packaging system of claim 35, wherein the nucleic acid transcript sequence comprises a cis-inhibition sequence.

38. A method for packaging a reporter nucleic acid molecule in a non-replicating transduced particle,
Providing conditions for said bacterial cell of any one of claims 1 to 31 inducing a cell lysate of said bacteriophage to produce said non-replicating transfected particles packaged with said reporter nucleic acid molecule ; And
And separating the non-replicate transduced particles comprising the reporter nucleic acid molecule.

39. 39. The method of claim 38, wherein said non-replicating transduced particles do not contain a replicated bacteriophage genome.

40. 39. The method of claim 38, wherein the induction of the cytolysin triggers the removal of the genomic DNA molecule from the genome of the bacterial cell.

41. 40. A composition comprising the replica of the reporter nucleic acid molecule produced from the method of any of claims 38-40, comprising the non-replicating transduced particle.

42. A bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle, said bacterial cell comprising
A baryonized bacteriophage genome comprising a first bacteriophage packaging initiation site sequence; And
A reporter nucleic acid molecule comprising a second bacteriophage packaging initiation site sequence
/ RTI >
Wherein the first bacteriophage packaging initiation site sequence comprises a mutation that blocks packaging of the bacteriophage nucleic acid molecule into the non-replicating transfected particle, wherein the second bacteriophage packaging initiation site sequence lacks the mutation and the non- Promoting packaging of the replicas of the reporter nucleic acid molecules into the introduced particles, wherein the replicas of the reporter nucleic acid molecules form replicons for packaging with the non-replicable transduced particles.

43. 43. The bacterial cell packaging system of claim 42, wherein said reporter nucleic acid molecule is operably linked to a promoter.

44. 44. The bacterial cell packaging system of claim 43, wherein the promoter is selected to contribute to the reactivity of the reporter molecule expressed from the reporter nucleic acid molecule in the bacterial cell.

45. 43. The bacterial cell packaging system of claim 42, wherein the reporter nucleic acid molecule comprises a source of replication.

46. 43. A bacterial cell packaging system according to claim 42, wherein said replicon comprises a concretamer capable of being packaged in said non-replicating transduced particles.

47. 43. The bacterial cell packaging system of claim 42, wherein the first and second bacteriophage packaging initiation site sequences each comprise a packaging initiation site sequence from a small terminally derived gene.

48. 43. The bacterial cell packaging system of claim 42, wherein the first and second bacteriophage packaging initiation site sequences each comprise a pac-site sequence from the pacA gene of bacteriophage P1 in the Enterobacteriaceae.

49. 43. The bacterial cell packaging system of claim 42, wherein the first bacteriophage packaging initiation site sequence comprises SEQ ID NO: 2.

50. 43. The bacterial cell packaging system of claim 42, wherein the second bacteriophage packaging initiation site sequence comprises SEQ ID NO: 1.

51. 43. The bacterial cell packaging system of claim 42, wherein the replicon comprises a bacteriophage P1 cell lysate replicon in the Enterobacteriaceae.

52. 52. The bacterial cell packaging system of claim 51, wherein the replicon comprises an in-frame deletion of a C1 inhibitor-regulated P53 promoter, a promoter P53 antisense, a repL gene, and a kilA gene.

53. 43. The bacterial cell packaging system of claim 42, wherein the replicon comprises the sequence of SEQ ID NO: 3.

54. 43. The method of claim 42, wherein the first and second bacteriophage packaging initiation site sequences are selected from the group consisting of: And a pac-site sequence from a small terrnase (terS) gene of Aureus bacteriophage? 11 or? 80?, Respectively.

55. 43. The method of claim 42, wherein the replicon is selected from the group consisting of S. A bacterial cell packaging system characterized in that it originates from the Aureus pT181 plasmid replication origin.

56. 43. The bacterial cell packaging system of claim 42, wherein the replicon comprises the sequence of SEQ ID NO: 5.

57. 43. The bacterial cell packaging system of claim 42, wherein the first bacteriophage packaging initiation site sequence comprises a sequence of SEQ ID NO: 2.

58. 43. The bacterial cell packaging system of claim 42, wherein the second bacteriophage packaging initiation site sequence comprises the sequence of SEQ ID NO: 1.

59. 43. The bacterial cell packaging system of claim 42, wherein the packaging initiation site sequence comprises a pac-site.

60. 43. The bacterial cell packaging system of claim 42, wherein the packaging initiation site sequence comprises a cos-site.

61. 43. The bacterial cell packaging system of claim 42, wherein the packaging initiation site sequence comprises a concatamer junction.

62. 43. The system of claim 42, wherein the mutation in the first bacteriophage packaging initiation site sequence comprises a silent mutation.

63. 43. The bacterial cell packaging system of claim 42, wherein said mutation in said first bacteriophage packaging initiation site sequence blocks cleavage of said packaging initiation sequence.

64. 43. The bacterial cell packaging system of claim 42, further comprising a plasmid comprising the reporter nucleic acid molecule.

65. 43. The bacterial cell packaging system of claim 42, wherein the bacteriophage comprises bacteriophage P1 in the Enterobacteriaceae.

66. 43. The method of claim 42, wherein the bacteriophage is selected from the group consisting of S. A bacteriophage < RTI ID = 0.0 > 11 < / RTI > or < RTI ID = 0.0 > 80a. ≪ / RTI >

67. 43. The method of claim 42, wherein the bacterial cell is selected from the group consisting of: Lt; RTI ID = 0.0 > cell < / RTI > cells.

68. 43. The method of claim 42, wherein the bacterial cell is selected from the group consisting of S. Aureus cells. ≪ / RTI >

69. 43. The bacterial cell packaging system of claim 42, wherein said bacterial cells comprise gram negative bacterial cells.

70. 43. The bacterial cell packaging system of claim 42, wherein the bacterial cell comprises Gram-positive bacterial cells.

71. 43. The bacterial cell packaging system of claim 42, wherein the reporter nucleic acid molecule comprises a reporter gene.

72. 72. The bacterial cell packaging system of claim 71, wherein the reporter gene encodes a detectable marker and / or a selectable marker.

73. 71. The method of claim 71, wherein the reporter gene is selected from the group consisting of genes (luxA, luxB, luxAB, luc, ruc, nluc) encoding an enzyme mediating a luminescent reaction, genes (lacZ, HRP) (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent protein) encoding a fluorescent protein, a nucleic acid molecule (His-tag, 3X-FLAG) encoding an affinity peptide and a gene encoding a selectable marker (ampC, tet (M), CAT, erm).

74. 72. The bacterial cell packaging system of claim 71, wherein the reporter nucleic acid molecule comprises an aptamer.

75. 43. The bacterial cell packaging system of claim 42, wherein the replicon is packaged in the non-replicating transduced particles by a bacteriophage packaging mechanism.

76. 43. The bacterial cell packaging system of claim 42, wherein the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to a second sequence of the reporter nucleic acid molecule.

77. 77. The bacterial cell packaging system of claim 76, wherein the nucleic acid transcript sequence is complementary to a cell transcript.

78. 43. The bacterial cell packaging system of claim 42, wherein the nucleic acid transcript sequence comprises a cis-suppression sequence.

79. 79. The method of any one of claims 42-78, wherein said replicas of said reporter nucleic acid molecule comprise a nucleic acid transcript sequence complementary to a second sequence of said replica of said reporter nucleic acid molecule. system.

80. 79. The bacterial cell packaging system of claim 79, wherein the nucleic acid transcript sequence is complementary to a cell transcript.

81. 80. The bacterial cell packaging system of claim 79, wherein the nucleic acid transcript sequence comprises a cis-inhibition sequence.

82. A method for packaging a reporter nucleic acid molecule in a non-replicating transduced particle,
Providing the bacterial cells of any one of claims 42 to 75 under conditions that induce a cytolysin of said bacteriophage to produce said non-cloned transgenic particles packaged with said reporter nucleic acid molecule; And
And separating the non-replicate transduced particles comprising the reporter nucleic acid molecule.

83. 83. The method of claim 82, wherein the non-replicating transfected particles do not contain a replicated bacteriophage genome.

84. 83. The method of claim 82, wherein the induction of the cytolysin triggers the removal of the genomic DNA molecule from the genome of the bacterial cell.

85. 84. A composition comprising the replica of the reporter nucleic acid molecule produced from the method of any one of claims 82-84.

86. A bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle, said bacterial cell comprising
A charged bacteriophage genome comprising a first bacteriophage gene comprising deletion of a packaging initiation site sequence of a first bacteriophage gene that blocks packaging of the bacteriophage nucleic acid molecule into the non-replicating transduced particle; And
A reporter nucleic acid molecule comprising a second bacteriophage gene comprising a second packaging initiation site sequence that promotes packaging of the replica of said reporter nucleic acid molecule into said non-
/ RTI >
Wherein said second bacteriophage gene encodes a protein and said replicas of said reporter nucleic acid molecules form replicons for packaging into said non-replicating transduced particles.

87. 83. The bacterial cell packaging system of claim 86, wherein the reporter nucleic acid molecule is operably linked to a promoter.

88. 90. The bacterial cell packaging system of claim 87, wherein the promoter is selected to contribute to the reactivity of the reporter molecule expressed from the reporter nucleic acid molecule in the bacterial cell.

89. 87. The bacterial cell packaging system of claim 86, wherein said reporter nucleic acid molecule comprises a source of replication.

90. 87. The bacterial cell packaging system of claim 86, wherein said replicon comprises a concretamer capable of being packaged in said non-replicating transduced particles.

91. 74. The bacterial cell packaging system of claim 74, wherein each of the first and second bacteriophage genes comprises the pacA gene of bacteriophage P1 in the Enterobacteriaceae and comprises the packaging initiation site sequence.

92. 87. The bacterial cell packaging system of claim 87, wherein the first bacteriophage gene comprises the sequence of SEQ ID NO: 6.

93. 87. The bacterial cell packaging system of claim 87, wherein the second bacteriophage gene comprises the sequence of SEQ ID NO: 7.

94. 87. The bacterial cell packaging system of claim 86, wherein the replicon comprises a bacteriophage P1 cell lysate replicon in the Enterobacteriaceae.

95. 96. The bacterial cell packaging system of claim 94, wherein the replicon comprises an in-frame deletion of a C1 inhibitor-regulated P53 promoter, a promoter P53 antisense, a repL gene, and a kilA gene.

96. 83. The bacterial cell packaging system of claim 86, wherein said replicon comprises the sequence of SEQ ID NO: 3.

97. 87. The bacterial cell packaging system of claim 86, wherein the first and second bacteriophage genes each comprise a small titer (terS) gene comprising the packaging initiation site sequence.

98. 98. The method of claim 97, wherein the terS gene is selected from the group consisting of S. A bacterial cell packaging system characterized in that it is a S. aureus bacteriophage? 11 or? 80? TerS gene.

99. 87. The bacterial cell packaging system of claim 86, wherein the first bacteriophage gene comprises the sequence of SEQ ID NO: 8.

100. 87. The bacterial cell packaging system of claim 86, wherein the second bacteriophage gene comprises the sequence of SEQ ID NO: 9.

101. 87. The method of claim 86, wherein the replicon is selected from the group consisting of S. A bacterial cell packaging system characterized in that it originates from the Aureus pT181 plasmid replication origin.

102. 87. The bacterial cell packaging system of claim 86, wherein the replicon comprises the sequence of SEQ ID NO: 5.

103. 87. The bacterial cell packaging system of claim 86, wherein the packaging initiation site sequence of the second bacteriophage gene comprises a pac-site.

104. 87. The bacterial cell packaging system of claim 86, wherein the packaging initiation site sequence of the second bacteriophage gene comprises a cos-site.

105. 87. The bacterial cell packaging system of claim 86, wherein the packaging initiation site sequence of the second bacteriophage gene comprises a concatamer junction.

106. 83. The bacterial cell packaging system of claim 86, further comprising a plasmid comprising the reporter nucleic acid molecule.

107. 87. The bacterial cell packaging system of claim 86, wherein said second bacteriophage gene is operably linked to a promoter.

108. 108. The bacterial cell packaging system of claim 107, wherein the promoter is an inducible promoter or a constitutive promoter.

109. 87. The bacterial cell packaging system of claim 86, wherein the bacteriophage comprises bacteriophage P1 in the Enterobacteriaceae.

110. 87. The method of claim 86, wherein the bacteriophage is selected from the group consisting of S. Aberrant bacteriophage? 80? Or bacteriophage? 11.

111. 87. The method of claim 86, wherein the bacterial cell is a. Lt; RTI ID = 0.0 > E. coli < / RTI > cells.

112. 87. The method of claim 86, wherein the bacterial cell is selected from the group consisting of S. Aureus cells. ≪ / RTI >

113. 87. The bacterial cell packaging system of claim 86, wherein said bacterial cells comprise gram negative cells.

114. 87. The bacterial cell packaging system of claim 86, wherein said bacterial cells comprise Gram-positive cells.

115. 87. The bacterial cell packaging system of claim 86, wherein the reporter nucleic acid molecule comprises a reporter gene.

116. 116. The bacterial cell packaging system of claim 115, wherein the reporter gene encodes a detectable marker and / or a selectable marker.

117. 65. The method of claim 65, wherein the reporter gene is selected from the group consisting of genes (luxA, luxB, luxAB, luc, ruc, nluc) encoding an enzyme mediating a luminescent reaction, genes (lacZ, HRP) (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent protein) encoding a fluorescent protein, a nucleic acid molecule (His-tag, 3X-FLAG) encoding an affinity peptide and a gene encoding a selectable marker (ampC, tet (M), CAT, erm).

118. 87. The bacterial cell packaging system of claim 86, wherein the reporter nucleic acid molecule comprises an aptamer.

119. 87. The bacterial cell packaging system of claim 86, wherein the replicon is packaged in the non-replicate transduced particles by a bacteriophage packaging mechanism.

120. 87. The bacterial cell packaging system of claim 86, wherein the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to a second sequence of the reporter nucleic acid molecule.

121. 121. The bacterial cell packaging system of claim 120, wherein the nucleic acid transcript sequence is complementary to a cell transcript.

122. 87. The bacterial cell packaging system of claim 86, wherein the nucleic acid transcript sequence comprises a cis-inhibition sequence.

123. 124. The method according to any one of claims 86 to 122, wherein the replicase of the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to a second sequence of the replicon of the reporter nucleic acid molecule. system.

124. 123. The bacterial cell packaging system of claim 123, wherein the nucleic acid transcript sequence is complementary to a cell transcript.

125. 123. The bacterial cell packaging system of claim 123, wherein the nucleic acid transcript sequence comprises a cis-suppression sequence.

126. A method for packaging a reporter nucleic acid molecule in a non-replicating transduced particle,
Providing the bacterial cell of any one of claims 86 to 125 under conditions that induce a cytolysin of the bacteriophage to produce the non-cloned transfected particle packaged with the reporter nucleic acid molecule; And
And separating the non-replicate transduced particles comprising the reporter nucleic acid molecule.

127. 126. The method of claim 126, wherein said non-replicating transduced particles do not contain a replicated bacteriophage genome.

128. 126. The method of claim 126, wherein said induction of said cytolysin triggers the elimination of said genomic DNA molecule from said genome of said bacterial cell.

129. A composition comprising the replica of the reporter nucleic acid molecule produced from the method of any one of claims 126 to 128.

130. A bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle, said bacterial cell comprising
A lytic bacteriophage genome comprising a gene that lacks a packaging gene and encodes a protein that forms the non-replicating transduced particle; And
Genomic DNA nucleic acid molecules containing reporter nucleic acid molecules and packaging genes
Wherein the bacterial cell packaging system comprises:

131. 130. The bacterial cell packaging system of claim 130, wherein the packaging gene comprises a small terminalase (terS) gene.

132. 132. The method according to claim 131, wherein the terS gene is selected from the group consisting of S. Atheros bacteriophage? 80? TerS gene or bacteriophage? 11 terS gene.

133. 131. The bacterial cell packaging system of claim 131, wherein the terS gene comprises the sequence of SEQ ID NO: 9.

134. 130. The bacterial cell packaging system of claim 130, wherein the genomic DNA nucleic acid molecule comprises a SaPIbov2 genomic region nucleic acid molecule.

135. 130. The bacterial cell packaging system of claim 130, wherein the genomic DNA molecule is selected from the group consisting of SaPI, SaPI1, SaPI2, SaPIbov1 and SaPibov2 genomic DNA.

136. 98. The bacterial cell packaging system of claim 98, wherein the reporter nucleic acid molecule is operably linked to a promoter.

137. 98. The bacterial cell packaging system of claim 98, wherein the reporter nucleic acid molecule comprises a source of replication.

138. 130. The method of claim 130, wherein the bacteriophage is selected from the group consisting of S. Aberrant bacteriophage? 80? Or bacteriophage? 11.

139. 130. The method of claim 130, wherein the bacterial cell is selected from the group consisting of S. Aureus cells. ≪ / RTI >

140. 130. The method of claim 130, wherein the genomic region nucleic acid molecule comprises an integrase gene, wherein the integrase gene comprises an integrase protein for the ablation and integration of the genomic region nucleic acid molecule outside and inside the bacterial genome of the bacterial cell Lt; RTI ID = 0.0 > cell < / RTI > packaging system.

141. 143. The bacterial cell packaging system of claim 140, wherein the integrase gene comprises the sequence of SEQ ID NO: 10.

142. 130. The bacterial cell packaging system of claim 130, wherein the genomic nucleotide molecule is integrated into the bacterial genome of the bacterial cell.

143. 130. The bacterial cell packaging system of claim 130, wherein the genomic DNA nucleic acid molecule is capable of replicating to form replicons, and replicons can be packaged by the bacteriophage packaging apparatus in the bacterial cells.

144. 143. The bacterial cell packaging system of claim 143, wherein the nucleic acid molecule forms a concatamer.

145. 143. The bacterial cell packaging system of claim 143, wherein the replicated genomic nucleotide molecule can be packaged in the non-cloned transfected particle.

146. 132. The bacterial cell packaging system of claim 130, wherein the packaging gene comprises a pac-site sequence.

147. 130. The bacterial cell packaging system of claim 130, wherein the packaging gene comprises a cos-site sequence.

148. 143. The bacterial cell packaging system of claim 130, wherein the packaging gene comprises a concatamer junction.

149. 130. The bacterial cell packaging system of claim 130, wherein the reporter nucleic acid molecule comprises a reporter gene.

150. 155. The bacterial cell packaging system of claim 149, wherein the reporter gene encodes a selectable marker and / or a selectable marker.

151. The reporter gene according to claim 149, wherein the reporter gene is selected from the group consisting of enzymes (luxA, luxB, luxAB, luc, ruc, nluc) mediating the luminescence reaction, enzyme (lacZ, HRP) (RFP, CFP, BFP, mCherry, near infrared fluorescent protein), affinity peptide (His-tag, 3X-FLAG) and selectable markers ampC, tet (M), CAT, erm A bacterial cell packaging system.

152. 132. The bacterial cell packaging system of claim 130, wherein the reporter nucleic acid molecule comprises an aptamer.

153. 130. The bacterial cell packaging system of claim 130, wherein the genomic DNA nucleic acid molecule lacks an integrase gene.

154. 154. The method of claim 153, further comprising a bacterial gene comprising an integrase gene operably linked to a promoter, wherein said integrase gene ablates and integrates said genomic DNA molecule outside and inside the bacterial genome of said bacterial cell And encodes the integrase protein to be used for

the cell culture.

155. 132. The bacterial cell packaging system of claim 130, wherein the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to a second sequence of the reporter nucleic acid molecule.

156. The bacterial cell packaging system of claim 155, wherein the nucleic acid transcript sequence is complementary to the cell transcript.

157. 155. The bacterial cell packaging system of claim 155, wherein the nucleic acid transcript sequence comprises a cis-inhibition sequence.

158. 155. The method according to any one of claims 130 to 157, wherein said replica of said reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to a second sequence of said replica of said reporter nucleic acid molecule. system.

159. 153. The bacterial cell packaging system of claim 158, wherein the nucleic acid transcript sequence is complementary to a cell transcript.

160. 153. The bacterial cell packaging system of claim 158, wherein the nucleic acid transcript sequence comprises a cis-inhibition sequence.

161. A method for packaging a reporter nucleic acid molecule in a non-replicating transduced particle,
Providing the bacterial cells of any one of claims 130 to 160 under conditions that induce the cytolysin of the bacteriophage to produce the non-cloned transfected particle packaged with the reporter nucleic acid molecule; And
And separating the non-replicate transduced particles comprising the reporter nucleic acid molecule.

162. 169. The method of claim 161, wherein said non-replicating transfected particles do not contain a replicated bacteriophage genome.

163. 160. The method of claim 161, wherein the induction of the cytolysin triggers the elimination of the genomic DNA molecule from the genome of the bacterial cell.

164. A composition comprising the replica of the reporter nucleic acid molecule produced from the method of any one of claims 161 to 163.

165. A method for detecting the presence or absence of bacterial cells in a sample,
A non-cloned transfected particle comprising a reporter gene encoding a reporter molecule and lacking a bacteriophage genome is introduced into the non-cloned transfected particle so that the non-cloned transgene particle transduces the bacterial cell and the reporter gene is expressed in the bacterial cell Introducing into a sample under the conditions of:
Providing conditions for activation of the reporter molecule; And
Detecting the presence or absence of a reporter signal delivered from said expressed reporter molecule, wherein the presence of said reporter signal accurately indicates the presence of said bacterial cell
≪ / RTI >

166. 169. The method of claim 165, wherein the method exhibits at least 80% detection specificity to a standard.

167. 174. The method of claim 165, wherein the method exhibits at least 90% detection specificity to the standard.

168. 169. The method of claim 165, wherein the method exhibits at least 95% detection specificity to the standard.

169. 169. The method of claim 165, wherein the method exhibits at least 80% detection sensitivity to a standard.

170. 169. The method of claim 165, wherein the method exhibits at least 85% detection sensitivity to a standard.

171. 169. The method of claim 165, wherein the method exhibits at least 90% detection sensitivity to a standard.

172. 169. The method of claim 165, wherein the method exhibits at least 95% detection sensitivity to a standard.

173. 169. The method of claim 165, wherein the method achieves at least 95% detection specificity for the standard and at least 90% detection sensitivity for the standard.

174. 173. The method of any one of claims 166 to 173, wherein the standard is a gold standard.

175. 165. The method of claim 165, wherein the bacterial cell is a methicillin resistant Staphylococcus aureus (MRSA) cell.

176. 165. The method of claim 165, wherein said bacterial cell is a methicillin sensitive staphylococcus aureus (MSSA) cell.

177. 174. The method of claim 165, wherein the reporter gene encodes a detectable marker or a selectable marker.

178. The reporter gene according to claim 165, wherein the reporter gene is a gene (luxA, luxB, luxAB, luc, ruc, nluc) encoding an enzyme mediating a luminescent reaction, a gene (lacZ, HRP) (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry, near-infrared fluorescent protein) encoding a fluorescent protein, a nucleic acid molecule (His-tag, 3X-FLAG) encoding an affinity peptide and a gene encoding a selectable marker (ampC, tet (M), CAT, erm).

179. 174. The method of claim 165, wherein the reporter gene is operably linked to a constitutive promoter.

180. 178. The method of claim 165, wherein the reporter signal is detectable from a sample at a detection limit (LoD) of less than 1,000 colony forming units (CFU).

181. 169. The method of claim 165, wherein the reporter signal is detectable from a sample at a detection limit (LoD) of less than 100 colony forming units (CFU).

182. 169. The method of claim 165, wherein the reporter signal is detectable from a sample at a detection limit (LoD) of less than 10 colony forming units (CFU).

183. 182. The method of claim 182, wherein the reporter signal is detectable from a sample at a LoD of less than 5 CFUs.

184. 191. The method of claim 183, wherein the reporter signal is detectable from a sample at a LoD of CFU of 3 or less.

185. 165. The method of claim 165, further comprising the step of providing an antibiotic to the sample at a predetermined concentration and detecting the presence or absence of the reporter signal to determine if the bacterial cell is resistant or susceptible to the antibiotic ≪ / RTI >

186. 165. The method of claim 165, further comprising providing the sample with a predetermined concentration of antibiotic, and detecting the amount of the reporter signal to determine a minimal inhibitory concentration of the bacterial cell for the antibiotic . ≪ / RTI >

187. As a composition,
A nucleic acid construct encoding a nucleic acid reporter transcript capable of forming at least two stereostructures, wherein the nucleic acid construct comprises a first stereostructure and a second stereostructure, wherein the first stereostructure comprises a first subsequence and a second Wherein the first stereostructure and the second stereostructure interfere with reporter expression comprising an intramolecular double-stranded region comprising a subsequence, the second stereostructure

lacks the intramolecular double-stranded region and allows reporter gene expression, Structure is mediated by competitive binding of the cell transcript to said first and / or said second subsequence.

188. 198. The composition of claim 187, further comprising a non-replicating transduction particle comprising the nucleic acid construct.

189. 198. The composition of claim 187, wherein said competitive binding of said first and / or said second subsequence and said cell transcript causes said second steric structure of said nucleic acid reporter construct.

190. 198. The composition of claim 187, wherein the first subsequence or the second subsequence comprises a cis-suppression sequence.

191. 203. The composition of claim 190, wherein the cis-suppression sequence comprises a sequence complementary or substantially complementary to a portion of the cell transcript.

192. 198. The composition of claim 187, wherein said first subsequence or said second subsequence comprises a reporter gene sequence.

193. 192. The composition of claim 192, wherein the reporter gene sequence comprises a ribosome binding site.

194. 192. The composition of claim 192, wherein the reporter gene sequence encodes a detectable molecule.

195. 198. The composition of claim 194, wherein the detectable marker comprises an enzyme capable of mediating fluorescent molecules or luminescent or colorimetric reactions.

196. 192. The composition of claim 192, wherein the reporter gene sequence encodes a selectable marker.

197. 198. The composition of claim 196, wherein the selectable marker comprises an antibiotic resistance gene.

198. 198. The composition of claim 187, wherein the first subsequence and the second subsequence are cis-positioned on the nucleic acid construct to form the intramolecular double stranded region.

199. 198. The composition of claim 187, wherein the first subsequence and the second subsequence are complementary or substantially complementary to each other to form the intramolecular double stranded region.

200. 198. The method of claim 187, wherein the first subsequence or the second subsequence of the first steric structure comprises a transcription enhancer sequence, wherein the transcription enhancer sequence is upstream from the coding region of the reporter gene sequence / RTI >

201. 198. The composition of claim 187, wherein said first stereostructure of said nucleic acid reporter transcript is capable of binding to a cleavage enzyme.

202. 198. The composition of claim 187, wherein the first steric structure of the nucleic acid reporter transcript is a target for degradation by a cellular enzyme.

203. 198. The composition of claim 187, wherein the first steric structure comprises a non-conjugated intramolecular region.

204. 203. The composition of claim 203, wherein the non-binding intramolecular region is located 3 ' of the first subsequence and 5 ' of the second subsequence.

205. 203. The composition of claim 203, wherein the non-binding intramolecular domain comprises the sequence YUNR, wherein Y is pyrimidine, U is uracil, N is any nucleotide, and R is purine.

206. 192. The composition of claim 187, wherein said first subsequence or said second subsequence comprises a modified sequence of said cell transcript.

207. 203. The composition of claim 206, wherein said modified sequence comprises nucleotide substitutions.

208. 203. The composition of claim 206, wherein said modified sequence comprises sequence insertion, deletion or inversion of said cell transcript.

209. As a composition,
A nucleic acid construct capable of encoding a nucleic acid reporter transcript comprising a gene reporter sequence and capable of forming at least two three-dimensional structures of said nucleic acid reporter transcript, said nucleic acid construct having a first unstable stereostructure and a second unstable stereostructure Wherein the first unstable steric structure blocks translation of the reporter gene sequence in the nucleic acid reporter transcript and the second stable steric structure results from the binding of the first labile steric structure to the cell transcript, Wherein the stable secondary sequence structure permits translation of said reporter gene sequence of said nucleic acid reporter transcript.

210. 34. The composition of claim 209, further comprising a non-replicating transduction particle comprising the nucleic acid construct.

211. 209. The composition of claim 209, wherein the cell transcript is linked to the 3 ' UTR sequence of the nucleic acid reporter transcript.

212. 31. The composition of claim 209, wherein the second stable secondary structure is formed by cleavage of a portion of the sequence of the first unstable secondary structure.

213. 209. The composition of claim 209, wherein the reporter gene sequence encodes a detectable molecule.

214. 214. The composition of claim 213, wherein the detectable marker comprises an enzyme capable of mediating fluorescent molecules or luminescent or colorimetric reactions.

215. 31. The composition of claim 209, wherein the reporter gene sequence encodes a selectable marker.

216. 22. The composition of claim 215, wherein the selectable marker comprises an antibiotic resistance gene.

217. As a composition,
A nucleic acid construct capable of encoding a nucleic acid reporter transcript comprising a reporter gene sequence and capable of forming at least two three-dimensional structures of said nucleic acid reporter transcript, said nucleic acid construct comprising a first stereostructure and a second stereostructure , Wherein the first steric structure blocks further transcription of the nucleic acid construct, and the second steric structure is formed upon binding of the first steric structure and the cell transcript, and the second steric structure allows transcription of the nucleic acid construct Composition.

218. 217. The composition of claim 217, further comprising a non-replicating transduction particle comprising the nucleic acid construct.

219. 218. The composition of claim 217, wherein the nucleic acid reporter transcript comprises a cis-inhibition sequence.

220. 218. The composition of claim 218, wherein the nucleic acid reporter transcript comprises a reporter gene sequence.

221. 223. The composition of claim 220, wherein the first steric structure is formed from a combination of the cis-suppression sequence and the reporter gene sequence.

222. 221. The composition of claim 221, wherein the first steric structure is a substrate of a cleavage enzyme.

223. 218. The composition of claim 218, wherein the first steric structure of the nucleic acid reporter transcript comprises a sequence that forms a transcription termination structure.

224. 233. The composition of claim 223, wherein the binding of the sequence with the cell transcript forming the transcription termination structure results in cleavage of a portion of the nucleic acid reporter transcript and formation of the second steric structure.

225. A vector comprising a regulatory sequence operably linked to a nucleic acid sequence encoding said nucleic acid reporter transcript according to any one of claims 187 to 224.

226. A method for detecting a target transcript in a cell,
Introducing the nucleic acid reporter construct of any one of claims 187 to 224 into the cell; And
Detecting the presence or absence of an output signal from the cell, wherein the presence of the output signal indicates the presence of a target transcript in the cell
≪ / RTI >

227. 226. The method of claim 226, further comprising detecting the presence of bacterial cells based on detecting the presence of the target transcript.

228. A method for detecting the presence of bacterial cells in a sample,
Introducing the nucleic acid reporter construct of any one of claims 187 to 224 into the sample; And
Detecting the presence or absence of an output signal from the sample, wherein the presence of the output signal indicates the presence of a bacterial cell in the sample
≪ / RTI >

229. A compartment for holding a sample comprising said nucleic acid reporter construct and cells according to any one of claims 187 to 224; And
A description for detecting the presence or absence of an output signal from the sample
Wherein the presence of an output signal indicates the presence of a target transcript in said cell.

230. As a composition,
Wherein the nucleic acid reporter construct comprises a first promoter operably linked to a reporter gene and wherein the first promoter is derived from an endogenous inducer protein in a bacterial cell, ≪ / RTI >

231. A method for detecting the presence of bacterial cells in a sample,
Contacting the sample with a non-replicative transduced particle comprising a nucleic acid reporter construct comprising a first promoter operably linked to a reporter gene, wherein the first promoter is expressed by an endogenous protein endogenous to the bacterial cell A step that can be induced; And
Detecting the presence or absence of an output signal from the reporter gene, wherein the presence of the output signal indicates the presence of bacteria in the sample
≪ / RTI >

232. 233. The method of claim 231, wherein said first promoter is identical to an inducible promoter operably linked to a target nucleic acid molecule in said bacterial cell.

233. A non-replicating transducing particle comprising a nucleic acid reporter construct comprising a reporter gene encoding a reporter molecule, the non-replicating transducing particle capable of penetrating into a bacterial cell; And
When the blockade is released as a blocked substrate exogenous to the bacterial cell, a substrate capable of reacting with the reporter molecule in the cell
≪ / RTI >

234. A method for detecting the presence of bacterial cells in a sample,
A non-cloned transfected particle comprising a nucleic acid reporter construct comprising a reporter gene encoding a reporter molecule, and a non-cloned transfected particle comprising a substrate capable of binding to said reporter molecule in said bacterial cell when said cell is released as an exogenous, And contacting the sample; And
Detecting the presence or absence of an output signal from the reporter molecule, wherein the presence of the output signal indicates the presence of the bacteria in the sample
≪ / RTI >

235. 233. The method of claim 234, wherein the target enzyme binds to the blocked substrate in the cell to produce an unlabeled substrate.

236. 233. The method of claim 235, wherein the unlabeled substrate reacts with the reporter molecule to produce the output signal.

237. Non-replicating transduction particles comprising a nucleic acid reporter construct encoding a switchable molecule capable of binding to a target molecule in a bacterial cell to form a complex; And
A substrate capable of penetrating the cell and capable of binding to the complex to produce a signal detectable from the cell
≪ / RTI >

238. A method for detecting the presence of bacterial cells in a sample,
A non-cloned transfected particle comprising a nucleic acid reporter construct encoding a switchable molecule capable of binding to a target molecule in said cell to form a complex, and a non-cloned transfected particle capable of binding to said complex to form a substrate- Contacting the sample with a substrate; And
Detecting the presence or absence of an output signal from the substrate-bound complex, wherein the presence of the output signal indicates the presence of the bacteria in the sample
≪ / RTI >

239. 233. The method of claim 238, wherein the combination of the switchable molecule and the target molecule causes a conformational change in the switchable molecule.

240. 233. The method of claim 239, wherein the steric configuration change in the switchable molecule allows the substrate to bind to the complex.

Description

[0001] NON-REPLICATIVE TRANSCRIPTION PARTICLE AND TRANSCRIPTION PARTICLE-BASED REPORTER SYSTEMS [0002]

Cross reference of related application

This application is a continuation-in-part of US provisional application no. 61 / 779,177, filed October 29, 2013, 61 / 897,040, filed February 12, 2014, 61 / 939,126, each of which is incorporated herein by reference in its entirety.

Technical field

The present invention relates to methods and compositions for packaging and delivery of non-replicative transducer molecules to cells for the detection of a target gene in the cell.

Transfected particles refer to viruses capable of delivering non-viral nucleic acid to cells. A virus-based reporter system is used to detect the presence of a cell and relies on the virus's ability to allow the expression of the reporter molecule from the cell. Virus-based reporter systems use replicating-competent transducing particles that express the reporter molecule and allow the target cell to emit a detectable signal.

However, the cell lysis cycle of the virus has been shown to adversely affect virus-based reporter assays (Carriere, C. et al., Conditionally replicating luciferase reporter phages: Improved sensitivity for rapid detection and assessment of drug susceptibility of Mycobacterium tuberculosis. Journal of Clinical Micro-biology, 1997. 35 (12): 3232-3239). Carriere et al., M., et < RTI ID = 0.0 > al., ≪ / RTI > Tober Coulosis / Bacillus Calmette-Guerin (BCG) developed Luciferase reporter phage. Using this system, Carriere et al. Demonstrated the detection of BCG using a reporter phage whose cell lysis cycle was inhibited.

However, there are some disadvantages associated with inhibiting the replication function of bacteriophages in bacteriophage-based reporter assays. First, when controlling the replication function of the bacteriophage, the analysis conditions are limited. For example, the cell lysis period of the reporter phage phAE40 used by Carriere et al. Was inhibited when this phage was used to infect cells at a non-permissive temperature of 30 < 0 > C. This temperature requirement was a limiting condition for reporter analysis in that the optimal temperature for the target bacteria was 37 ° C. These restrictive conditions hinder the optimal analytical performance.

Moreover, the replication function of viruses is difficult to control. Replication of the virus should be suppressed during use of the transduced particles as a reporter system. For example, the cytolytic activity of the reporter phage phAE40 reported by Carriere et al. Was reduced but not eliminated, resulting in a weaker luciferase signal in the assay. Carriere et al. Emphasized the ultimate weakening of reporter signals such as intact phage-expressing genes and temperature limits of analysis, all of which are due to the fact that the cell lysis cycle of the phage reporter was not eliminated.

Reporter assays dependent on the natural longevity of the phage can be expected to sporadically exhibit cytolytic activity. In addition, analysis that relies on the phage cycle of the phage may tend to show redundant infection immunity from a naturally occurring host restriction system targeting target nucleic acids already invaded with similar phage and invading viral nucleic acid, Limit the range.

In another example, the transduced particle production system is designed to pack foreign nucleic acid molecules, but the transduced particles mainly contain a combination of foreign nucleic acid molecules and native progeny viral nucleic acid molecules. Native viruses may exhibit cytolytic activity that interferes with the analytical performance. In order to purify the transformed particles, the cytolytic activity of the virus should be removed. However, such purification is generally impossible. Quot; Reporter Plasmid Packaging System for Detection of Bacteria " by Scholl et al. 2009/0155768 A reported the development of these transduced particle systems. The product of this system is a combination of reporter transfected particles and native bacteriophage (see Figure 8 of the reference). Although the authors suggested that the transduced particles and the native bacteriophage could be separated by ultracentrifugation, this separation is only possible in systems where the transducing particles and native viruses exhibit different densities allowing separation by ultracentrifugation. These attributes are presented by the bacteriophage T7-based packaging system described in the reference, but this is not an attribute that is generally applicable to other virus systems. Virus packaging machinery exhibits head pool packaging resulting in native viruses and transduced particles, which often exhibit indistinguishable density that can not be separated by ultracentrifugation. In addition, the viral packaging system relies on a minimal amount of packaging as a requirement for proper virus structure assembly leading to indigenous viruses with undifferentiated densities and transduced particles.

Thus, adverse effects from the cell lysis function of the virus, which can limit the performance of the reporter assay by increasing the detection limit and bringing false negative results, and the host restriction mechanism targeting overlapping infection immunity and viral nucleic acid molecules and viral function Non-replicating transduced particles that are not affected by the likelihood of being limited are desired.

Even when the transfected particles are manipulated, there are some limitations on how to use the transfected particles to detect and report target nucleic acid molecules in the cells. Some methods require complex techniques for destroying cells and for separating and detecting transcripts from cell lysates. Detection methods include using labeled probes such as antibodies, aptamers, or nucleic acid probes. The labeled probe assigned to the target gene may result in a non-specific binding to an unintended target or a signal with a high signal-to-noise ratio. Therefore, there is a need for a specific, effective and accurate method for detection and reporting of endogenous nucleic acid molecules in cells.

Therefore, there is a need for a method and system for generating non-replicating transgenic particles that permit packaging and expression of reporter molecules in cells while eliminating replication-competent offspring viruses. In addition, effective and accurate methods for detecting molecules in cells using expressed reporter molecules are also desired.

A bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle is disclosed herein, the bacterial cell comprising a charged bacteriophage genome lacking a bacteriophage gene encoding a packaging initiation site sequence; And a reporter nucleic acid molecule comprising a second bacteriophage gene, wherein deletion of the bacteriophage gene blocks packaging of the bacteriophage nucleic acid molecule in the non-transfected transgenic particle, and wherein the second bacteriophage gene encodes the packaging initiation site sequence And the second bacteriophage gene is capable of expressing a protein encoded by the gene, wherein the replicase of the reporter nucleic acid molecule is the non-replicating transfected particle, - Form replicons that can be packaged in replicate transduced particles.

In some embodiments, the reporter nucleic acid molecule is operably linked to a promoter. In other embodiments, the promoter is selected to contribute to the reactivity of the reporter molecule expressed from the reporter nucleic acid molecule in the bacterial cell. In one embodiment, the reporter nucleic acid molecule comprises a source of replication. In another embodiment, the replicon includes a conchamer that can be packaged in the non-replicate transduced particle.

In one embodiment, the first and second bacteriophage genes each comprise the pacA gene of bacteriophage P1 in the Enterobacteriaceae and comprise the packaging initiation site sequence. In one embodiment, the second bacteriophage gene comprises the sequence of SEQ ID NO: 9. In another embodiment, the replicon is a bacteriophage P1 cell lysate replicon in an Enterobacteriaceae. In certain embodiments, the replicon comprises an in-frame deletion of the C1 repressor-regulated P53 promoter, the promoter P53 antisense, the repL gene, and the kilA gene. In one embodiment, the replicon comprises the sequence of SEQ ID NO: 3.

In yet another embodiment, the first and second bacteriophage genes each comprise a small ternary (terS) gene comprising the packaging initiation site sequence. In one embodiment, the terS gene is selected from the group consisting of S. Aureus bacteriophage? 11 or? 80? TerS gene.

In another embodiment, the replicon is selected from the group consisting of S. Aureus pT181 plasmid origin of replication. In another embodiment, the replicon comprises the sequence of SEQ ID NO: 5. In some embodiments, the packaging initiation site sequence of the second bacteriophage gene comprises a pac-site. In another embodiment, the pac-region of the second bacteriophage gene comprises the sequence of SEQ ID NO: 7. In one embodiment, the packaging initiation site sequence of the second bacteriophage gene comprises a cos-site. In another embodiment, the packaging initiation site sequence of the second bacteriophage gene comprises a concatamer junction.

In another embodiment, the plasmid comprises the reporter nucleic acid molecule. In one embodiment, the second bacteriophage gene is operably linked to a promoter. In other embodiments, the promoter is an inducible promoter or a constitutive promoter. In one embodiment, the bacteriophage comprises bacteriophage P1 in the

Enterobacteriaceae. In another embodiment, the bacteriophage is selected from the group consisting of S. Aurus bacteriophage? 80? Or bacteriophage? 11. In one embodiment, the bacterial cell is a < RTI ID = 0.0 > ≪ / RTI > In another embodiment, the bacterial cell is S. cerevisiae. Aureus cells. In another embodiment, the bacterial cell comprises a gram negative cell. In another embodiment, the bacterial cell comprises Gram-positive cells.

In another embodiment, the reporter nucleic acid molecule comprises a reporter gene. In one embodiment, the reporter gene encodes a detectable marker and / or a selectable marker. (LacZ, HRP), fluorescent proteins (GFP, eGFP, YFP, RFP, luc, ruc, nluc) CFP, BFP, mCherry, near infrared fluorescent protein), an affinity peptide (His-tag, 3X-FLAG) and a selectable marker (ampC, tet (M), CAT, erm). In another embodiment, the reporter nucleic acid molecule comprises an aptamer. In another embodiment, the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to the second sequence in the reporter nucleic acid molecule.

In one embodiment, the nucleic acid transcript sequence is complementary to the cell transcript. In another embodiment, the nucleic acid transcript sequence comprises a cis-suppressing sequence. In another embodiment, the replicase of the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to a second sequence in the replicas of the reporter nucleic acid molecule, wherein the nucleic acid transcript sequence is complementary to the cell transcript, Transcript sequences include cis-suppression sequences.

In some embodiments, there are provided conditions for inducing a cytolysin of a bacteriophage to the bacterial cells disclosed herein to produce packaged non-replicating transfected particles with the reporter nucleic acid molecule; And separating the non-replicate transduced particles comprising the reporter nucleic acid molecule. The present invention also provides a method for packaging a reporter nucleic acid molecule. In one embodiment, the non-replicating transduced particle does not contain a replicated bacteriophage genome. In another embodiment, induction of the cytolysin triggers ablation of the genomic DNA molecule from the genome of the bacterial cell.

In another embodiment, there is provided a composition comprising the non-replicative transduced particles comprising a replica of the reporter nucleic acid molecule produced from the methods disclosed herein.

The present invention comprises a bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle, wherein the bacterial cell comprises a circulating bacteriophage genome comprising a first bacteriophage packaging initiation site sequence, and a second bacteriophage packaging initiation site Wherein the first bacteriophage packaging initiation site sequence comprises a mutation that blocks packaging of the bacteriophage nucleic acid molecule in the non-replicating transfected particle, and wherein the second bacteriophage packaging initiation site sequence Replicable transfection particle lacks the mutation and promotes packaging of the replica of the reporter nucleic acid molecule in the non-replicating transduced particle, and the replica of the reporter nucleic acid molecule is replicable for packaging into the non- .

In one embodiment, the reporter nucleic acid molecule is operably linked to a promoter. In other embodiments, the promoter is selected to contribute to the reactivity of the reporter molecule expressed from the reporter nucleic acid molecule in the bacterial cell. In another embodiment, the reporter nucleic acid molecule comprises a replication origin. In one embodiment, the replicon includes a con- catamer that can be packaged in the non-replicate transduced particle. In another embodiment, the first and second bacteriophage packaging initiation site sequences each comprise a packaging initiation site sequence from a small terminally derived gene. In one embodiment, the first and second bacteriophage packaging initiation site sequences each comprise a pac-site sequence from the pacA gene of bacteriophage P1 in the Enterobacteriaceae. In another embodiment, the first bacteriophage packaging initiation site sequence comprises SEQ ID NO: 2. In another embodiment, the second bacteriophage packaging initiation site sequence comprises SEQ ID NO: 1. In one embodiment, the replicon comprises a bacteriophage P1 cell lysate replicon in the Enterobacteriaceae. In another embodiment, the replicon comprises an in-frame deletion of the C1 repressor-regulated P53 promoter, the promoter P53 antisense, the repL gene, and the kilA gene. In another embodiment, the replicon comprises the sequence of SEQ ID NO: 3. In a particular embodiment, the first and second bacteriophage packaging initiation site sequences are selected from the group consisting of S. And a pac-site sequence from the small teratogenic (terS) gene of Aureus bacteriophage < RTI ID = 0.0 > 11 or < / RTI > In another embodiment, Aureus pT181 plasmid origin of replication. In another embodiment, the replicon comprises the sequence of SEQ ID NO: 5. In one embodiment, the first bacteriophage packaging initiation site sequence comprises the sequence of SEQ ID NO: 2. In some embodiments, the second bacteriophage packaging initiation site sequence comprises the sequence of SEQ ID NO: 1. In another embodiment, the packaging initiation site sequence comprises a pac-site. In another embodiment, the packaging initiation site sequence comprises a cos-site. In another embodiment, the packaging initiation site sequence comprises a concatamer junction. In some embodiments, the mutation in the first bacteriophage packaging initiation site sequence comprises a silent mutation. In another embodiment, the mutation in the first bacteriophage packaging initiation site sequence blocks cleavage of the packaging initiation sequence. In another embodiment, the plasmid comprises the reporter nucleic acid molecule. In one embodiment, the bacteriophage comprises bacteriophage P1 in the Enterobacteriaceae.

In another embodiment, the bacteriophage is selected from the group consisting of S. Aureus bacteriophage < RTI ID = 0.0 > 11 < / RTI > In one embodiment, the bacterial cell is a < RTI ID = 0.0 > ≪ / RTI > In another embodiment, the bacterial cell is S. cerevisiae. Aureus cells. In some embodiments, the bacterial cell comprises a gram negative bacterial cell. In one embodiment, the bacterial cell comprises a Gram-positive bacterial cell. In another embodiment, the reporter nucleic acid molecule comprises a reporter gene. In another embodiment, the reporter gene encodes a detectable marker and / or a selectable marker.

In another embodiment, the reporter gene is a gene (luxA, luxB, luxAB, luc, ruc, nluc) encoding an enzyme that mediates a luminescent reaction, a gene (lacZ, HRP) (His-tag, 3X-FLAG) encoding an affinity peptide and a gene (ampC, SEQ ID NO: 2) encoding a selectable marker (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry and near infrared fluorescent protein) tet (M), CAT, erm). In another embodiment, the reporter nucleic acid molecule comprises an aptamer. In another embodiment, the replicon is packaged in the non-replicating transduced particle by a bacteriophage packaging mechanism. In some embodiments, the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to the second sequence in the reporter nucleic acid molecule. In another embodiment, the nucleic acid transcript sequence is complementary to the cell transcript.

In one embodiment, the nucleic acid transcript sequence comprises a cis-suppressing sequence. In another embodiment, the replicase of the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to the second sequence in the replicas of the reporter nucleic acid molecule, wherein the nucleic acid transcript sequence is complementary to the cell transcript, Sequences include cis-suppression sequences.

In certain embodiments, providing a condition for inducing a cytolysin of the bacteriophage to the bacterial cells disclosed herein to produce packaged non-replicating transfected particles with a reporter nucleic acid molecule; And separating the non-replicate transduced particles comprising the reporter nucleic acid molecule.

In another embodiment, the non-replicating transduced particle does not contain a replicated bacteriophage genome. In one embodiment, the introduction of the cytolysin triggers ablation of the genomic DNA molecule from the genome of the bacterial cell.

In another aspect, the invention includes a composition comprising the non-replicative transduced particles comprising a replica of the reporter nucleic acid molecule produced from the method disclosed herein.

In one embodiment, the invention includes a bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle, wherein the bacterial cell is capable of blocking packaging of the bacteriophage nucleic acid molecule to the non- A solubilized bacteriophage genome comprising a first bacteriophage gene comprising deletion of a packaging initiation site sequence of a first bacteriophage gene; And a reporter nucleic acid molecule comprising a second bacteriophage gene comprising a second packaging initiation site sequence promoting packaging of the replicase of the reporter nucleic acid molecule in the non-replicating transduced particle, wherein the second bacteriophage gene is a protein And the replicas of the reporter nucleic acid molecules form replicons for packaging the non-replicating transduced particles.

In another embodiment, the reporter nucleic acid molecule is operably linked to a promoter. In one embodiment, the promoter is selected to contribute to the reactivity of the reporter molecule expressed from said reporter nucleic acid molecule in said bacterial cell. In certain embodiments, the reporter nucleic acid comprises a source of

replication. In another embodiment, the replicon includes a conchamer that can be packaged in the non-replicate transduced particle. In one embodiment, the first and second bacteriophage genes each comprise the pacA gene of bacteriophage P1 in the Enterobacteriaceae and comprise the packaging initiation site sequence. In another embodiment, the first bacteriophage gene comprises the sequence of SEQ ID NO: 6. In a particular embodiment, the second bacteriophage gene comprises the sequence of SEQ ID NO: 7. In one embodiment, the replicon comprises a bacteriophage P1 cell lysate replicon in an Enterobacteriaceae. In another embodiment, replicons include in-frame deletions of the C1 repressor-controlled P53 promoter, the promoter P53 antisense, the repL gene, and the kilA gene. In another embodiment, the replicon comprises the sequence of SEQ ID NO: 3. In another embodiment, the first and second bacteriophage genes each comprise a small ternary (terS) gene comprising the packaging initiation site sequence. In one embodiment, the terS gene is selected from the group consisting of S. Aureus bacteriophage? 11 or? 80? TerS gene. In another embodiment, the first bacteriophage gene comprises the sequence of SEQ ID NO: 8. In another embodiment, the second bacteriophage gene comprises the sequence of SEQ ID NO: 9. In one embodiment, the replicon is S. < RTI ID = 0.0 > Aureus pT181 plasmid origin of replication. In one embodiment, the replicon comprises the sequence of SEQ ID NO: 5. In another embodiment, the packaging initiation site sequence of the second bacteriophage gene comprises a pac-site. In another embodiment, the packaging initiation site sequence of the second bacteriophage gene comprises a cos-site.

In certain embodiments, the packaging initiation site sequence of the second bacteriophage gene comprises a concatamer junction. In one embodiment, the plasmid comprises the reporter nucleic acid molecule. In another embodiment, the second bacteriophage gene is operably linked to a promoter. In yet another embodiment, the promoter is an inducible promoter or a constitutive promoter. In certain embodiments, the bacteriophage comprises bacteriophage P1 in the Enterobacteriaceae. In one embodiment, the bacteriophage is selected from the group consisting of S. Aurus bacteriophage? 80? Or bacteriophage? 11. In another embodiment, the bacterial cell is a < RTI ID = 0.0 > ≪ / RTI > In another embodiment, the bacterial cell is S. cerevisiae. Aureus cells. In one embodiment, the bacterial cells comprise gram negative cells. In another embodiment, the bacterial cell comprises Gram-positive cells.

In another embodiment, the reporter nucleic acid molecule comprises a reporter gene. In one embodiment, the reporter gene encodes a detectable marker and / or a selectable marker. In another embodiment, the reporter gene is a gene (luxA, luxB, luxAB, luc, ruc, nluc) encoding an enzyme that mediates a luminescent reaction, a gene (lacZ, HRP) (His-tag, 3X-FLAG) encoding an affinity peptide and a gene (ampC, SEQ ID NO: 2) encoding a selectable marker (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry and near infrared fluorescent protein) tet (M), CAT, erm). In one embodiment, the reporter nucleic acid molecule comprises an aptamer. In another embodiment, the replicon is packaged in the non-replicate transduced particle by a bacteriophage packaging mechanism. In another embodiment, the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to the second sequence in the reporter nucleic acid molecule. In one embodiment, the nucleic acid transcript sequence is complementary to the cell transcript. In another embodiment, the nucleic acid transcript sequence comprises a cis-suppressing sequence. In certain embodiments, the replicase of the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to a second sequence in the replicas of the reporter nucleic acid molecule, wherein the nucleic acid transcript sequence is complementary to the cell transcript, Cucumber sequences include cis-suppression sequences.

The present invention includes a method of packaging a reporter nucleic acid molecule in a non-replicating transduced particle, which comprises introducing into the bacterial cell a cytolysin of the bacteriophage to produce a non-replicating transduced particle packaged with the reporter nucleic acid molecule Providing a condition to be performed; And separating the non-replicate transduced particles comprising the reporter nucleic acid molecule. In one embodiment, the non-replicating transduced particle does not contain a replicated bacteriophage genome. In another embodiment, induction of the cytolysin triggers ablation of the genomic DNA molecule from the genome of the bacterial cell.

In some embodiments, the invention comprises a composition comprising said non-replicating transduced particles comprising a replica of said reporter nucleic acid molecule produced from said method disclosed herein.

In another aspect, the invention includes a bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle, wherein the bacterial cell lacks a packaging gene and encodes a protein that forms the non-replicating transduced particle A lytic bacteriophage genome comprising a gene encoding the gene; And a genomic region nucleic acid molecule comprising a reporter nucleic acid molecule and a packaging gene. In one embodiment, the packaging gene comprises a small terminalase (terS) gene. The terS gene is available from S. Aureus bacteriophage? 80? TerS gene or bacteriophage? 11 terS gene.

In one embodiment, the terS gene comprises the sequence of SEQ ID NO: 9. In another embodiment, the genomic DNA nucleic acid molecule comprises a SaPIbov2 genomic region nucleic acid molecule. In another embodiment, the genomic DNA nucleic acid molecule is selected from the group consisting of SaPI, SaPI1, SaPI2, SaPIbov1 and SaPibov2 genomic region nucleic acid molecules. In another embodiment, the reporter nucleic acid molecule is operably linked to a promoter. In another embodiment, the reporter nucleic acid molecule comprises a replication origin. In some embodiments, the bacteriophage is selected from the group consisting of S. Aurus bacteriophage? 80? Or bacteriophage? 11. In another embodiment, the bacterial cell is S. cerevisiae. Aureus cells. In one embodiment, the genomic region nucleic acid molecule comprises an integrase gene, wherein the integrase gene encodes an integrase protein for the ablation and integration of the genomic region nucleic acid molecule outside and within the bacterial genome of the bacterial cell . In another embodiment, the integrase gene comprises the sequence of SEQ ID NO: 10. In another embodiment, the genomic DNA nucleic acid molecule is integrated into the bacterial genome of the bacterial cell.

In certain embodiments, genomic DNA nucleic acid molecules can be cloned and form molecular replicons that can be packaged by the bacterial phage packaging device in the bacterial cells. In another embodiment, the nucleic acid molecule forms a concatamer. In another embodiment, the replicated genomic region nucleic acid molecule can be packaged in the non-replicating transduced particle. In certain embodiments, the packaging gene comprises a pac-site sequence. In another embodiment, the packaging gene comprises a cos-site sequence. In another embodiment, the packaging gene comprises a concatamer junction.

In another embodiment, the reporter nucleic acid molecule comprises a reporter gene. In some embodiments, the reporter gene encodes a selectable marker and / or a selectable marker. (LacZ, HRP), a fluorescent protein (GFP, eGFP, YFP, RFP), which mediates a color reaction, , CFP, BFP, mCherry, near infrared fluorescent protein), an affinity peptide (His-tag, 3X-FLAG) and a selectable marker (ampC, tet (M), CAT, erm). In certain embodiments, the reporter nucleic acid molecule comprises an aptamer. In another embodiment, the genomic DNA nucleic acid molecule lacks the integrase gene. In another embodiment, the invention comprises a bacterial gene comprising an integrase gene operably linked to a promoter, wherein the integrase gene is capable of modulating and integrating the genomic DNA molecule within or outside the bacterial genome of the bacterial cell Lt; RTI ID = 0.0 > integrase < / RTI > In one embodiment, the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to the second sequence in the reporter nucleic acid molecule. In another embodiment, the nucleic acid transcript sequence is complementary to the cell transcript. In another embodiment, the nucleic acid transcript sequence comprises a cis-suppressing sequence. In another embodiment, the replicase of the reporter nucleic acid molecule comprises a nucleic acid transcript sequence complementary to the second sequence in the replicon of the reporter nucleic acid molecule. In another embodiment, the nucleic acid transcript sequence is complementary to the cell transcript. In another embodiment, the nucleic acid transcript sequence comprises a cis-suppressing sequence.

The present invention includes a method of packaging a reporter nucleic acid molecule in a non-replicating transduced particle, which comprises bringing into contact with the reporter nucleic acid molecule a nucleic acid molecule capable of expressing a condition inducing a cytolysin of the bacteriophage to provide packaged non-Providing to a bacterial cell; And separating the non-replicate transduced particles comprising the reporter nucleic acid molecule. In some embodiments, the non-replicating transduced particles do not contain a replicated bacteriophage genome. In one embodiment, induction of the cytolysin triggers ablation of the genomic DNA molecule from the genome of the bacterial cell.

In another embodiment, the invention comprises a composition comprising said non-replicative transduced particles comprising a replica of said reporter nucleic acid molecule produced from said method disclosed herein.

The invention also encompasses a method for detecting the presence or absence of bacterial cells in a sample, which comprises encoding non-replicating transgenic particles comprising a reporter gene encoding a reporter molecule and lacking a bacteriophage genome, Introducing into the sample a particle capable of transducing said bacterial cell and said reporter gene being expressible in said bacterial cell; Providing conditions for activation of the reporter molecule; And detecting the presence

or absence of a reporter signal delivered from the expressed reporter molecule, wherein the presence of the reporter signal accurately represents the presence of the bacterial cell.

In one embodiment, the method achieves at least 80% detection specificity based on a standard, at least 90% detection specificity based on a standard, or at least 95% detection specificity based on a standard. In another embodiment, the method achieves at least 80% detection sensitivity based on the standard, at least 85% detection sensitivity based on the standard, at least 90% detection sensitivity based on the standard, and at least 95% detection sensitivity based on the standard . In another embodiment, the method achieves at least 95% detection specificity and at least 90% detection sensitivity based on standards. In another embodiment, the standard is a gold standard. In yet another embodiment, the bacterial cell comprises methicillin resistant Staphylococcus aureus (MRSA) cells. In another embodiment, the bacterial cell comprises a methicillin sensitive staphylococcus aureus (MSSA) cell.

In another embodiment, the reporter gene encodes a detectable marker or a selectable marker. In one embodiment, the reporter gene is a gene (luxA, luxB, luxAB, luc, ruc, nluc) encoding an enzyme mediating a luminescent reaction, a gene (lacZ, HRP) (His-tag, 3X-FLAG) encoding an affinity peptide and a gene (ampC) encoding a selectable marker (GFP, eGFP, YFP, RFP, CFP, BFP, mCherry and near infrared fluorescent protein) , tet (M), CAT, erm). In one embodiment, the reporter gene is operably linked to a constitutive promoter.

In another embodiment, the reporter signal can be detected from the sample at a detection limit (LoD) of less than 1,000 colony forming units (CFU). In another embodiment, the reporter signal can be detected from the sample at a detection limit (LoD) of less than 100 colony forming units (CFU). In one embodiment, the reporter signal can be detected from the sample at a detection limit (LoD) of less than 10 colony forming units (CFU). In another embodiment, the reporter signal can be detected from the sample at a LoD of less than 5 CFU. In another aspect, the reporter signal can be detected from the sample at a LoD of 3 or less CFUs.

In one embodiment, the method comprises the steps of providing an antibiotic to the sample at a predetermined concentration and detecting the presence or absence of the reporter signal to determine if the bacterial cell is resistant or susceptible to the antibiotic do. In another embodiment, the method comprises the steps of providing the sample with a predetermined concentration of antibiotic, and detecting the amount of the reporter signal for determining the minimal inhibitory concentration of the bacterial cell against the antibiotic .

In one embodiment, the invention includes a composition comprising a nucleic acid construct encoding a nucleic acid reporter transcript capable of forming at least two stereostructures, wherein the stereostructure comprises a first subsequence and a second subsequence A first steric structure that blocks reporter expression comprising an intramolecular double stranded region, and a second steric structure that lacks the intramolecular double stranded region and allows reporter gene expression, wherein the first steric structure and the second steric structure Conversion between stereostructures is mediated by competitive binding of the first and / or the second subsequence to the cell transcript.

In another aspect, the present invention includes a non-replicating transduced particle comprising the nucleic acid construct. In another embodiment, competing binding of said first and / or said second subsequence with said cell transcript causes said second stereostructure of said nucleic acid reporter construct. In one embodiment, the first subsequence or the second subsequence comprises a cis-suppression sequence. In another embodiment, the cis-suppression sequence comprises a sequence complementary or substantially complementary to a portion of the cell transcript. In another embodiment, the first subsequence or said second subsequence comprises a reporter gene sequence. In another embodiment, the reporter gene sequence comprises a ribosome binding site. In another embodiment, the reporter gene sequence encodes a detectable molecule. In another embodiment, the detectable marker comprises an enzyme capable of mediating fluorescent molecules or luminescent or colorimetric reactions. In one embodiment, the reporter gene sequence encodes a selectable marker. In another embodiment, the selectable marker comprises an antibiotic resistance gene.

In another embodiment, the first subsequence and the second subsequence are cis positioned relative to each other in the nucleic acid construct to form the intramolecular double stranded region. In certain embodiments, the first subsequence and the second subsequence are complementary or substantially complementary to each other to form an intramolecular double stranded region. In one embodiment, the first subsequence or the second subsequence of the first steric structure comprises a transcription enhancer sequence, wherein the transcription enhancer sequence is upstream from the coding region of the reporter gene sequence. In another embodiment, the first steric structure of the nucleic acid reporter transcript may bind to a cleavage enzyme. In another embodiment, the first steric structure of the nucleic acid reporter transcript is a target for degradation by a cellular enzyme. In another embodiment, the first steric structure comprises a non-binding intramolecular domain. In another embodiment, the non-binding intramolecular domain is located 3 ' of the first subsequence and 5 ' of the second subsequence. In another embodiment, the non-binding intramolecular domain comprises the sequence YUNR, wherein Y is pyrimidine, U is uracil, N is any nucleotide, and R is purine.

In one embodiment, the first subsequence or the second subsequence comprises a modified sequence of the cell transcript. In another embodiment, the modified sequence comprises a nucleotide substitution. In another embodiment, the modified sequence comprises sequence insertion, deletion or inversion of the cell transcript.

The method comprises a nucleic acid construct encoding a nucleic acid reporter transcript comprising a genetic reporter sequence and capable of forming at least two stereostructures of the nucleic acid reporter transcript, wherein the two stereostructures comprise a nucleic acid reporter transcript And a second stable three-dimensional structure resulting from the combination of the cell transcript and the first unstable three-dimensional structure, wherein the second stable three-dimensional structure is a nucleotide sequence of the nucleic acid reporter Allowing translation of the reporter gene sequence of the transcript.

In one embodiment, the composition comprises non-replicating transduced particles comprising said nucleic acid construct. In another embodiment, the cell transcript is bound in the 3 ' UTR sequence of the nucleic acid reporter transcript. In one embodiment, the second stable secondary stereostructure is formed by cleavage of a portion of the sequence of the first unstable secondary stereostructure. In another embodiment, the reporter gene sequence encodes a detectable molecule. In some embodiments, the detectable marker comprises an enzyme capable of mediating fluorescent molecules or luminescent or colorimetric reactions. In another embodiment, the reporter gene sequence encodes a selectable marker. In another embodiment, the selectable marker comprises an antibiotic resistance gene.

The invention also encompasses a composition comprising a nucleic acid construct capable of encoding a nucleic acid reporter transcript comprising a reporter gene sequence and capable of forming at least two stereostructures of the nucleic acid reporter transcript, A first steric structure that blocks additional transcription of the construct, and a second steric structure that is formed upon binding of the cell transcript and the first steric structure, wherein the second steric structure allows transcription of the nucleic acid construct. In some embodiments, the composition comprises a non-replicating transduced particle comprising the nucleic acid construct. In another embodiment, the nucleic acid reporter transcript comprises a cis-suppressing sequence.

In one embodiment, the nucleic acid reporter transcript comprises a reporter gene sequence. In another embodiment, the first steric structure is formed from the binding of said cis-suppressing sequence to said reporter gene sequence. In some embodiments, the first steric structure is a substrate for a cleavage enzyme. In one embodiment, the first steric structure of the nucleic acid reporter transcript comprises a sequence that forms a transcription termination structure. In another embodiment, binding of the cell transcript to the structure that forms the transcription termination structure results in cleavage of a portion of the nucleic acid reporter transcript and formation of the second steric structure.

The invention includes a vector comprising a regulatory sequence operably linked to a nucleic acid sequence encoding said nucleic acid reporter transcript disclosed herein.

The invention includes a method of detecting a target transcript in a cell, said method comprising the steps of: introducing into said cell a nucleic acid reporter construct as disclosed herein; And detecting the presence or absence of an output signal from the cell, wherein the presence of the output signal indicates the presence of a target transcript in the cell. The method comprises detecting the presence of bacterial cells based on detecting said presence of said target transcript.

In one embodiment, a method for detecting the presence of a bacterial cell in a sample comprises the steps of: introducing the nucleic acid reporter construct disclosed herein to the sample; And detecting the presence or absence of an output signal from the sample, wherein the presence of the output signal indicates the presence of a bacterial cell in the sample.

The invention provides a kit comprising: a compartment for holding a sample comprising said nucleic acid reporter construct and cells as disclosed herein; And a kit including instructions for detecting the presence or absence of an output signal from the sample, the presence of an output signal indicating the presence of a target transcript in the cell.

The present invention includes a composition comprising a non-replicative transduced particle comprising a nucleic acid reporter construct comprising a first promoter operably linked to a reporter gene, wherein the first promoter is derived from an inducer protein endogenous to the bacterial cell .

The present invention includes a method of detecting the presence of a bacterial cell in a sample comprising contacting said sample with a reporter gene comprising a first promoter operably linked to an inducer protein endogenous to said bacterial cell With a non-replicating transducing particle comprising a nucleic acid reporter construct; And detecting the presence or absence of an output signal from the reporter gene, wherein the presence of the output signal indicates the presence of the bacterial cell in the sample.

In one embodiment, the first promoter is identical to an inducible promoter operably linked to a target nucleic acid molecule in the bacterial cell.

The present invention provides a non-cloned transgenic particle comprising a nucleic acid reporter construct comprising a reporter gene encoding a reporter molecule and capable of entering a bacterial cell; And a substrate capable of reacting with the reporter molecule in the cell when the containment is released as a blocked substrate exogenous to the bacterial cell.

The invention includes a method of detecting the presence of a bacterial cell in a sample comprising contacting the sample with a non-replicating transduced particle comprising a nucleic acid reporter construct comprising a reporter gene encoding a reporter molecule and a non- Contacting the substrate with a substrate capable of binding to the reporter molecule in the bacterial cell when the containment is released as a substrate; And detecting the presence or absence of an output signal from the reporter molecule, wherein the presence of the output signal indicates the presence of the bacterial cell in the sample.

In one embodiment, the target enzyme in the cell binds to the blocked substrate to produce a substrate that is unblocked. In some embodiments, an unsealed substrate is reacted with the reporter molecule to produce the output signal.

The invention also provides a non-cloned transfected particle comprising a nucleic acid reporter construct encoding a switchable molecule capable of binding to a target molecule in a bacterial cell to form a complex; And a substrate capable of penetrating the cell to bind to the complex to produce a signal detectable from the cell.

The invention includes a method of detecting the presence of a bacterial cell in a sample comprising contacting the cell with a non-replicating transduced particle comprising a nucleic acid reporter construct encoding a switchable molecule capable of binding a target molecule in the cell to form a complex And contacting the sample with a substrate capable of binding to the complex to form a substrate-binding complex; And detecting the presence or absence of an output signal from the substrate-binding complex, wherein the presence of the output signal indicates the presence of the bacterial cell in the sample. In one embodiment, the binding of the switchable molecule to the target molecule causes a conformational change in the switchable molecule. In another embodiment, a conformational change in the switchable molecule allows the substrate to bind to the complex.

These and other features, aspects, and advantages of the present invention will become better understood with regard to the following description and accompanying drawings.
Figure 1 shows an example of the design and function of a silent mutation / compensation-based P1 plasmid packaging system according to embodiments of the present invention.
Figure 2 shows an illustration of the pGWP10001 vector according to embodiments of the present invention.
Figure 3 illustrates an example of the design and function of a pac-site deletion / compensation plasmid packaging system in accordance with embodiments of the present invention.
Figure 4 shows an illustration of the pGW80A0001 vector according to embodiments of the present invention.
Figure 5 illustrates a process for genome island (GI) packaging by bacteriophage according to embodiments of the present invention.
Figure 6 shows an example of the design and function of a GI-based packaging system according to embodiments of the present invention.
Figure 7 illustrates the design and function of a GI-based packaging system lacking the integrase gene according to embodiments of the present invention.
Figure 8 shows the design and function of a SaPIbov2-based packaging system lacking integrase genes according to embodiments of the present invention.
Figure 9 shows a system for use of NRTP for detection of an inducer for targeting a gene promoter in a live-growing cell according to an embodiment of the present invention.
Figure 10 is a schematic diagram of a reporter nucleic acid molecule (for example, SEQ ID NO: 2) constructed to detect VanR, an inducer of the vancomycin resistant (vanA) gene promoter in Enterococcus faecium For example, a plasmid). This reporter plasmid carries a reporter gene operably linked to the vanA gene promoter.
11 shows a seed according to an embodiment of the present invention. (E. G., A plasmid) constructed to detect TcdD, an inducer of the promoters of the diphylloxyl toxin A and B genes (tcdA and tcdB, respectively). This reporter nucleic acid molecule contains a reporter gene operably linked to a tcdA gene promoter.
Fig. 12 is a block diagram of an embodiment of the present invention. A reporter system comprising a reporter nucleic acid molecule (e.g., a plasmid) configured to detect SarS, an inducer of the promoter of the protein A gene (spa) of Aureus. This reporter nucleic acid molecule comprises the bacterial luciferase genes luxA and luxB operably linked to the spa gene promoter.
Figure 13 shows a reporter system comprising a system for the detection of enzymes in live cells using blocked substrate molecules in which the containment can be released by the target intracellular enzyme according to embodiments of the present invention.
Figure 14 shows the design and function of the? -Lactamase enzyme detection system according to embodiments of the present invention.
Figure 15 shows a reporter system for detection of enzymes in viable cells using switchable molecules capable of producing a detectable signal when combined with a target molecule according to embodiments of the present invention.
Figure 16 illustrates the design and function of a bacteriophage / switchable-aptamer (SA) -based intracellular molecular reporter system according to embodiments of the present invention.
Figure 17 shows an example of a system using a cis-suppression mechanism capable of targeting the 5 ' UTR (untranslated region) of a reporter sequence on a reporter transcript according to embodiments of the present invention.
Figure 18 shows an example of a system for detecting the presence of a target transcript in a cell based on a cis-suppression mechanism that targets a ribosome binding site (RBS) of a reporter sequence in a reporter transcript according to an embodiment of the present invention.
Figure 19 illustrates an exemplary system for detecting the presence of a target transcript in a cell based on a cis-suppression mechanism that targets the coding region ("AUG") of the reporter sequence in a reporter transcript according to embodiments of the present invention .
Figure 20 illustrates an exemplary system for detecting the presence of a target transcript in a cell based on an inhibitory mechanism using an unstable reporter transcript according to embodiments of the present invention.
Figure 21 shows the results of a transduction assay according to an embodiment of the present invention in which 36 tetracycline-sensitive MRSAs were exposed to the transducing particles with pGW80A0001 and then incubated in a culture plate containing 5 ug / ml of tetracycline Spatting.
FIG. 22 is a graph showing the activity of methicillin sensitive susceptible S. cerevisiae transduced with the transduced particles according to an embodiment of the present invention. The luminescence measured from 28 clinical isolates of Aureus (MSSA) and 80 clinical isolates of MRSA is shown.
Figure 23 shows SEM images of S < RTI ID = 0.0 > 8, < / RTI > The results of Aureus are shown.

Figure 24 shows the RLU values obtained by NRTP analysis in the presence of 4, 8, 16, 32, 64, and 128 ug / mL of hypoxicin. In FIG. 24, the x-axis is set to the MSSA RLU cutoff value.
Figure 25 shows the secondary structure of a mecA transcript generated based on the lowest energy steric structure computed by MFold and visualized as VARNA.
Figure 26 shows the end loop 23 (T23) of the mecA transcript containing the YUNR consensus sequence.
Figure 27 shows a cis-suppression sequence designed to form a stem-loop structure blocking the RBS sequence of the luxA gene ("AAGGAA") and added to the 5 'end of the luxAB gene.
28 shows a diagram of a base pair of a cis-suppression sequence of a target transcript and a reporter transcript.
29 shows an example of a target mecA gene sequence according to an embodiment of the present invention.
Figure 30 shows an exemplary mecA transcript sequence that can be used to design a reporter transcript (SEQ ID NO: 16) according to embodiments of the present invention.
Figure 31 shows an example of a luxAB locus DNA sequence that can be used to design a reporter transcript according to embodiments of the present invention.
Figure 32 shows an example of a luxAB transcript sequence that can be used to design a reporter transcript (SEQ ID NO: 18) according to embodiments of the present invention.
Figure 33 shows an example of a luxAB cis-suppressed transcript sequence that can be used to design a reporter transcript (SEQ ID NO: 19) according to embodiments of the present invention.
Figure 34 shows an example of a cell containing a vector encoding a reporter transcript according to an embodiment of the invention, wherein the cell lacks an endogenous mecA transcript.
Figure 35 shows the vector introduced into the cell, which encodes a reporter transcript comprising a cis-suppressing sequence and a reporter sequence (luxA and luxB genes). When the mecA transcript present in the cell binds to the cis-suppressing sequence, the inhibitory hairpin loop is opened and the RBS for the luxA gene is exposed. In this case, the translation of the reporter sequences (luxA and luxB) can proceed and the luxAB enzyme is formed. The luxAB enzyme produces a detectable emission signal. In this way, transcriptor reporter vectors report the presence of endogenous mecA transfectants in the cells.

I. Definition

Terms used in the claims and specification are defined as indicated below unless otherwise stated.

As used herein, "reporter nucleic acid molecule" refers to a nucleotide sequence comprising DNA or RNA molecules. The reporter nucleic acid molecule may naturally occur, or it may be an artificial or synthetic molecule. In some embodiments, the reporter nucleic acid molecule is foreign to the host cell and may be introduced into the host cell as part of a foreign nucleic acid molecule, such as a plasmid or vector. In certain embodiments, the reporter nucleic acid molecule may be complementary to the target gene in the cell. In another embodiment, the reporter nucleic acid molecule comprises a reporter gene encoding a reporter molecule (e. G., A reporter enzyme, a protein). In some embodiments, the reporter nucleic acid molecule is referred to as a "reporter construct" or a "nucleic acid reporter construct."

"Reporter molecule" or "reporter" refers to a molecule (e.g., nucleic acid or protein) that confers a detectable or selective phenotype to an organism. The detectable phenotype can be, for example, colorimetric, fluorescent or luminescent. Reporter molecules include reporter genes (luxA, luxB, luxAB, luc, ruc, and nluc) that encode enzymes that mediate the luminescence reaction, genes that encode enzymes that mediate the color reaction (lacZ, HRP) (His-tag, 3X-FLAG) encoding an affinity peptide and a gene (ampC, tet (M-FLAG) encoding a selectable marker)), CAT, erm). Reporter molecules can be used as markers for successful absorption of nucleic acid molecules or foreign sequences (plasmids) into cells. In addition, reporter molecules may be used to indicate the presence of a target gene, a target nucleic acid molecule, a target cell molecule, or a cell as disclosed herein. Alternatively, the reporter molecule may be a nucleic acid such as an aptamer or ribozyme.

In some embodiments of the invention, the reporter nucleic acid molecule is operably linked to a promoter. In another aspect of the invention, the promoter may be selected or designed to contribute to the reactivity and cross-reactivity of the reporter system based on the activity of the promoter only in a particular cell (e.g., a particular species). In certain embodiments, the reporter nucleic acid molecule comprises a source of replication. In another embodiment, when the replication of the reporter nucleic acid molecule in the target cell contributes to the reporter signal generation based on the activity of the replication origin in the particular cell (e. G., A particular species) Reactive < / RTI > and cross-reactivity. In some embodiments, the reporter nucleic acid molecule forms a replicon that can be packaged as conchamer DNA in the offspring virus during viral replication.

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

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

Transcripts derived from reporter nucleic acid molecules may be referred to as "reporter transcripts ". The reporter transcript may comprise a reporter sequence and a cis-suppression sequence. The reporter transcript may have a sequence that forms a complementary region, whereby the transcript comprises two regions that form a duplex (e. G., An intermolecular duplex region). One region may be referred to as a "cis-suppressing sequence " and has complementarity to some or all of the target transcript and / or reporter sequence. The second region of the transcript is referred to as a "reporter sequence " and may have complementarity to a cis-suppressing sequence. Complementarity may be complete complementarity or substantial complementarity. The presence and / or binding of the cis-suppressing sequence and the reporter sequence may form any conformation in the reporter transcript, which may block further expression of the reporter molecule. The reporter transcript can form a secondary structure, e.g., a hairpin structure, so that regions within the reporter transcript that are complementary to each other can hybridize to each other.

When referring to a nucleic acid molecule or a foreign sequence (e. G., A plasmid, vector, construct), "introducing into a cell" means facilitating uptake into the cell as understood by those skilled in the art. Absorption of nucleic acid constructs or transcripts may occur via non-co-diffusion or active cellular processes, or by adjuvants or devices, including the use of bacteriophages, viruses, and transduced particles. The meaning of this term is not limited to cells in vitro, and even if the cell is part of a living organism, the nucleic acid molecule can be "introduced into the cell ". In this example, introduction into the cell will involve delivery to the organism. For example, for in vivo delivery, the nucleic acid molecule, construct or vector of the invention may be injected or administered systemically to the tissue site. In vitro introduction into cells involves methods known in the art, such as electroporation and lipofection. Additional approaches are also disclosed herein or known in the art.

"Transduced particle" refers to a virus capable of delivering non-viral nucleic acid molecules to a cell. The virus may be a bacteriophage, an adenovirus, or the like.

Refers to a virus that can deliver a non-viral nucleic acid molecule to a cell, but does not package its replicated viral genome into the transducing particle. The virus may be a bacteriophage, an adenovirus, or the like.

A "plasmid" is a small DNA molecule that is physically discrete and can be replicated independently of chromosomal DNA in a cell. Small circular double-stranded DNA molecules are most commonly found in bacteria, and plasmids are sometimes present in archaea and eukaryotic organisms. Plasmids are considered replicons that can replicate themselves in the appropriate host.

A "vector" is a nucleic acid molecule used as a vehicle capable of artificially delivering a foreign genetic material to another cell, which can be cloned and / or expressed.

A "virus" is a small infectious agent that replicates only within living cells of other organisms. Viral particles (known as virions) contain two or three parts: i) a genetic material made from DNA or RNA molecules with genetic information; ii) protein coatings to protect these genes; And in some cases a lipid envelope surrounding the protein coating.

"MRSA" refers to methicillin-resistant Staphylococcus aureus.

"MSSA" refers to methicillin-sensitive staphylococcus aureus.

The term " alleviating "refers to any therapeutically beneficial consequence of a disease state, including any beneficial consequences in the treatment of a disease, e. G., Reduction, improvement, or cure, of prevention, severity or progression.

The term "in situ" refers to a process that occurs in a live cell that is growing separately from a living organism, for example, growing in a tissue culture.

The term "in vivo" refers to the process that takes place in living organisms.

The term "mammal " as used herein includes, but is not limited to, human and non-human primates, canine, feline, murine, bovine, horse and porcine.

"G", "C", "A" and "U" respectively denote nucleotides each containing guanine, cytosine, adenine and uracil as bases, respectively. "T" and "dT" are used interchangeably herein and refer to a deoxyribonucleotide in which the nucleobase is thymidine, such as deoxyribothimidine. It will be appreciated, however, that the terms "ribonucleotide "," nucleotide "or" deoxyribonucleotide "may also refer to a modified nucleotide, or substitute substitutional portion, Those skilled in the art are well aware that guanine, cytosine, adenine, and uracil can be replaced by other moieties without substantial alteration of the base pair property of oligonucleotides comprising nucleotides with such substitution moieties. For example, and not by way of limitation, nucleotides containing inosine as a base can be base-paired with nucleotides containing adenine, cytosine or uracil. Thus, nucleotides containing uracil, guanine or adenine can be substituted in the nucleotide sequence of the present invention with, for example, nucleotides containing inosine. Sequences comprising such substitutions are also embodiments of the invention.

As used herein, the term "complementarity" when used to describe a first nucleotide sequence in conjunction with a second nucleotide sequence means that the oligonucleotide or polynucleotide comprising the second nucleotide sequence hybridizes under specific conditions to a duplex Refers to the ability of an oligonucleotide or polynucleotide comprising a first nucleotide sequence capable of forming a structure. Complementarity sequences are also disclosed as binding to each other and are characterized by binding affinity.

For example, the first nucleotide sequence can be described as being complementary to the second nucleotide sequence when the two sequences are hybridized (e. G., Annealed) under the conditions of the tandem hybridization. Hybridization conditions include temperature, ionic strength, pH, and organic solvent concentration for annealing and / or washing steps. The term " tight hybridization conditions "refers to conditions in which a first nucleotide sequence hybridizes preferentially to its target sequence, e.g., a second nucleotide sequence, to a lesser extent with other sequences, or to no hybridization at all. Tight hybridization conditions are sequence dependent and are different in different environments. Generally, the co-hybridization conditions are selected to be about 5 [deg.] C lower than the thermal melting point (Tm) for the nucleotide sequence at the defined ionic strength and pH. The Tm is the temperature (at the specified ionic strength and pH) at which 50% of the first nucleotide sequence hybridizes to the fully matched target sequence. Broad guidelines for the hybridization of nucleic acids can be found, for example, in Tijssen (1993) Laboratory Techniques in Biochemistry and Molecular Biology - Hybridization with Nucleic Acid Probes part I, chap. 2, "Overview of the principles of hybridization and the strategy of nucleic acid probe assays," Elsevier, N. Y. ("Tijssen"). Other conditions such as physiologically relevant conditions that may occur in the organism may also be applied. One skilled in the art can set conditions that are most suitable for the complementarity test of two sequences, depending on the end use of the hybridized nucleotide.

Complementarity includes the base pairing of oligonucleotides or polynucleotides comprising a first nucleotide sequence with an oligonucleotide or polynucleotide comprising a second nucleotide sequence over the entire length of the first and second nucleotide sequences. Such sequences may be referred to herein as being "completely complementary" with respect to each other. However, where the first sequence is referred to herein as being "substantially complementary" with respect to the second sequence, both sequences may be fully complementary, or may hybridize to one or more , Typically up to 4, 3 or 2 mismatched base pairs. However, when two oligonucleotides are designed to form one or more single strand overhangs upon hybridization, such overhangs should not be considered mismatches in relation to complementarity determinations. For example, a dsRNA comprising one oligonucleotide of 21 nucleotides in length and another oligonucleotide of 23 nucleotides in length, even when the longer oligonucleotide comprises a sequence of 21 nucleotides that is completely complementary to the shorter oligonucleotide May be referred to as being "fully complementary" as purposed herein.

The term "complementarity" as used herein also includes base pairs and / or non-Watson-Creek base pairs formed from non-natural and modified nucleotides, as long as the above requirements relating to the ability to hybridize are met, have. Such non-Watson-Crick base pairs include, but are not limited to, G: U Wobble or Hoogstein base pairs.

As used herein, the terms "complementarity "," fully complementary ", and "substantially complementary" refer to the distance between two strands of a dsRNA, or between an antisense strand and a target sequence of a dsRNA, Can be used in connection with base matching between RNA sequences and complementary strands of a single stranded DNA sequence.

As used herein, a "duplex structure" includes two reverse-parallel and substantially complementary nucleic acid sequences. A complementary sequence between two transcripts in a nucleic acid construct, between two regions in a transcript, or between a transcript and a target sequence can form a "duplex structure ". Generally, most of the nucleotides of each strand are ribonucleotides, but as disclosed in detail herein, each strand or both strands also includes at least one non-ribonucleotide, such as deoxyribonucleotides and / or modified nucleotides can do. The two strands forming the duplex structure may be different portions of one large RNA molecule, or they may be separate RNA molecules. If two strands are part of one large molecule and the 3'-end of one strand and the 5'-end of each of the remaining strands are joined by an uninterrupted chain of nucleotides to form a duplex structure, the connecting RNA The chain is referred to as a "hairpin loop ". When two strands are covalently linked to form a duplex structure by means other than the uninterrupted chain of nucleotides between the 3'-end of one strand and the 5'-end of each of the remaining strands, Linker ". The RNA strands may have the same or different numbers of nucleotides. The maximum number of base pairs is the number of nucleotides in the shortest strand of the duplex minus any overhang present in the duplex. Generally, the duplex structure is 15 to 30 or 25 to 30, or 18 to 25, or 19 to 24, or 19 to 21, or 19, 20, or 21 base pair lengths. In one embodiment, the duplex is 19 base pairs in length. In another embodiment, the duplex is 21 base pairs in length. Duplex lengths may be the same or different when two different siRNAs are used in combination.

The term "complementarity region" as used herein refers to a region on an antisense strand that is substantially complementary to a sequence, e. G., A target sequence, as defined herein. If the region of complementarity is not entirely complementary to the target sequence, the mismatch is most favored in the terminal region and is generally flanked in the terminal region or other regions, for example at the 5 ' and / or 3 ' , 3, or 2 nucleotides.

The term "identity" with respect to two or more nucleic acid or polypeptide sequences can be determined using one of the sequence comparison algorithms described below (e.g., BLASTP and BLASTN or other algorithms available to those skilled in the art) Refers to two or more sequences or subsequences that are the same percent nucleotides or nucleic acid residues when compared and aligned for sex. Depending on the application, the "identity" percentage may be across any region of the sequence being compared, for example over the functional domain, or over the entire length of the two sequences being compared.

For sequence comparison, typically one sequence acts as a reference sequence against which the test sequences are compared. When using a sequence comparison algorithm, test sequences and reference sequences are entered into the computer, subsequence coordinates are specified if necessary, and sequence algorithm program variables are specified. Next, the sequence comparison algorithm calculates the percent sequence identity of the test sequence (s) to the reference sequence based on the specified program variable.

Optimal sequence alignments for comparison are described, for example, in Smith & Waterman, Adv. Appl. Math. 2: 482 (1981), Needleman & Wunsch, J. Mol. Biol. 48: 443 (1970), Pearson & Lipman, Proc. Nat'l. Acad. Sci. (GAP, BESTFIT, FASTA, and TFASTA, the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison, Wis.), (See generally Ausubel et al.).

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

The term "sufficient amount" means an amount sufficient to produce the desired effect, for example, an amount sufficient to produce a detectable signal from the cell.

The term "therapeutically effective amount" is an amount effective to alleviate the symptoms of the disease. If the prophylaxis has a therapeutic effect, the therapeutically effective amount may be a "prophylactically effective amount ".

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

II. Virus Yongwon And Cell lysis Cycle

The virus undergoes a lysis cycle and a cell lysis cycle in the host cell. When the longevity cycle is initiated, the phage chromosomes can be integrated into the bacterial chromosome, or can establish a plasmid that is self-stable in the host, in which case it can remain dormant for extended periods of time. When the source is induced, the phage genome is excised from the bacterial chromosome and initiates the cell lysis cycle, which terminates upon lysis of the cells and release of the phage particles. The cell lysis cycle results in the generation of new phage particles that are released by cell lysis of the host.

Certain average phage can exhibit cytolytic activity, and this tendency can change as the host bacteria change. To demonstrate this phenomenon, two bases in 10 MRSA clinical isolates. The cell lysis activity of Aureus phage was tested by plaque analysis (Table 1). Phage φ11 showed cell lysis activity in 10 out of 10 clinical MRSA isolates and φ80α showed cell lysis activity in 6 out of 10 clinical MRSA isolates. Therefore, reporter assays dependent on the natural longevity of the phage can be expected to sporadically exhibit cytolytic activity.

In 10 clinical MRSA isolates, The cell lysis activity (denoted by the letter "x ") of the aureus primer phage 11 and &

MRSA isolate	φ11	φ80α
One	x	
2	x	
3	x	x
4	x	x
5	x	x
6	x	
7	x	x
8	x	
9	x	x
10	x	x

In addition, virus-based reporter assays such as phage-based reporters suffer from limited reactivity (i.e., analytical inclusiveness) due to restriction of the phage host range caused by host-based and prophage-derived phage resistance mechanisms . These resistance mechanisms can target or inhibit native phage DNA to denature or inhibit phage DNA and function. This resistance mechanism includes restriction systems that cleave phage DNA and CRISPR systems that suppress phage-derived transcripts.

Both cell lytic activity and phage resistance may be inhibitory in an assay based on reporter phage. The cytolytic activity can suppress the signal by destroying or inhibiting the cell that produces the detectable signal, thereby affecting the detection limit by reducing the amount of detectable signal or by blocking the generation of detectable signal. The phage resistance mechanism may limit the host range of the phage and limit the inclusion of the phage-based reporter, which may also reduce the amount of detectable signal or block the generation of detectable signal, . The cytolytic activity and phage resistance caused by the integration of phage DNA in the reporter phage can result in false-negative results in a phage reporter-integrated assay.

III. Non-replicating transduced particles (NRTP)

A. Disruption / compensation-based methods for producing non-replicating transduced particles

1) Silent mutation / compensation packaging system

The present invention includes a method for generating NRTP using a silent mutation / compensation-based method.

A non-replicating transduction particle packaging system is based on introducing a silent mutation into the genome component of the virus that is recognized by the viral packaging machinery as an element in which genome packaging is initiated during viral production. Examples of such elements include the pac-site sequence of pac-type bacteriophage and the cos-site sequence of cos-type bacteriophage.

Since these packaging initiation sites are found primarily in the coding region of the gene essential for viral production, silent mutations are introduced such that the pac-site is no longer recognized as a packaging initiation site by the viral packaging machinery. At the same time, the mutation does not destroy the gene whose region is encoded. By destroying the packaging site sequence, the mutated virus can undergo a cell lysis cycle, but the genome DNA can not be packaged in the packaging unit.

Foreign reporter nucleic acid molecules, such as plasmid DNA, may be introduced into the pooled host cells as viral genomes with mutated packaging initiation site sequences. The foreign reporter nucleic acid molecule may comprise a native packaging initiation site sequence. Foreign reporter nucleic acid molecules can be introduced into cells and replicated in cells. When the mutated virus undergoes a cell lysis cycle, the expressed viral packaging machinery packages the foreign reporter nucleic acid molecule with a native packaging initiation site sequence in the viral packaging unit. The viral genome is not packaged in a packaging unit because the packaging initiation site sequence has been mutated. In certain embodiments, the mutation in the packaging initiation site sequence comprises a silent mutation that blocks cleavage of the packaging initiation sequence and does not inhibit expression of the gene product comprising the packaging initiation site sequence. This produces a viral structural component with non-replicating transduced particles, e. G., Replicated foreign nucleic acid molecules.

An example of such a system is based on bacteriophage P1, pac-type phage. In one embodiment, the plasmid comprising the native P1 pac-site is transformed into a cell. Cells are primed with the P1 promoter genome. The P1 promoter genome contains a silent mutation in the pac-site sequence encoded in the pacA gene of P1. Once the cell lysis cycle of the prophage is induced, the system produces P1-based transduced particles with plasmid DNA. Examples of silent mutations suitable for this system are described in U.S. Patent Nos. 5,104,508, 7,900, 7,2002, filed November 7,2002, which is incorporated herein by reference in its entirety. 2005/0118719. An example is also found in SEQ ID NO: 2 presented below (P1 pac-site with silent mutation, lower case indicates mutated base).

Figure 1 illustrates an example of the design and function of a silent mutation / compensation-based P1 plasmid packaging system 100 in accordance with embodiments of the present invention. In this system, The E. coli cell 101 is enriched with the P1 prophage 102 containing a silent mutation in the packaging initiation site sequence (e.g., the pac-site). This cell is transformed into a plasmid 103 containing a native pac-site, which is replicated in the cell to form plasmid conchamer 104. The plasmid may also contain a reporter gene encoding the reporter molecule. When the cell lysis cycle of P1 protease is induced, P1 protease is ablated from the

bacterial genome and P1 structural component 105, such as a capsid protein, is expressed. The P1 structural component only packages DNA containing the native pac-site (e. G., Plasmid DNA) and thus produces a non-replicating transgenic particle 106 with plasmid DNA (e. G., A reporter gene) .

An exemplary vector for use in a silent mutation / compensation-based Pl plasmid packaging system is shown in FIG. Details of how strains and vectors in a silent mutation / compensation-based P1 plasmid packaging system are constructed are described in detail in Example 1 below.

2) Fruiting / compensation-based packaging systems

The present invention includes a method for generating NRTP using a loss / compensation-based method.

This non-replicating transduction particle packaging system is based on the deletion of the genomic component of the virus that is recognized by the viral packaging machinery as a factor in which the genomic packaging is initiated during viral production. Examples of such elements include the pac-site sequence of pac-type bacteriophage and the cos-site sequence of cos-type bacteriophage. These packaging initiation sites are found primarily in the coding region of the gene essential for virus production. In some embodiments, only the packaging initiation site is deleted so that the mutated virus undergoes a cell lysis cycle but can not package the genomic DNA. For example, SEQ ID NO: 6 is an example of a P1 pacA gene in which the pac-site sequence is deleted (the lower case is deleted pac-site sequence). In another embodiment, the entire gene including the packaging initiation site is deleted. For example, SEQ ID NO: 8 represents the deletion of the terS gene (lower case is the deleted sequence).

In one example, the genome of a cell is enriched with a viral genome in which the packaging initiation site has been deleted. A compensating plasmid is introduced into the cell, and the plasmid DNA contains a gene with a packaging initiation site sequence that compensates for the packaging initiation site sequence deleted in the viral genome. When the mutated virus undergoes a cell lysis cycle, the viral packaging protein is packaged into a packaging unit of the plasmid DNA due to the packaging initiation site, and non-replicating transduced particles with the cloned plasmid DNA are produced.

In some embodiments, the deletion / reward is preferably designed such that there is no homology between the mutated viral DNA and the compensating exogenous DNA. This is because the lack of homology between the mutated viral DNA and the compensating exogenous DNA can avoid the possibility of homologous recombination between two DNA molecules into which the packaging sequence can be reintroduced into the viral genome. One strategy for achieving homozygosity is to delete the entire gene containing the packaging initiation site sequence from the viral genome and then to compensate for this gene with a foreign DNA molecule containing fewer sequences than the DNA sequence deleted from the virus will be. In this strategy, the compensating DNA molecule is designed to express the gene deleted from the virus.

Another example of such a system is provided using bacteriophage phi 80 alpha, pac-type phage. This phage genome is small in terminal bacterial cells and contains a small terminally derived gene with a deletion of the pac-site of the pac-type protein A.phi.80. A plasmid containing a complementary small ternase gene with a native pac-site is transformed into a cell. When the cell lysis cycle of the enriched protease is induced, the bacteriophage packaging system does not package the plasmid DNA into the native bacteriophage DNA but packages it into the offspring bacteriophage structural component. Thus, this packaging system produces non-replicating transgenic particles with plasmid DNA.

Figure 3 illustrates an example of the design and function of a pac-site deletion / compensation plasmid packaging system 300 in accordance with embodiments of the present invention. The bacterial cell 301 is enriched with a pac-type phage 302 in which a small terminalase (terS) gene has been deleted. This cell is transformed into a rolling circle replicating plasmid (303) containing a small terminal gene that compensates for the terS gene deletion in the phage. The small terminator gene contains the packaging initiation site sequence, e.g., the pac-site. Plasmid 303 may also contain a reporter gene encoding the reporter molecule.

Protein complexes, including small tTNase and large TTN protein, can recognize and cleave double-stranded DNA molecules at or near the pac-site, allowing the plasmid DNA molecules to be packaged in a phage capsid. When the protein is induced in the cell, the cell lysis cycle of the phage produces the phage's structural protein (304) and the phage's large terminal protein (305). The compensatory plasmid is replicated and a small terminal antigen protein 306 is expressed. The replicated plasmid DNA 307 containing the terS gene (and the reporter gene) is packaged in a phage capsid to generate non-replicating transfected particles 308 having only the plasmid DNA. Figure 4 shows an example of a vector used in a pac-site deletion / compensation plasmid packaging system. Further details of the components and construction of the pac-site deletion / compensation plasmid packaging system are set forth in Example 2 below.

B. Pathogenic island-based packaging system

Pathogenic islets (PTI) are a subset of the horizontally transferred gene elements known as the genomic islands. There is a family of highly mobile PTIs in Staphylococcus aureus that is induced to be resected and replicated by certain residual proteins. These PTIs are packaged in small headed phage-like particles and delivered at a frequency corresponding to the plaque-forming potency of the phage. This process is referred to as the SaPI ablation replica-packaging (ERP) cycle, and high-frequency SaPI delivery is referred to as SaPI-specific delivery (SPST) to distinguish it from authentic generalized transduction (CGT). SaPI is parallel to bacteriophages and has a highly conserved genetic organization that is distinct from all other horizontally acquired genomic islands. SaPII -encrypted and SaPIbov2-encoded integrase are required for ablation and integration of the corresponding elements, which is presumed to be the same for other SaPIs. Phage 80α can induce several different SaPIs including SaPI1, SaPI2, and SaPIbov1, and φ11 can induce SaPIbov1 but not two other SaPIs.

Figure 5 shows the natural process for genome island (GI) packaging 500 by bacteriophage. Naturally, bacterial cells 501, which have been rounded off with a suitable protease 503 and have GI 504, can produce phage particles 512 with GI conchalamer. In this process, when the phage is introduced into the cell lysis cycle, the phage genome is excised (not shown) from the bacterial genome 502, and then the capsid component 505 and the large terminal antigen protein (TerL) Express the bacteriophage protein. Induction of the prophage also triggers GI excision through expression of the GI integrase protein (int) (507). In a manner similar to the truncated phage genome (not shown), GI is circularized (508), expressed a small terminal protein (TerS) (509), and cloned to begin to generate GI conchamer (510) . Next, the GI conchamer is cut through the pac-site sequence in the GI genome by combining and binding the TerL gene with the GI TerS gene, and then the GI conchatamer is packaged (511) in the phage capsid to form the GI conchamer Thereby generating the holding phage particle 512.

In the natural system shown in Fig. 5, the cell lysate produced from phage contains both native and GI-containing phage particles. The native phage particles are the result of the packaging of the native phage genome due to the recognition of the pac-site within the phage genome concomitant.

1) Design of genome island (GI) packaging system And function

The method of the present invention for generating NRTP includes a GI based-packaging system.

Compared to plasmid packaging systems, natural GI-packaging systems have the advantage that the DNA being packaged is derived from the genomic region in the bacterial genome and thus does not require a plasmid by the bacterial host.

In some embodiments, the invention includes a bacterial cell packaging system for packaging a reporter nucleic acid molecule in a non-replicating transduced particle, wherein the bacterial cell comprises a charged bacteriophage genome lacking a packaging gene, Phage, or bacteriophage (e.g., helper phage) for the movement of nucleic acid molecules, and includes other nucleic acid molecules including reporter nucleic acid molecules and packaging genes. Genome island-based systems are described, for example, in S. Aureus Pathogenic Island (SaPI), this. The collision concealment grasp P4 and the helper grasp P2, and the enterococcal concealment grasp P7 and the helper grasp P1.

Using a GI-packaging system, foreign nucleic acid sequences can be packaged by bacteriophage. This can be accomplished by integrating the foreign nucleic acid sequence into GI.

In order to remove the native phage in this process, a small terminal gene of the phage can be deleted. The small ternary gene sequence contains the pac-site sequence of the native phage and this deletion has the effect of blocking the packaging of the native phage DNA. In other embodiments, only the pac-site of the small terminalase gene can be deleted. The GI to be packaged contains a small terminal glutamate gene that expresses a pac-site and a suitable small terminal protease, and only GI DNA can be packaged in the system.

Figure 6 illustrates an example of the design and functionality of a GI-based packaging system 600 in accordance with embodiments of the present invention. In this system, the bacterial cell 601 has a genome 603 that has been appropriately digested with a suitable protease from which a small terminal gene has been deleted, and the genome 602 of the cell has a GI 604. If the phage is induced into the cell lysis cycle, the phage genome is excised from the bacterial genome 602 (not shown). The phage genome expresses a bacteriophage protein comprising a capsid component (605) and a large terminal antigen protein (TerL) (606). Induction of the prophage also triggers GI excision through the expression of the GI integrase protein (int) (607). In a manner similar to the truncated phage genome (not shown), GI is circularized (608), expressed in small terminal antigenic protein (TerS) (609) and cloned to form GI conchamer (610). Next, the phage TerL gene and the GI TerS gene are combined and bound and the GI conchamer is cut through the pac-site sequence in GI DNA. Next, the GI conchalamer is packaged (611) in the phage capsid to obtain the phage particle 612 having the concretamer. In this system, phage DNA will not be packaged in phage particles because it lacks the terS gene containing the pac-site sequence of the phage, and therefore can not be recognized by the expressed GI TerS and phage TerL proteins.

When the phage particle containing the packaged GI DNA is administered to the recipient cell, the phage will bind to the surface of the recipient cell and introduce the packaged GI DNA conchamer into the cell. GI will once again integrate into the specific part of the recipient cell's genome, once again expressing the integrase protein inside the cell. If the foreign DNA sequence is included in the GI prior to packaging, the packaging system will deliver the foreign DNA sequence to the recipient cell and integrate the foreign DNA sequence into the genome of the recipient cell.

2) Integraze Missing GI-based packaging system

In another embodiment, the packaging system is designed such that the packaged GI DNA can not be integrated into the genome of the recipient cell. This can be achieved by compensating for the deletion by deleting the integrase gene in GI and causing expression of the integrase gene in trans from GI. In this way, integrase proteins can be used for the ablation of GI in packaging host cells, and GI DNA packaged in bacteriophage can not express the integrase protein because it does not contain the integrase gene, and therefore the integration of the delivered GI .

Figure 7 illustrates the design and functionality of a GI-based packaging system lacking the int gene 700 according to embodiments of the present invention. In this system, the bacterial cells 701 are rendered usable with a suitable protease 703 from which a small terminal gene has been deleted. Cell genome 702 has a deleted int gene 705 operably linked to a suitable promoter with GI 704 in which the integrase protein (Int) has been deleted. Thus, the int gene can express the integrase protein (Int) (706) trans from GI. When the phage is induced into the cell lysis cycle, the phage genome is expressed (not shown) from the bacterial genome 702 and then expressed in the bacteriophage protein containing the capsid component 707 and the large terminal antigen protein (TerL) 708 do. In addition, phage induction triggers GI excision through expression of integrase protein (706). In a manner similar to the truncated phage genome (not shown), the rescued GI is circularized (709), expresses small tiniase protein (TerS), and replicates to begin to form GI conchamer (711). The GI terat gene and the GI TerS gene can be combined and combined to cleave the GI con- catamer through the pac-site sequence in the GI DNA. Next, the GI con- catamer is packaged in the phage capsid (712) It is possible to generate the holding phage particles 713. In this system, phage DNA will not be packaged because it lacks the terS gene containing the pac-site sequence of the phage and thus can not be recognized by the expressed GI TerS and phage TerL proteins.

When a phage particle containing packaged GI DNA lacking the int gene is administered to the recipient cell, the phage will bind to the surface of the recipient cell and then introduce the packaged GI DNA conchamer into the cell. GI can not express an integrase protein because of the absence of integrase gene in the cell and can not be integrated into a specific site in the genome of a recipient cell. If the foreign DNA sequence is included in the GI before packaging, the packaging system delivers the foreign DNA sequence to the recipient cell, and the delivered DNA sequence is not integrated into the genome of the recipient cell at a specific site for GI integration.

3) Integraze Lacking SaPIbov2 - Design of the base packaging And function

In some embodiments, the NRTP generation method uses GI SaPIbov2 and bacteriophage < RTI ID = 0.0 ># 11 < / RTI > in a GI-based packaging system. Other embodiments may utilize SaPIbov1 and SaPIbov2 of SaPI with other SaPI GI and other suitable bacteriophages including SaPI1, SaPI2, SaPIbov1 and SaPIbov2 of SaPI, and bacteriophage φ11 with bacteriophage 80α. Those skilled in the art will know how to develop a GI-based packaging system that lacks the int gene as disclosed in Section IIA.

FIG. 8 illustrates the design and functionality of a SaPIbov2-based packaging system 800 lacking an int gene according to embodiments of the present invention. In this system, Aureus cells (801) are elaborated by φ11 (803) in which a small terminal gene is deleted. The cell's genome 802 has a deleted int gene (805) with SaPIbov2 (804) in which the integrase (int) gene is deleted and also operatively linked to the constitutively expressed PclpB gene promoter. The int gene can express the integrase protein (Int) (806) as a trans from SaPIbov2. When the phage is induced into the cell lysis cycle, the phage genome expresses the bacteriophage protein including the capsid component 807 and the large terminal antigen protein (TerL) after ablation (not shown) from the bacterial genome 802. Prophage induction triggers SaPIbov2 resection through the expression of integrase protein (807) (808). In a similar fashion to the ablated phage genome (not shown), the rescued SaPIbov2 was circularized 809, expressed a small terminally expressed protein (TerS) 810, replicated and started to form SaPIbov2 concatamer 811 do. The SaPIbov2 concatamer can be cleaved through the pac-site sequence in the SaPIbov2 DNA, followed by the SaPIbov2 concatamer being packaged in the phage capsid (812) and the SaPIbov2 concatamer Gt; 813 < / RTI > In this system, the phage DNA will not be packaged because it lacks the terS gene containing the pac-site sequence of the phage and thus can not be recognized by the expressed SaPIbov2 TerS and phage TerL proteins.

IV. Reporter

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

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

In other embodiments, the reporter molecule may be an enzyme that mediates the luminescent reaction (luxA, luxB, luxAB, luc, ruc, nluc, etc.). The reporter molecule may be selected from the group consisting of bacterial luciferase, eukaryotic luciferase, enzymes suitable for colorimetric detection (lacZ, HRP), proteins suitable for immunodetection such as affinity peptides (His-tag, 3X-FLAG) (Ribozyme) or a selectable marker such as an antibiotic resistance gene (ampC, tet (M), CAT, erm). Other reporter molecules that are well known in the art can also be used to generate signals for detecting target nucleic acids or cells.

In another embodiment, the reporter molecule comprises a nucleic acid molecule. In some embodiments, the reporter molecule is an aptamer that has a specific binding activity or exhibits enzymatic activity (e. G., Aptazyme, DNAzyme, ribozyme).

Reporter and reporter analyzes are described in more detail in Section V of this application.

V. NRTP And Reporter Analysis

A. Inducer Reporter Analysis

The present invention encompasses methods of using NRTP as reporter molecules for use with intrinsic or naturally occurring ducers that target gene promoters in viable cells. The NRTP of the present invention can be engineered using the method disclosed in Section III and Examples 1-6 below.

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

Figure 9 shows the genomic locus (900) of the target cell with the gene (902) encoding the two genes, the inducer and the target gene (903). Also shown is a reporter nucleic acid molecule 904 comprising a reporter gene 905 operably linked to a target gene promoter 906 of the target cell. The reporter nucleic acid molecule 904 can be introduced into cells via NRTP.

In the native cells, when the inducer gene 902 is expressed and produces the inducer protein 907, the inducer protein 907 can induce a target gene promoter 906 operatively linked to the target gene, Thus producing the expression of the target gene and the target gene product 908.

If the reporter nucleic acid molecule 904 is present in the target organism, then the inducer 907 can also induce the target gene promoter 906 present in the reporter nucleic acid molecule 904, thus producing a detectable signal Resulting in the expression of reporter gene 905, which produces reporter molecule 909.

Thus, the generation of a detectable signal from the reporter molecule 909 indicates the presence of the cell based on the presence of the inducer protein 907 in the target cell.

One) VanR Reporter system

In one embodiment, the reporter system comprises an NRTP comprising a reporter nucleic acid molecule (e.g., a plasmid). The reporter nucleic acid molecule can be configured to detect VanR, an inducer of the promoter of the vancomycin resistance (vanA) gene in Enterococcus faecium (or E. faecalis). This reporter plasmid has a reporter gene operably linked to the vanA gene promoter.

Figure 10 outlines the design and function of the VanR Reporter system. Fig. Describes the region of transposon Tn1546 (1001) that may be present in fauce. The Tn1546 transposon may comprise the vanR inducer gene (1002) and the vanA target gene (1003). Also depicted in this figure is a reporter nucleic acid molecule 1004 packaged in NRTP that can be introduced into cells. The reporter nucleic acid molecule 1004 comprises a reporter gene 1005 operably linked to a promoter PH (1006) that controls the expression of the vanHAX operon comprising the vanA gene. In wild-type cells, when the vanR gene (1002) is expressed to produce the VanR protein (1007), VanR can induce PH (1006) in the Tn1546 transposon and thus induce the expression of the vanA gene,). When the reporter nucleic acid molecule 1003 (vector) is present in the target organism, VanR can induce PH 1006 in the reporter nucleic acid molecule 1003, thus causing expression of the reporter molecule 1009. Thus, the production of the reporter molecule indicates the presence of VanR in the target cell.

Examples of promoters suitable for the development of VRE assays include the vanA gene promoter and the vanB gene promoter (Arthur, M., et al., The VanS sensor negatively controls VanR-mediated transcriptional activation of glycopeptide resistance genes of Tn1546 and related elements in J. Bacte-riol., 1997. 179 (1): p. 97-106).

2) TcdD Reporter system

In another embodiment of this system, the reporter nucleic acid molecule is introduced into the cell using NRTP. The reporter nucleic acid molecule is Mr. And TcdD, which is an inducer of the promoter of the toxin A and B genes of Dipicyl (tcdA and tcdB, respectively). The reporter nucleic acid molecule comprises a reporter gene operably linked to a tcdA gene promoter.

Figure 11 outlines the design and functionality of a TcdD reporter system in accordance with embodiments of the present invention. Fig. Describes the region of the transposon PaLoc 1101 that may be present in the diphenyl. The PaLoc transposon may contain the tcdD gene (1102) and the tcdA target gene (1103). Also depicted in this figure are reporter nucleic acid molecules 1104 (e.g., vectors) that can be introduced into cells using NRTP. The reporter nucleic acid molecule 1104 comprises a reporter gene 1105 operably linked to a tcdA gene promoter (PtcdA) 1106.

In the native cells, when the tcdD gene is expressed to produce the TcdD protein 1107, TcdD can induce PtcdA (1106) in the PaLoc transposon 1101, thus causing the expression of the tcdD gene 1103, A protein (1108).

When the reporter nucleic acid molecule 1104 is present in the target organism, TcdD can induce PtcdA (1106) in the reporter vector, thus causing the expression of the reporter molecule 1109. Thus, the production of reporter molecule 1109 indicates the presence of TcdD in the target cell.

Seed. Examples of promoters suitable for the development of the diptheric assay include the tcdA gene promoter and the tcdB gene promoter (Karlsson, S., et al., Expression of Clo-stridium difficile Toxins A and B and Their Sigma Factor TcdD Is Controlled by Temperature Infect. Immun., 2003. 71 (4): p. 1784-1793).

Target Cells and Inducers : Target cells can include inducers related to eukaryotic and prokaryotic targets.

Vector delivery system : The delivery of the vector containing the recombinant DNA can be performed by a non-biological or biological system. But are not limited to, liposomes, virus-like particles, transgenic particles derived from phages or viruses, and conjugation.

3) Bacteriophage-based SarS Reporter system

In another embodiment of the invention, the reporter nucleic acid molecule is selected from the group consisting of S. Is constructed to detect SarS, the inducer of the promoter of the protein A gene (spa) in Aureus. The reporter nucleic acid molecule can be introduced into cells in NRTP and includes the bacterial luciferase genes luxA and luxB operatively linked to the spa gene promoter (Pspa). The reporter nucleic acid molecule may, for example, It is delivered to Aureus. If SarS is present in the cell, it will induce the expression of the luxAB gene, thus producing a luciferase enzyme capable of producing a luminescent signal.

Figure 12 outlines the design and function of the SarS Reporter system according to embodiments of the present invention. Fig. 12 is a graph showing the relationship between the SarS gene (1202) and the Ssp gene (1203). Describes the region of the Aureus genome 1201. Also depicted is a reporter nucleic acid molecule (e. G., A vector) 1204 that is delivered to the cell by NRTP, which is operably linked to a promoter Pspa 1206 that controls the expression of the spa gene 1203 luxAB reporter gene (1205).

In the native cells, when the sarS gene 1202 is expressed to generate the SarS protein 1207, Can induce Pspa 1206 in the Aureus genome transposon, thus inducing the expression of the spa gene 1203 and producing protein A 1208.

When the reporter nucleic acid molecule 1204 is present in the target organism, SarS 1207 is capable of inducing Pspa 1206 in the reporter nucleic acid molecule 1204 and thus is capable of inducing luciferase enzyme 1209 Lt; RTI ID = 0.0 > luxAB. ≪ / RTI > Thus, the production of luciferase indicates the presence of SarS in the target cells.

B. Enzyme Reporter Assay

The present invention includes a system for the detection of enzymes in viable cells using a blocked substrate capable of being released by an enzyme in a target cell according to embodiments of the present invention.

Figure 13 shows the design and function of an intracellular enzyme detection system. The reporter molecule-expression vector 1301 is delivered to the target cell 1302 by NRTP (not shown). The reporter molecule-expression vector 1301 penetrates the target cell 1302 through NRTP to deliver the reporter molecule gene 1303 to the target cell 1302 and then the reporter molecule 1304 is transferred from the reporter molecule gene 1303 Lt; / RTI > In addition, a blocked substrate 1305 can be added to the target cell 1302 and infiltrated. When the target intracellular enzyme 1307 is present in the target cell 1302, the enzyme 1307 can remove the containment component of the occluded substrate 1305, thus producing an unsealed substrate 1308. The unlabeled substrate 1308 then reacts with the reporter molecule 1304 inside the cell 1302 and the product of this reaction produces a detectable signal 1309.

Target Cells and Enzymes: Target cells are enzymes associated with eukaryotic and prokaryotic targets, e. G. Lt; RTI ID = 0.0 > a-lactase. ≪ / RTI >

Vector delivery system: The delivery of the vector containing the recombinant DNA can be performed by a non-biological or biological system. But are not limited to, liposomes, virus-like particles, transgenic particles derived from phages or viruses, and conjugation.

Reporter Molecules and Blocked Substrates: Various reporter molecules and blocked substrates may be those disclosed in Daniel Sobek, JR, Enzyme detection system with caged substrates, 2007 Zymera, Inc.

1) Bacteriophage-based < RTI ID = 0.0 > Lactamase Reporter

In one embodiment, the reporter molecule-expression vector can be carried by NRTP, whereby the vector can be delivered to the bacterial cell. The expressed reporter molecule may be renilla luciferase, and the blocked substrate may be blocked Reilera luciferin, thereby cleaving the blocking compound from luciferin, which is endogenous to the target cell, to block the [beta] -lactamase enzyme, Lt; RTI ID = 0.0 > luciferin. ≪ / RTI >

Figure 14 shows the design and function of the? -Lactamase enzyme detection system according to embodiments of the present invention. The Renilla luciferase-expression vector carried by the bacteriophage-based NRTP 1401 is the target S. Is added to the Aureus cells (1402). The renilla luciferase-expression vector can infiltrate target cells 1402 using NRTP comprising a vector. NRTP can express Renilla luciferase 1404 from a gene after delivering Renilla luciferase gene (1403) to target cell (1402). Also, the blocked Renilla luciferin (1405) can be added to the target cell (1402) and infiltrated. When an intracellular [beta] -lactamase (1407) is present in the target cell (1402), the enzyme can remove the containment component of the blocked luciferin (1406) and thus produce the unlabeled luciferin (1408). Next, the unlabeled luciferin 1408 can react with the renilla luciferase 1404 inside the cell 1402, and the product of this reaction generates the luminescence 1409.

In this manner, when target cells containing beta -lactamase are exposed to NRTP and blocked luciferin, the cells will display a luminescent signal indicative of the presence of beta-lactamase present in the cell.

C. Intracellular Molecular Reporter

The present invention includes a system for the detection of molecules in viable cells using switchable molecules capable of producing a detectable signal upon binding to the target molecule.

Figure 15 shows the design and function of a switchable molecular (SM) -based intracellular molecular detection system. The SM-expression vector (1501) is delivered to the target cell (1502) in NRTP. The SM-expression vector 1501 can penetrate the target cell 1502 and can deliver the SM gene 1503 to the target cell 1502. Next, the SM protein 1504 can be expressed from the SM gene 1503. Next, the SM protein 1504 can bind to the target molecule 1505 inside the cell, and thus form the SM-target molecule complex 1506. The combination of the SM 1504 and the target molecule 1505 alters the steric structure of the SM 1504 so that the SM can be coupled to the substrate. Substrate 1508 can be added to penetrate cells 1502. The bound SM inside the cell 1502 can also bind to the substrate to form the SM-target molecule-substrate complex 1509. Finally, the binding of the target molecule-SM conjugate to the substrate 1508 produces a detectable signal 1510. Thus, a detectable signal generated by this system indicates the presence of a target molecule inside the cell.

Target Cells and Molecules: Various eukaryotic and prokaryotic targets can be used, and switchable aptamer-based SMs can be used as described in Samie Jaffrey, JP, Coupled recognition / detection system for in vivo and in vitro use, 2010, Cornell University Can be designed to target various nucleic acid and amino acid-based intracellular molecular targets.

Vector delivery system: The delivery of the vector containing the recombinant DNA can be performed by a non-biological or biological system. But are not limited to, liposomes, virus-like particles, transgenic particles derived from phages or viruses, and conjugation.

1) non-replicating transgenic particles / switchable Aptamer -base Intracellular Molecular Reporter System

In one example of this method, a switchable molecular-expression vector can be carried by the bacteriophage-based transducing particles such that the vector can be delivered to the bacterial cell. The expressed switchable molecule may be a switchable aptamer designed to undergo a conformational change upon binding to an intracellular target molecule. The conformational change causes the aptamer to continue to associate with the fluorophore, and the fluorophore exhibits enhanced fluorescence when bound by the aptamer.

Figure 16 shows the design and function of a bacteriophage / switchable-aptamer (SA) -based intracellular molecular reporter system. The SA-expression vector 1601 carried by the NRTP is added to the target cell 1602. NRTP can deliver the SA-expression vector 1601 and the SA-expression gene 1603 to the target cell 1602. Next, the SA protein 1604 can be expressed from the SA gene 1603. Next, the SA protein 1604 can bind to the target molecule 1605 inside the cell to form the SA-target molecule complex 1606. The combination of the SA 1604 and the target molecule 1605 changes the steric structure of the SA so that the SA can bind (1608) with the fluorophore. The fluorescence terminal 1607 can be added to the cells and infiltrated (1608). Bound SA inside the cell can also bind to the fluorescent moiety to form the SA-target molecule-fluorophore complex (1609). Finally, the binding of the conjugate of the target molecule-SA to the fluorophore enhances the fluorescence 1610 of the fluorophore. Thus, a detectable fluorescent signal generated by this system indicates the presence of a target molecule inside the cell.

D. Transcript Reporter Analysis

The invention includes reporter assays that include an antisense RNA-based method for detecting target transcripts in viable cells by causing the expression of the reporter molecule when the target transcript is present in the cell.

Certain intracellular methods in the art for inhibiting gene expression use small interfering RNAs, such as double-stranded RNA (dsRNA), to target genes transcribed in cells. dsRNA comprises antisense and sense strand delivered to or expressed in a cell, strands of dsRNA acting through a trans-action inhibiting mechanism, and one strand (typically an antisense strand) comprises a target gene sequence (RNA transcript) To inhibit the expression of the target gene sequence. Double-stranded RNA molecules have been shown to block (degrade) gene expression in a highly conserved regulatory mechanism known as RNA interference (RNAi). WO 99/32619 (Fire et al. Disclosed is the use of dsRNA of at least 25 nucleotides in length to inhibit the expression of a gene in an elegance. In addition, dsRNA can be expressed in plants (WO 99/53050, Waterhouse et al .; and WO 99/61631, Heifetz et al.), Fruit flies (Yang, D., et al., Curr. -1200), and mammals (see WO 00/44895, Limmer; and DE 101 00 586.5, Kreutzer et al.). However, the binding of any strand of the dsRNA to the target gene may be non-specific. If a similar mechanism has been applied to the detection system, this non-specific binding can lead to a high false positive rate, and this is therefore unsuitable for the development of clinically useful detection systems.

Existing trans-action inhibitory mechanisms have been found to be unsuitable for the development of clinically useful detection systems. For example, some methods cause a false positive rate of up to 90% when achieving a high level of non-specific signal and 90% analytical sensitivity (see U.S. Patent No. 8,329,889). A specific method for post-transcriptional control of gene expression using a cis-repressed marker transcript, such as a green fluorescent protein marker, has been developed in which the ribosome binding site of the marker with the trans-activated RNA transcript is located in the cis- . When the trans-activated RNA transcript is bound to a cis-suppression marker transcript, the hairpin structure of the cis-suppression marker transcript is altered and the upstream ribosome binding site of the marker gene is

exposed and the marker gene is transcribed and expressed. However, these methods have never been used for the detection of endogenous transcripts, and have never been above the basic switching mechanism for controlling the expression of genes in cells.

1) Nucleic acid molecule interaction and mechanism

The methods of the present invention utilize the advantages of a transposon-level regulatory mechanism involving antisense RNA (asRNA) machinery in cells to deliver nucleic acid molecules to cells. Antisense mechanisms include all forms of sequence-specific mRNA recognition resulting in reduced, eliminated, increased, activated, or altered expression of target transcripts (Good, L., Translated By Antisense Sequences. Cellular and Lioliou, E., RNA-mediated regulation in bacteria: from natural to artificial systems, New Biotechnology 2010. 27 (3): p 222- 235). Spontaneous asRNA is found in all three areas of life and affects RNA processing and transcription as well as messenger RNA (mRNA) destruction, inhibition and activation (Sabine, B., Antisense-RNA regulation and RNA interference. Biochimica et Biophysica Acta (BBA) - Gene Structure and Expression, 2001. 1575 (1-3): p. This mechanism has been investigated in the process of inhibiting protein synthesis for therapeutic use.

Antisense RNA is single stranded RNA complementary to the messenger RNA (mRNA) strand that is transcribed in the cell. The asRNA can be introduced into the cell to inhibit the translation of the complementary mRNA by physically blocking the translation machinery by base pairing with it. Antisense RNA annealing to the complementary mRNA target sequence, and translation of the mRNA target sequence, is disrupted as a result of the steric hindrance of ribosome access or ribosome reading.

The antisense RNA mechanism is catalyzed by silencing of catalyzed genes by targeting RNA-induced silencing complexes (RISCs), most typically double stranded RNA fragments (dsRNA, also referred to as small interfering RNAs (siRNAs) (RNAi), which is a related process that triggers the initiation of RNA interference. annealing of strands of dsRNA molecules to mRNA or DNA can be accomplished either by a ribonuclease in the cell or by cleavage of the target RNA by the antisense compound itself to form a duplex RNA, a hybrid RNA / DNA duplex, or a duplex RNA reassembly precursor tRNA Can be rapidly degraded.

The RNAi pathway is found in many eukaryotes and is initiated by enzyme dicers, which cut long double-stranded RNA (dsRNA) molecules into short double-stranded fragments of about 20 nucleotides, called siRNA. Each siRNA is unwound with two single-stranded RNA (ssRNA), the passenger strand and the guide strand. The recipient strand is disassembled and the guide strand is integrated into an RNA-induced silencing complex (RISC). In posttranscriptional gene silencing, the guiding strand forms a base pair with the complementary sequence in the messenger RNA molecule and is cleaved by a protein called the agonist, a catalytic component of the RISC complex.

With respect to the nucleic acid interactions of the mechanism of the present invention, the interaction between the reporter transcript and the target transcript may be based on base pairing (e.g., "kissing complex") between loops present in two transcripts, Can depend on base pairing between single strand (ss) regions. In some cases, kissing complex formation is sufficient to mediate the desired effect of the interaction, and in other cases the propagation of the primary contact will result in the interaction leading to the desired result.

2) Transcript - translation of reporter structure through level control Cis Mechanisms for inhibition and trans-activation

The following description illustrates a transcriptor reporter system based on various inhibition / activation mechanisms that may be used in accordance with embodiments of the present invention. In each of Figures 17-20, the vector comprises a reporter construct comprising a reporter sequence, and a reporter construct in each of the figures, including regions that can be targeted for inhibition by cis-suppression sequences. The following disclosures provide non-limiting examples of various inhibitory mechanisms including transcriptional attenuation, translation attenuation, and transcript destabilization, and various activation mechanisms including steric conformational changes and cleavage.

Figure 17 shows an example of a system 1700 using a cis-suppression mechanism capable of targeting the 5 'UTR (untranslated region) of the reporter sequence 1702 on the reporter transcript 1703. The regions (5 'UTR 1701, RBS, coding region and 3' UTR) in the reporter sequence 1702 are also shown. The cis-suppression sequence 1705 is at the 5 'UTR (1701) of the maximum reporter sequence upstream of the reporter sequence. RNA polymerase 1704 transcribes the sequence of reporter construct 1703 from vector 1706.

At some point during transcription, the transcription process is terminated by the formation of a transcription termination (TT) stem-loop structure 1707 in the reporter transcript 1703 due to interactions within the transcribed cis-suppression sequence 1705. The transcription termination structure 1707 stops the RNA polymerase 1704 from transcribing the vector 1706 (1708). In some embodiments, a transcription termination protein (e. G., NusA in E. coli) binds with an RNA polymerase and / or transcription termination structure 1707 to stop transcription of the reporter construct.

When the target transcript 1709 is present in the cell, the target transcript 1709 binds with the reporter transcript 1703. In some embodiments, the binding between the target transcript and the reporter transcript is accomplished by base pairing of the nucleotides of each sequence. The interaction between the target transcript 1709 and the reporter transcript 1703 causes the transcription termination (TT) stem-and-loop structure 1707 to be cut 1710. Cleavage of the reporter transcript 1703 may occur by a cellular enzyme such as RNase III. In this case, the secondary structure of the target transcript is analyzed for the presence of the RNAse III consensus sequence of the ssRNA region of the secondary structure, for example 5'-nnWAWGNNNUUN-3 'or 5'-NAGNNNNCWUWnn-3' N "and" n "are some nucleotides," W "is A or U," N "represents a relatively stringent requirement for Watson- . When such a consensus sequence is found in the target transcript, the loop of transcription termination structure 1707 can be designed to be complementary to the RNAse III consensus sequence so that the ssRNA is hybridized in each RNA molecule, (1707). In the mecA transcript, loop T23 starting at nucleotide 1,404 has the sequence CAGAUAACAUUUU suitable for this approach.

In some embodiments, the cleavage site is manipulated in the reporter construct such that the reporter transcript is cleaved after transcription. In the example provided, the cutting can occur immediately adjacent to the position of the loop in the transfer terminator structure. The transcription is initiated again by RNA polymerase 1704 (1711). The truncation of the transcription termination (TT) stem-loop structure 1707 causes the remainder of the reporter sequence 1702 to be transcribed and subsequently translated. This produces detectable markers or selectable markers from the translated reporter molecule.

The transcription termination structure (1707) in prokaryotes includes a Rho-independent mechanism with a stem-loop structure that is 7-20 base pair lengths hatched with a cytosine-guanine base pair followed by the uracil residue chain. NusA binds to the transcription termination loop-loop structure 1707 and confers RNA polymerase during transcription of the poly-uracil sequence. The weak adenine-uracyl bond lowers the destabilizing energy for the RNA-DNA duplex, thereby releasing it from the RNA polymerase and dissociating. In eukaryotes, transcription termination structure 1707 is recognized by protein factors and includes cleavage of the new transcript followed by polyadenylation.

18 depicts a system 1800 for detecting the presence of a target transcript in a cell based on a cis-suppression mechanism that targets the ribosome binding site (RBS) 1801 of the reporter sequence 1702 in the reporter transcript 1703. [FIG. RBS 1801 is the sequence of mRNA that is joined by ribosome 1802 when initiating protein translation. The cis-suppression sequence 1705 is designed to associate with the RBS 1801 (e.g., the cis-suppression sequence 1705 is complementary to the RBS 1801 sequence). RBS 1801 binds to cis-suppression sequence 1705 and is isolated (inaccessible by ribosome 1802) to block translation of reporter transcript 1703. When the target transcript 1709 from the cell binds to the reporter transcript 1703, the target transcript 1709 has a higher binding affinity for the RBS sequence 1801 and the cis- And the RBS sequence 1801 is released, the steric structure of the reporter transcript 1703 is changed. This allows the ribosome 1802 to engage with the RBS 1801 to enable translation of the reporter transcript 1703.

19 depicts an exemplary system 1900 for detecting the presence of a target transcript in a cell based on a cis-suppression mechanism that targets the coding region ("AUG") 1901 of the reporter sequence 1702 in the reporter transcript 1703. [ ). The cis-inhibition sequence 1705 is configured (e. G., Complementary) to associate with the coding region 1901 of the reporter sequence 1702. The "AUG" start codon is shown as part of the coding region 1901. The combination of the cis-suppression

sequence 1705 and the coding region 1901 results in a steric structure resulting in the cleavage 1902 of the reporter structure 1703. Cutting of the reporter transcript 1703 blocks translation.

When the target transcript 1709 is present in the cell, the target transcript 1709 binds to the cis-suppression sequence 1705 in such a manner as to change the steric structure of the reporter transcript 1703. This change in steric structure blocks or eliminates the interaction between the cis-suppression sequence 1705 and the coding region 1901 of the reporter sequence 1702, thereby enabling translation of the reporter sequence 1702.

Figure 20 shows an exemplary system 2000 for detecting the presence of a target transcript in a cell based on a repressor mechanism using an unstable reporter transcript 2001. The reporter transcript 2001 is designed to be unstable to form an unstable three-dimensional structure that blocks translation. Reporter transcripts (2001) are defined as unstable if they exhibit a tendency for rapid degradation due to a variety of factors such as the activity of the exosome complex or digastosome. In the cell, the target transcript 1709 binds to a portion of the unstable reporter transcript 2001. In this example, the portion that results in the destabilization of the transcript is located in the 3 'UTR (2005) of the reporter sequence, which acts like a cis-suppression sequence of reporter construct 1703. The combination of the target transcript 1709 and the 3 'UTR 2005 of the reporter sequence results in truncation 2003 which stabilizes the reporter transcript 2001 and translates the translation 2004 of the reporter transcript 2001 . The cleavage occurs upon binding of the target transcript 1709, and acts to remove a part of the sequence which causes destabilization of the transcript. In this example, the target transcript 1709 binds to the 3 'UTR 405 of the reporter sequence, but the system 400 also allows the binding and cleavage to occur upstream of the 5' UTR, 5 'UTR, or downstream of the 3' UTR As shown in FIG. Binding and cleavage can occur outside the region required for translation of the reporter sequence (1702).

In some embodiments, the cis-suppression sequence itself comprises two sequences that are capable of binding to each other (e.g., are complementary to each other), and the stereostructure of the reporter transcript resulting from the combination of the two sequences is identical to that of the reporter sequence . ≪ / RTI >

3) Natural occurrence and synthesis system for inhibition / activation mechanism

Several naturally occurring and synthetically produced transcript-level mechanisms have been disclosed to demonstrate the individual mechanisms (i.e., conformational changes and cleavage) used in each of the examples shown in Figures 17-20.

Transcription termination was observed upon antisense RNA (asRNA) -mediated transcriptional attenuation. In one example, a stable duplex continues to form after two loop-loop interactions between RNAIII / repR mRNA. This complex stabilizes the Rho-independent terminator structure to stop elongation by RNA polymerase (RNAP).

The RBS isolation mechanism was initiated through the development of synthetic riboswitch systems. In this system, a sequence complementary to the RBS is located upstream of the RBS, resulting in a linker sequence between the two regions. After transcription of the mRNA, two complementary regions hybridize to produce a hairpin that blocks docking of the ribosome. To activate translation, a synthetic trans-activated RNA with an RBS sequence binds to the hybridized RNA, resulting in the RBS being exposed and available for translation.

In addition, translation blocking due to cleavage of the RNA has been described in a natural system in which asRNA MicC targets sequences within the coding region of ompD mRNA. Interaction promoted by Hfq causes cleavage of mRNA by RNase E.

Another natural mechanism proves cleavage to activate, not inhibit translation. this. The E. coli GadY asRNA targets mutual regions between the two genes of the gadXW operon. After formation of a stable helix between GadY and the 3 'UTR of gadX, RNase cleavage occurs in the transcript, stabilizing the gadX transcript and translating it.

4) Reporter sequence Cis - inhibition and target Transpositional Mechanism of steric structure change by bonding

The general mechanism used in the present invention is intermolecular nucleic acid molecule interactions that can lead to two consecutive mechanisms: (1) the steric transition of the secondary structure of the nucleic acid molecule, and (2) cleavage. A method of designing a reporter transcript capable of causing a conformational change between a cis-restrained stereostructure and a de-repressed stereostructure is disclosed herein wherein the stereostructure change is induced by the combination of a reporter transcript and a target transcript .

As described above, the reporter transcript may comprise a reporter sequence and the translation of the reporter gene sequence may be designed to be blocked by cis-suppression of the ribosome binding site (RBS) of the reporter gene.

In some embodiments, the following scheme can be used to design the reporter transcript of the present invention.

1) RNA Institute of Arts and Sciences, University at Albany, State University of New York (Mfold web server for nucleic acid folding and hybridization prediction. Nucleic Acids Res. 31 (13), 3406-15 (2003) // mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form) is used to calculate the RNA secondary structure using a secondary structure program such as Mfold.

2) Use on servers maintained by Graduate School of Information Science, Nara Institute of Science and Technology (NAIST), Department of Biosciences and Informatics, Keio Unive-rsity Japan (http://rna.naist.jp/ractip/) Intermolecular RNA interactions are calculated using software programs such as RNA-RNA Interaction Prediction using possible Integer Programming (RactIP).

3) Secondary Structure of RNA Visualization of the RNA secondary structure using visualization applet (VARNA) (http://varna.lri.fr/) for RNA, a Java lightweight applet dedicated to drawing.

The secondary structure of the target transcript is generated based on the lowest energy steric structure computed by MFold and can be visualized as VARNA.

The ssRNA region or target region can be identified in a target transcript that may be ideal for binding to a reporter transcript. In some examples, the secondary structure of the target transcript comprises a consensus sequence or loop sequence that is capable of binding to a portion of the reporter sequence. For example, methicillin resistant S. The mecA transcript of Aureus has an end loop containing a consensus YUNR sequence ("UUGG") that can be used to bind to the cis-suppression sequence of a reporter transcript. Analysis of the secondary structure of the target transcript reveals one or more ssRNA regions that may be suitable for binding to the cis-suppressing sequences. The cis-suppression sequence of the reporter transcript may be designed to bind to one or more ssRNA regions.

In some embodiments, the cis-suppressing sequence may be designed to bind to the RBS of the reporter sequence in a reporter transcript and to form a stem-loop structure within the reporter transcript, whereby the cis- Thereby blocking the binding of the enzyme. Upon binding of the cis-suppressing sequence to the ssRNA region of the target transcript, the RBS of the reporter sequence may be exposed and translation of the reporter sequence may be initiated.

In some embodiments, the cis-suppression sequence of the reporter transcript is designed to be located at the 5 ' end of the reporter sequence and may be designed to generate a stem-loop structure in the reporter sequence, thereby blocking the RBS sequence of the reporter sequence. The cis-restricted stem-loop structure can be designed to block the RBS sequence based on the lowest energy steric structure of the reporter transcript when calculated by MFold and visualized as VARNA. The predicted intermolecular interaction between the target transcript and the cis-inhibition sequence of the reporter transcript can be calculated by RactIP and visualized by VARNA. A diagram for visualizing the base pairing between the target transcript and the cis-suppression sequence of the reporter transcript as shown in Fig. 28 below can be drawn.

Interactions were obtained at the 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24 in the target and cis- , 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, , Or 50 or more nucleotides. The complementarity between the two sequences may be fully complementary, substantially complementary, or

partially complementary. The base pairing may be carried out across consecutive nucleotide sequences or regions within the target and cis-suppression sequences, e. G., As shown in FIG.

5) Cis -control Transcript Or reporter On the transcript Cutting mechanism for

The general mechanism used in the present invention is intermolecular nucleic acid molecule interactions that can lead to two consecutive mechanisms: (1) the steric transition of the secondary structure of the nucleic acid molecule, and (2) cleavage. Methods and systems for designing a reporter transcript using cutting are disclosed herein.

In some embodiments, the cleavage mechanism can be used in the systems and methods of the present invention for cis-inhibition or trans-activation. For example, as described above with reference to Figures 17, 19 and 20, the system can be used to expose a nucleic acid sequence of a reporter transcript to a cleavage enzyme (RNase) or isolate a single strand sequence recognized by a sequence specific RNAase Can be designed to take advantage of the cleavage mechanism.

In one example, a ribonuclease E (RNAse E) site can be designed in a reporter transcript (* denotes the cleavage site): (G, A) N (C, A) N , U, A) * (A, U) (C, U) N (C, A) (C, A). Kaberdin et al., Probeing substrate specificity of E. coli RNase E using a novel oligonucleotide-based assay. Nucleic Acids Research, 2003, Vol. 31, No. 16 (doi: 10.1093 / nar / gkg690).

In a cis-suppression system, the cis-suppression sequence can be incorporated into the design of the reporter transcript, thereby providing a single stranded region containing the sequence RNAse E recognition motif at the desired site where the stereostructure of the reporter transcript will be transcribed Exposed. In some embodiments, the cleavage site may be included in the transcriptional repression of the reporter transcript, for example, when the cleavage site is within the coding region of the reporter gene.

In the case of trans-deparment suppression systems, the cis-inhibitory transcript can be engineered to bind to the target transcript, thereby causing the interaction to cause a conformational change in the reporter transcript, isolating the single stranded region containing the RNAse E site.

The system can be designed such that the cis-suppression mechanism is due to the specific secondary structure generated by the steric structure of the cis-suppression sequence, such as the transcription termination structure disclosed above. In this example, cleavage serves to depressurize the reporter sequence. This can be achieved by designing the cis-suppressing sequences to interact (bind) with naturally-occurring plasmids or other cell transcripts, whereby the interactions can be cleaved and thus eliminate cis-suppression sequences from the reporter transcript A single stranded region containing the RNAse E site is generated.

In some embodiments, when cleavage is used to express a reporter, the RNAse E site is designed to be outside the coding region of the reporter sequence with sufficient sequence length in the 5 'and 3' UTRs to allow for a bred reporter transcript . In this case, the RNAse E site has at least 0, 1, 2, 3, 4, 5, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500 or more upstream of the start codon in the eukaryotic system. Or more base pairs or at least 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 200, 300, 400, 500, or more base pairs downstream of the stop codon . In other embodiments, where cleavage is used to inhibit the reporter, the RNAse E site is designed to be within the coding region of the reporter sequence, or positioned so as to inhibit the expression of the reporter.

6) Transcript

As described above, the transcript is the length of the nucleotide sequence (DNA or RNA) transcribed from the DNA or RNA template sequence or gene. The transcript may be a cDNA sequence transcribed from an RNA template or an mRNA sequence transcribed from a DNA template. The transcript may be transcribed from the engineered nucleic acid construct. The transcript may have a complementary region in itself, whereby the transcript contains two regions that can form an intra-molecular duplex. One region may be referred to as a "cis-suppressing sequence" that binds to a reporter sequence and blocks translation. The second sequence of the transcript is referred to as a "reporter sequence" that encodes a reporter molecule such as a detectable marker or a selectable marker.

The transcripts of the present invention can be used in combination with any one or more of the following nucleotides: 5,6,7,8,9,10,11,12,13,14,15,16,17,18,19,20,21,22,23,24 or 25 nucleotides Lt; RTI ID = 0.0 > length. ≪ / RTI > In other embodiments, the transcript may 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-suppressing sequence and the reporter sequence may be the same length or different lengths.

In some embodiments, the cis-suppression sequence is 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 25, 26, 27, 28, 29, 30, 35, 40, 45, 50, 55, 60 or more spacer nucleotides.

7) Vector

In other embodiments, the transcripts (including antisense and sense sequences) of the invention are expressed from a transcription unit inserted into a DNA or RNA vector (see, e.g., Couture, A, et al., TIG. (1996) 5-10; Skillern, A., et al., International PCT Publication No. WO 00/22113, Conrad, International PCT Publication No. WO 00/22114, and Conrad, US Patent No. 6,054,299). These sequences can be introduced as viral vectors containing linear structures, circular plasmids, or bacteriophage-based vectors, which can be integrated and inherited as transgenes integrated into the host genome. The transcript may also be constructed to allow it to be inherited as an extrachromosomal plasmid (see Gassmann, et al., Proc. Natl. Acad Sci USA (1995) 92: 1292).

The transcript sequence can be translated by a promoter located on the expression plasmid. In one embodiment, the cis-suppression and reporter sequences are expressed as inverted repeats followed by a linker polynucleotide sequence such that the transcript has a stem and loop structure.

Recombinant expression vectors may be used to express the transcripts of the invention. Recombinant expression vectors are generally DNA plasmids or viral vectors. Virus vectors expressing transcripts include, but are not limited to, adeno-associated viruses (see Muzyczka, et al., Curr. Topics Micro. Immunol. (1992) 158: 97-129); Adenovirus (see 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 alpha viruses, as well as others known in the art. Retroviruses have been used to introduce a variety of genes into many different cell types including endothelial cells in vitro and in vivo (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: 6141-6145; Huber et al., 1991, Proc Natl Acad Sci USA 88: 8039-8043; Ferry et al., 1991, Proc. 1992, Proc Natl Acad Sci USA 89: 7640-19; Kay et al., 1992; Chowdhury et al., 1991, Science 254: 1802-1805; van Beusechem 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; PCT Application WO 89/07366; PCT Application WO 89/02468; PCT Application WO 89/05345; and PCT Application WO 92/07573). A recombinant retroviral vector capable of transducing and expressing the gene inserted into the cell genome can be generated by transfecting the recombinant retroviral genome with a suitable packaging cell line 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., rats, hamsters, dogs and chimpanzees) (Hsu et al., 1992, J. Infectious Disease, 166: 769) And also has the advantage that no mitogen-activated cells are required for infection.

Any viral vector capable of containing the coding sequence for the expressed transcript (s) may be used, for example adenovirus (AV); Adeno-associated virus (AAV); Retroviruses (e. G., Lentivirus (LV), Rabdovirus, murine leukemia virus); Herpes viruses, and the like. The tropism of the viral vector can be modified by also typing the vector into a coat protein or other surface antigen from another virus, or, if appropriate, by substituting different viral capsid proteins.

For example, the lentiviral vectors specified in the present invention can also be typed as surface proteins from vesostomatitis virus (VSV), rubies, ebola, mokola, and the like. The AAV vectors specified in the present invention can be prepared to target different cells by manipulating the vectors to express different capsid protein serotypes.

Techniques for constructing AAV vectors expressing different capsid protein serotypes are within the skill of the art; See, for example, Rabinowitz JE et al., Which is incorporated herein by reference in its entirety. (2002), J Virol 76: 791-801.

Methods for inserting nucleic acid sequences to express transcripts in vectors, and for delivering viral vectors to cells of interest, are well within the skill of the art, including selection of recombinant viral vectors suitable for use in the present invention. For example, Dornburg R (1995), Gene Therap. 2: 301-310; Eglitis MA (1988), Biotechniques 6: 608-614; Miller 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, the entire contents of which are incorporated herein by reference.

Viral vectors may be derived from AV and AAV. Suitable AV vectors for expressing the transcripts specified in the present invention, methods for constructing recombinant AV vectors, and methods for delivering vectors to target cells are described in Xia H et al. (2002), Nat. Biotech. 20: 1006-1010. Also suitable AAV vectors for expressing the transcripts specified in the present invention, methods for constructing recombinant AV vectors, and methods for delivering vectors to target cells are described in Samulski R et al. (1987), J. Virol. 61: 3096-3101; Fisher KJ et al. (1996), J. Virol., 70: 520-532; Samulski R et al. (1989), J. Virol. 63: 3822-3826; U.S. Pat. 5,252,479; U.S. Pat. 5,139,941; International patent application no. WO 94/13788; And international patent application no. WO 93/24641, the entire contents of which are incorporated herein by reference.

The promoter that promotes transcript expression in the DNA plasmid or viral vector specified in the present invention may be an eukaryotic RNA polymerase I (for example, a ribosome RNA promoter), an RNA polymerase II (for example, a CMV early promoter or an actin promoter or U1 snRNA promoter) or an RNA polymerase III promoter (e.g., a U6 snRNA or a 7SK RNA promoter) or a prokaryotic promoter, such as a T7 promoter, although the expression plasmid may also encode a T7 RNA polymerisation Encrypt the enzyme. Promoters can also direct transgene expression to the pancreas (see, for example, the insulin regulatory sequence in the 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 expression systems such as inducible regulatory sequences and specific physiological regulatory factors, such as cyclic glucose levels, or hormone-modulating regulatory sequences (Docherty et al., 1994, FASEB J. 8: 20-24). Such an inducible expression system suitable for the control of transgene expression in a cell or mammal is selected from the group consisting of exdysone, estrogen, progesterone, tetracycline, a chemical inducer of dimerization, and isopropyl-beta-D-1-thiogalactopyranose (IPTG). ≪ / RTI > One skilled in the art will be able to select appropriate regulatory / promoter sequences based on the intended use of the dsRNA transgenes.

Generally, recombinant vectors capable of expressing transcript molecules are delivered as described below and persist in the target cells. Alternatively, viral vectors that provide for the transient expression of transcript molecules can be used. Such a vector may be repeatedly administered as needed. Once administered, the transcript binds to the target RNA and regulates function or expression. Delivery of the transgene expression vector can be systemically performed, for example, by intravenous or intramuscular administration, administration to the subject cell that has been eaten out of the patient, reintroduction into the patient, or any other means that allows introduction into the desired target cell.

Transcript-expressing DNA plasmids are typically transfected into target cells as a complex with a cationic lipid carrier (e.g., oligopectamine) or with a non-cationic lipid-based carrier (e.g., transit-TKOTM). In addition, multiple lipid transfection for dsRNA-mediated knockdown targeting different regions of a single PROC gene or multiple PROC genes over a period of more than one week is presented by the present invention. Successful introduction of the vector into the host cell can be monitored using a variety of known methods. For example, a fluorescent marker such as a green fluorescent protein (GFP), which is a transient transfection. Stable transfection of the ex vivo cells can be ensured using markers that provide transfected cells that are resistant to certain environmental factors such as hygromycin B resistance (e. G. Antibiotics and drugs).

The delivery of the vector containing the recombinant DNA can be performed by a non-biological or biological system. But are not limited to, liposomes, virus-like particles, transgenic particles derived from phages or viruses, and conjugation.

8) Transcript Reporter for analysis

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 it is expressed in the cell. In some embodiments, the reporter molecule may be a detectable marker or a selectable marker. In certain embodiments, the reporter molecule can be a fluorescent reporter molecule such as a green fluorescent protein (GFP), a yellow fluorescent protein (YFP), a cyan fluorescent protein (CFP), a blue fluorescent protein (BFP), or a red fluorescent protein have. In other embodiments, the reporter molecule may be a chemiluminescent protein.

The reporter molecule may 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 immunological detection, or a nucleic acid functioning as an aptamer or exhibiting enzymatic activity.

Selectivity markers can also be used as reporters. The selectivity marker may be, for example, an antibiotic resistance gene.

9) Transcript Cells and target genes for reporter analysis

Examples of cells that can be used for detection include, but are not limited to, gram-positive and gram-negative bacteria, Aureus, this. Collai, Kay. Fungi such as Streptomyces coelicolor, and other eukaryotic cells including cells from humans, other mammals, insects, invertebrates, or plants.

The target transcript may comprise any endogenous transcript that is either coding or non-coding. The target transcript may be from progressive and prokaryotic cells, for example, S. MecA transcript of aureus cells (MRSA mark), seeds. Epidermal tcdB transcripts (indicative of toxic seeds. Dipicyl), and HPV E6 / E7 transcripts of cervical endothelial cells (indicia of cervical cancer). Genes associated with infectious agents, such as viruses, including HIV and HPV, may also be targets. Other examples of target genes include RNAs such as snoRNA, microRNA, siRNA, snRNA, exRNA, and piRNA and ncRNA as well as non-coding RNAs such as transcriptional RNA (tRNA) and ribosomal RNA (rRNA).

Example

The following examples are illustrative examples of specific embodiments for carrying out the present invention. These embodiments are provided for illustrative purposes only, and are not intended to limit the scope of the invention in any way. Efforts have been made to ensure accuracy with respect to the numbers used (eg, quantity, temperature, etc.), but some experimental errors and deviations should, of course, be allowed.

The practice of the present invention will employ conventional methods in the field of protein chemistry, biochemistry, recombinant DNA technology, and pharmacology, within the skill of the art, unless otherwise indicated. These techniques are disclosed in detail in the literature. For example, T.E. Creighton, Proteins: Structures and Molecular Properties (W. H. Freeman and Company, 1993); A.L. Lehninger, Biochemistry (Worth Publishers, Inc., current addition); Sambrook, et al., Molecular Cloning: A Laboratory Manual (2nd Edition, 1989); Methods In Enzymology (S. Colowick and N. Kaplan, eds., Academic Press, Inc.); Remington ' s Pharmaceutical Sciences, 18th Edition (Easton, Pennsylvania: Mack Publishing Company, 1990); See Carey and Sundberg Advanced Organic Chemistry 3rd Ed. (Plenum Press) Vols A and B (1992).

Example 1: Silent mutation / compensation packaging system

The following is an example of the design and construction of a silent mutation / compensation-based packaging system to produce non-replicating transgenic particles.

The materials used in the development of the packaging system are:

Bacterial strain:

- N1706, Lee. Cola K-12 P1 c1-100 Tn9 Solvent

vector:

- Y14439 (pBHR1 backbone)

The following GenBank accession numbers (N.B., sequences cited as accession numbers are those recorded in the database as of the priority date of the present application) or SEQ ID NOs. Can be used in the vector backbone and cassette sequences:

- X06758 (bacterial luciferase gene luxAB)

- SEQ ID NO: 1 (native P1 pac-site)

- P1 cell lysis replicon containing the in-frame deletion of SEQ ID NO: 3 (Cl repressor-control P53 promoter, promoter P53 antisense, repL gene, and kilA gene)

- SEQ ID NO: 4 (Pblast promoter promoting luxAB expression)

Configuration of N1706 (pac): pacA The mutant strain: pacA an exemplary sequence of the mutated sequence SEQ ID NO: 2 are presented in, it is proposed to informal sequence listing. This mutation can be accomplished by constructing the mutated sequence through gene synthesis, and then replacing the N1706 wild-type sequence with the mutated sequence through an allotransformation approach.

Construction of the GWP10001 reporter vector: The GWP10001 vector is operably linked to the pBHR1 origin of replication, which exhibits broad Gram negative activity, two selectable markers for kanamycin and chloramphenicol, a native bacteriophage P1 pac-site sequence, and a constitutive blasticin promoter (Pblast) A P1 cell lysis replicon containing the luxA and luxB genes from the connected Vibrio hyeii and the C1 repressor-regulated P53 promoter, the promoter P53 antisense, the repL gene, and the in-frame deletion of the kilA gene.

Figure 2 can be constructed in a number of ways known to those of skill in the art, including obtaining cassettes from PCRs or by gene synthesis from a native source and assembling vectors through alternative techniques such as conventional restriction enzyme-based cloning or Gibson assembly (GWP10001, SEQ ID NO: 11).

Silent / Compensated Packaging System: This packaging system contains the pacA mutant strain N1706 (pac), compensated with the vector pGWP10001. As known to those skilled in the art, the manner of constructing this system can be accomplished by N1706 (pac) transformation into the vector pGWP10001. The vector pGWP10001 can be maintained in a culture of transformed N1706 (pac) by growing the transformant in the presence of 50 ug / mL kanamycin.

Generation of Transfected Particles Having Plasmid DNA: Non-replicate transduced particles with vector pGWP10001 can be generated from N1706 (pac) transformants through heat induction at 42 ° C. Incubation at 42 캠 induces a P1 cell lysis cycle, and as shown in Fig. 1, the prophage is excised from the N1706 genome to produce a phage structural element, and the pGWP10001 concatamer formed by the cell lysate replicon in the progeny phage particle Package the DNA. Next, the resulting cell lysate is collected, which contains non-replicating transduced particles, each particle consisting of bacteriophage P1 particles with a linear con- catamer of pGWP10001 DNA.

Example 2: Fulfillment / Compensation Packaging System

The following is an example of the design and construction of a deletion / compensation-based packaging system for generating non-replicating transduced particles.

The materials used in the development of the packaging system are:

Bacterial strain:

RN4220 is a non-bulky derivative of NCTC 8325, It is aureus strain, and this is. It is an effective recipe for Molly DNA. This is Kreiswirth, B.N. < / RTI > et al., The toxic shock syndrome exotoxin structural gene is not detectably transmitted by a prophage. Nature, 1983, 305 (5936): p. 709-712.

RN10616 is induced by ligation of RN4220 with bacteriophage < RTI ID = 0.0 > 80A < / RTI > (Ubeda C. et al., Specificity of staphylococcal phage and SaPI DNA packaging as revealed by integrase and terminase mutations. Molecular Microbiology, 2009. 72 Reference).

ST24 is induced by deletion of the small terminalogenic gene terS from the lytic bacteriophage φ80α of RN10616 (Ubeda C. et al., Specificity of staphylococcal phage and SaPIDNA packaging as integrase and terminase mutations. Molecular Microbiology, 2009. 72 1): p. 98-108).

vector:

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

The following GenBank accession numbers may be used in the cassette sequence:

- SEQ ID NO: 5 (S. aureus pT181 plasmid origin or cloned copy number variant pT181cop-623 repC)

- M21136 (tetA (M))

- SEQ ID NO: 12 (PclpB promoter sequence)

- SEQ ID NO: 9 (? 11 small terminalase (terS) gene sequence)

- L09137 (amp ColE1 ori)

- X06758 (luxAB)

- M62650 (End of Warrior)

terS deletion: The construction of the terS knockout strain ST24 can be achieved by an allele-exchange-based strategy that results in intra-frame deletion by removing most of the coding sequence of the? 80? small terminal gene. Details of this strategy can be found in Ubeda C. et al., Specificity of staphylococcal phage and SaPI DNA packaging as integrase and terminase mutations. Molecular Microbiology, 2009. 72 (1): p. 98-108.

An exemplary sequence of the terS knockout strain is shown in SEQ ID NO: 13 (shown in the Sequence Listing below). SEQ ID NO: 13 is the RN10616 genomic sequence locus indicating ϕ 80 alpha terS deletion and compensation.

Vector composition: The GW80A0001 vector is the E. coli / S. aureus shuttle vector. This vector contains ampicillin (amp) and tetracycline (tet (M)) for selection in S. aureus (pT181cop-623 repC) and E. coli (E. Coliori) replication origin, E. coli and S. aureus,) Selectivity marker for resistance, the? 11 small terminalase (terS) gene

sequence containing its own promoter, Contains the luxA and luxB genes and transcription termination sequence (TT) from Vibrio hybaii operably linked to the Aureus PclpB promoter.

Figure 4 shows a vector (pGW80A0001, SEQ ID NO: 14) that may be constructed in a number of ways known to those skilled in the art. In one example, the tet (M) cassette and luxAB gene can be obtained by PCR amplification from publicly available pCN36 and pCN58 vectors (Charpentier, E., et al.). PclpB S. Can be obtained from PCR amplification from Aureus RN4220, and terS can be obtained from PCR amplification from RN10616. The vector backbone can be obtained by removing the ermC gene from the publicly available vector pCN48 (Charpentier E., et al.), And the various components of the final vector pGW80A0001 are assembled via suitably designed restriction enzyme-based cloning on this vector backbone .

Defect / Compensation Packaging System: This packaging system may contain a terS knockout strain ST24 compensated with vector pGW80A0001 to generate strain GW24. As is known to those skilled in the art, the manner in which this system is constructed can be accomplished by ST24 transfection into vector pGW80A0001. The vector pGW80A0001 can be maintained in a culture of transformed ST24 by growing the transformant in the presence of 50 ug / mL tetracycline.

Generation of Transfected Particles Having Plasmid DNA : Non-transfected transgenic particles with the vector pGW80A0001 were obtained. Can be produced from GW24 through the mitomycin C-inducible method, which was first demonstrated in E. coli and is currently the standard technique for obtaining a protease from a solubilized bacteria (Otsuji, N. et al., Induction of Phage Formation in the Lysogenic Escherichia coli K-12 by Mitomycin C. Nature, 1959. 184 (4692): p. 1079-1080). This prophage induction method induces a cell lysis cycle of? 80 ?. As shown in FIG. 2, the prophage is excised from the GW24 genome to generate a phage structural element, and the pGW80A0001 concatamer DNA is packaged into the progeny phage particle. Next, the obtained cell lysate is collected, which contains non-replicating transduced particles, and each particle is composed of bacteriophage? 80? Particles having a linear con- catamer of pGW80A0001 DNA.

Example_3: Integraze_Lacking SaPIbov2 - based packaging system

The following is an example of the design and construction of a SaPIbov2-based packaging system to produce non-replicating transduced particles.

The materials used in the development of the packaging system are:

The following materials can be used to develop a SaPIbov2-based packaging system that lacks integrase.

Bacterial strain:

RN451 is a bacteriophage, It is aureus strain.

JP2131 is RN451, which is termed SaPIbov2 (Maiques, E. et al., Role of Staphylococcal Phage and SaPI Integrase in Intra- and Interspecies SaPI Transfer. J. Bacteriol., 2007. 189 (15): p. 5608-5616 Reference).

JP2488 is a strain JP2131 (SaPIbov2? Int) in which an int gene has been deleted from SapIbov2. Maiques, E. et al., Role of Staphylococcal Phage and SaPI Integrase in Intra- and Interspecies SaPI Transfer. J. Bacteriol., 2007. 189 (15): p. 5608-5616.

Bacteriophage:

The bacteriophage φ11 is this. It has been proved for the first time in E. coli and is now available through Mitomycin C-induction, a standard technique for obtaining probes from dissolved bacteria. (Otsuji N. et al., Induction of Phage Formation in the Lysogenic Escherichia coli K-12 by Mitomycin C. Nature, 1959. 184 (4692): p. 1079-1080).

Promoter:

PclpB can be used as a promoter in this embodiment. The clpB gene promoter is a constitutive promoter used to control the expression of the int gene. s. Aureus clpB (PclpB) gene promoter sequences were first identified in 2004 (Frees, D., et al., Clp ATPases are required for stress tolerance, intracellular reprogramming and biofilm formation in Staphylococcus aureus. Molecular Micro-biology , 2004. 54 (5): p. 1445-1462). In addition, this was first used to control gene expression in plasmids in 2004 (Arnaud, M., A. Chastanet and M. Debarbouille, New Vector for Efficient Allele Replacement in Naturally Non-transformable, -Positive Bacteria. Appl. Environ., Micro-biol., 2004. 70 (11): 6887-6891). This promoter was used as primer in 2004, Can be obtained from Aureus RN4220 (see same data).

φ11 / SaPIbov2Δint common - the creation of Longyuan (RN451 (φ11 SaPIbov2Δint)): By enriching JP2488 with φ11, strain JP2488 (φ11 SaPIbov2Δint) can be generated.

φ11 The deletion of terS (RN451 (φ11ΔterS SaPIbov2Δint): Tormo, MA et al., Staphylococcus aureus Pathogenicity Island DNA Is Packaged in Particles Composed of Phage Proteins. J. Bacteriol., 2008. 190 (7): p. Strain RN451 (? 11? InterSs SaPIbov2? Int) can be generated by deleting the? 11 terS gene from RN451 (? 11SaPIbov2? Int), as disclosed in WO98 / 2434-2440.

Es. Integration of PclpB - int into Aureus genome (RN451 (φ11ΔterS SaPIbov2Δint PclpB-int)): First, PclpB and int were first fused through standard molecular biology techniques. Then, PclpB-int fusions were inserted into the genome of RN451 (φ11ΔterS SaPIbov2Δint), followed by insertion of PclpB- RN451 (? 11? interS SaPIbov2? int PclpB-int) can be generated by selecting a clone with int.

SaPIbov2Δint PclpB - int Φ11 generation of particles with only cones Kata Murray:. Otsuji, N. et al, Induction of Phage Formation in the Lysogenic Escherichia coliK-12 by Mitomycin C. Nature, 1959. 184 (4692): p. 1079-1080, φ11 particles with SaPIbov2Δint PclpB-int concatamers can be generated through mitomycin-C induction of RN451 (φ11ΔterS SaPIbov2Δintint PclpB-int). The cell lysate contains non-replicating transduced particles, each particle consisting of a bacteriophage < RTI ID = 0.0 > 11 < / RTI > structural protein with a linear con- catamer of GI-derived DNA.

Those skilled in the art will be able to understand how to construct the NRTP of the present invention using the above mentioned materials and molecular biology and gene technology well known in the art.

Example_4: terS_Fruit / Rewards - Based SarS_Reporter Transfection Particles

The following is an embodiment of an inducer reporter-based SarS reporter system using terS deletion / compensation-based non-replicating transduced particles.

Reporter gene: Bacterial luciferase (luxAB). The luxA and luxB genes originate from Vibrio Harvey. They lack the transcriptional promoter and contain ribosome binding sites, respectively.

Spa gene promoter (Pspa): spa gene promoter will be used to control the expression of the luxAB gene.

Pspa - luxAB Construction of fusions : The luxAB gene can be fused with the Pspa promoter sequence so that the luxAB gene is operably linked to the Pspa promoter.

luxAB - Construction of Expression Reporter Vector:

The luxAB-expression reporter vector can be generated through standard molecular biology techniques by incorporating the Pspa-luxAB fusion product into the MCS of the shuttle vector described below.

E. Collie / S. Aureus Shuttle Vector Aureus (pT181cop-623 repC) and this. A small polynucleotide (MCS) gene, a pol111 small terminalase (terS) gene under the control of ColE1ori replication origin, ampicillin (amp) and tetracycline (tet (M) resistance gene, constitutive promoter (PclpB) And has a transcription termination sequence (TT).

For the cassette sequence GenBank Access number:

- J01764 (pT181 replicon)

- M21136 (tetA (M))

- Access number not yet available (PclpB)

- AF424781 REGION: 16526..16966 (terS)

- L09137 (amp ColE1 ori)

- M62650 (TT)

The multiplication of the vector for the performance of the in vitro manipulation and the verification of the manipulation is shown in Fig. Can be accomplished through the Top 10, and the final transformed vector is then transformed into S. Can be introduced into Aureus RN0451? TerS. Transfected particles with shuttle vectors were found in 1959. Can be produced from RN0451 [Delta] terS transformants for the first time in E. coli and now through the mitomycin C-induction method, which is the standard technique for obtaining proteases from dissolved bacteria (Otsuji, N., et al., Induction of Phage Formation in the Lysogenic Esche-richia coli K-12 by Mitomycin C. Nature, 1959. 184 (4692): p. 1079-1080). Next, a cell lysate containing the non-replicating transduced particles is collected, It is composed of bacteriophage &phis; 11 structural protein with linear con- catamer of plasmid DNA which can be confirmed for the presence of SarS in Aureus cells.

Example 5: terS The deletion / compensation-based < RTI ID = 0.0 & Lactamase Reporter Transfection Particles

The following is an example of an intracellular enzyme reporter-based beta-lactamase reporter system using terS deletion / compensation-based non-replicating transduced particles.

Reporter gene: Renilla luciferase (ruc)

Promoter: The promoter may be PblaZ. Constitutive beta-lactamase may be used to promote expression of the ruc gene.

Blocked Substrate: Blocked coelenterazine-phosphate is disclosed by Daniel Sobek, JR, Enzyme detection system with caged substrates, 2007, Zymera, Inc.

PblaZ - ruc The fusion configuration: ruc genes may be fused with a promoter sequence such that PblaZ ruc gene is operably linked to a promoter PblaZ.

Construction of the ruc -expression reporter vector: The ruc -expression reporter vector can be generated through standard molecular biology techniques by incorporating the PblaZ-ruc fusion product into the MCS of the shuttle vector described in section V, A, 3), i) above .

The multiplication of the vector for the performance of the in vitro manipulation and the verification of the manipulation is shown in Fig. Can be accomplished through the Top 10, and the final transformed vector is then transformed into S. Can be introduced into Aureus RN0451? TerS. Transfected particles with shuttle vectors were found in 1959. Can be produced from RN0451 [Delta] terS transformants for the first time in E. coli and now through the mitomycin C-induction method, which is the standard technique for obtaining proteases from dissolved bacteria (Otsuji, N., et al., Induction of Phage Formation in the Lysogenic Esche-richia coli K-12 by Mitomycin C. Nature, 1959. 184 (4692): p. 1079-1080). Next, a cell lysate containing NRTP is collected, and each particle is cultivated in a bioinducing range < RTI ID = 0.0 > And a bacteriophage < RTI ID = 0.0 > 11 < / RTI > structural protein with a linear con- catamer of plasmid DNA capable of expressing renilla luciferase in aureus.

Example 6: terS Fruit / Rewards - Based Intracellular Molecular Reporter Transfection Particles

The following is an example of an intracellular molecular reporter-based reporter system using terS deletion / compensation-based non-transgenic transgenic particles.

Promoter: The promoter may be PblaZ. Constitutive beta-lactamase may be used to promote expression of the ruc gene.

Switchable aptamer: it can be designed as described in the switchable aepta meoga Samie Jaffrey, JP, Coupled recognition / detection system for in vivo and in vitro use, 2010, Cornell University and configured.

Fluorophore substrate: There is a fluorophore and the substrate corresponding to the switchable aptamer may be designed and configured as disclosed in Jaffrey Samie, JP, Coupled recognition / detection system in vivo and in vitro for use, 2010, Cornell University.

Construction of PblaZ- SA fusions : The SA gene can be fused with the PblaZ promoter sequence so that the SA gene is operably linked to the PblaZ promoter.

Construction of SA-Expression Reporter Vectors : SA-Expression Reporter Vectors can be generated through standard molecular biology techniques by incorporating the PblaZ-SA fusion product into the MCS of the shuttle vector described in Example 4. [

The multiplication of the vector for the performance of the in vitro manipulation and the verification of the manipulation is shown in Fig. Can be accomplished through the Top 10, and the final transformed vector is then transformed into S. Can be introduced into Aureus RN0451? TerS. Transfected particles with shuttle vectors were found in 1959. Can be produced from RN0451 [Delta] terS transformants for the first time in E. coli and now through the mitomycin C-induction method, which is the standard technique for obtaining proteases from dissolved bacteria (Otsuji, N., et al., Induction of Phage Formation in the Lysogenic Esche-richia coli K-12 by Mitomycin C. Nature, 1959. 184 (4692): p. 1079-1080). Next, cell lysates containing non-replicate transduced particles are collected, and each particle is cultivated in the range of viability within the? 11 host range. And a bacteriophage < RTI ID = 0.0 > 11 < / RTI > structural protein with a linear con- catamer of plasmid DNA capable of expressing SA within the aureus.

Example 7: Non-replicating transduced particle-based reporter system

The non-replicating transduced particles described above can be used in a reporter system for detecting the presence of live breeding bacteria through the expression of reporter molecules (e.g., luxAB). When the transduction particle introduces a reporter vector (for example, pGW80A0001) into a cell within the host range of the transduced particle, the cell in which the promoter (for example, PclpB) is recognized by the cell transcription mechanism, Expression of the reporter molecule can be promoted.

s. A variety of MSSA / MRSA reporter assays have been developed to test the functionality of non-replicating transgenic particles as reporters for detecting the presence of Aureus cells. In one embodiment, the non-replicating transduction particles are S. cerevisiae. Was developed from Aureus-specific bacteriophage and incorporated the bacterial luciferase gene luxAB under the control of constitutive promoters. Wherein the non-replicating transduction particles are S. When the reporter nucleic acid was delivered to the aureus, the constitutive promoter was a live-grown promoter. Expressing luxAB suitable for confirming the presence of Aureus.

In addition, the antibiotic Sepoxytin is available from S. Was added before, simultaneously with, or after the addition of the transducing particles to the sample containing the Aureus cells. If the cells are not phenotypically resistant to hypoxicin (ie not MRSA), the luminescence is not reduced or detected, indicating that the cells are MSSA. However, if the cell is phenotypically resistant to hypoxia (i. E., MRSA) the luminescence is increased or detectable indicating that the cell is MRSA.

Non-replicating transgenic particle-based Live birth Cell Reporter Analysis Capability

The function of the non-replicating transduced particles as a reporter was analyzed. The range of the transduction host for the bacteriophage ϕ 80 [alpha] -based non-replicating transduced particles was tested in 101 clinical MRSA isolates. The transfection assay was performed by exposing each bacterial isolating culture grown in TSB modified to a GW24 cell lysate containing non-replicating transduced particles, and culturing the mixture on a solid medium containing tetracycline.

In this example, the non-replicating transduced particles had a tetracycline selectable marker. Cells transduced with non-replicating transduced particles were expected to be resistant to tetracycline. Transduction was also tested by exposing each bacterial isolate in a liquid culture to a cell lysate containing non-replicating transducing particles and then by evaluating the mixture for bacterial luciferase luminescent activity after incubation .

This transfection assay showed that the < RTI ID = 0.0 > phi80a-based < / RTI > non-replicating transduction particles were able to transfect all 101 clinical isolates of MRSA, Aureus staphylococci showed that none could transduce it.

Figure 21 shows the results of a transfection assay spotted onto a culture plate containing 5 ug / mL tetracycline after 36 tetracycline-sensitive MRSA were exposed to the transducing particles with pGW80A0001. This result indicates that all 36 MRSA strains have grown due to transduction into pGW80A0001 in a medium containing tetracycline. Control experiments in which the MRSA isolate strain was spotted on the tetracycline containing medium without exposure to the transduced particles showed no growth (not shown). Plasmid isolation from the transfected MRSA strain also demonstrated recovery of the pGW80A0001 plasmid as identified through sequencing of the isolated plasmid. Thus, the transfection results demonstrate that the replication origin of the reporter plasmid is active against all MRSA isolates tested.

Figure 22 shows that 80 clinical isolates of MRSA transduced with the transduced particles and methicillin sensitive S. Lt; RTI ID = 0.0 > (MSSA) < / RTI > In this experiment, cultures of MRSA and MSSA were grown to an optical density of 0.1 at 600 nm, then 100 uL of the cultures grown in the modified TSB were mixed with 10 uL of GW24 cell lysate containing the transduced particles, Lt; RTI ID = 0.0 > 37 C < / RTI > for 4 hours. Luminescence measurements were performed by adding 10 uL of a 1 mM Decanal solution, which is an aldehyde that triggers a luminescent reaction in cells expressing bacterial luciferase. As expected, the luminescence is not reflected in the s. Was observed from both MRSA and MSSA transduced with aureus-specific, non-replicating transduced particles. In addition, when the cytoxytin was added to the cell culture at the same time as the addition of the transfected particles, luminescence was observed from MRSA but no luminescence was observed in MSSA, indicating that the transduced particles Proves the ability of. Thus, the luminescence results show that the promoter that promoted luxAB expression was tested. RTI ID = 0.0 > Aureus < / RTI > isolate.

Non-replicating transgenic particle-based Live birth Optimization of cell reporter MRSA assays - Transfection particle reagent preparation

The preparation and formulation of non-replicating transduction particle reagents was optimized for the final formulation. In summary, 15 L scale fermentation was performed using TSB medium containing peroxide induction of GW24. A 15 L fermentor batch was inoculated with 200 mL overnight saline seed culture (1.3% (v / v) inoculum ratio). The culture was induced at 0.8 OD with hydrogen peroxide and cooled to 25 DEG C after induction without pH or DO control. The next morning, the culture supernatant was collected by tangential flow filtration (TFF) in order to purify the phage-transduced particles from the cell debris. Next, the material was concentrated and dialyzed in gelatin-free SM buffer, and stored at 2-8 ° C prior to final sterile filtration and storage.

A detailed summary of this process is as follows:

Seed flask growth:

(1) Inoculate 200 mL of TSB containing 5 ug / mL tetracycline with GW24.

(2) Incubate at 37 [deg.] C, 200 RPM for 10-18 hours.

Fermented inoculation (15 L TSB, 5 ug / mL tetracycline):

(1) Prepare fermentors with the following fermentation conditions: 37 ° C, 250 RPM agitation, 15 LPM airflow, and 3 psig back pressure.

(2) Inoculate the fermentation apparatus using 200 mL overnight saline seed culture.

Culture induction:

(1) Once the OD600nm reaches 0.8 (0.6 to 0.9) leads to the culture to 0.5mM H_2O_2.

(2) Increase the temperature set point of the fermenter to 42 ° C.

Post induction conditions and monitoring :

(1) Once the 30-minute induction is completed, the temperature target of the fermenter is reset to 25 ° C.

(2) After 1 hour of cooling, stop supplying air to the fermenter and set stirring to zero.

(3) Monitor the fermentation culture more frequently at 1 hour intervals, or as needed, until the OD600nm decreases to less than 0.40.

Collection / Purification:

(1) After the fermentation culture OD600 reaches the minimum value of 0.40 or less, take a 20 mL sterile sample in the fermentation apparatus and add 30 μL of Benzonase.

(2) The agitation is reset to 250 rpm. It is stirred for 60 minutes during benzoinase incubation.

(3) Centrifuge at 3000g for 15 minutes to purify the EOF sample.

(4) The purified material is passed through a 0.45 uM membrane filter.

Concentration and buffer exchange:

(1) The purified culture is concentrated using a 500 kDa flat sheet membrane 10-fold and the culture purified by TFF.

(2) Using a 500 kDa TFF membrane used for concentration, dialyze the cultured medium at a constant volume for SM buffer without gelatin.

Final filtration:

(1) The concentrated buffer exchange material is filtered through a 0.2 mu m filter.

(2) Store the final filtered material at 2-8 ℃.

A variety of other reagents and agents known to those skilled in the art may be used to obtain the agent.

Non-replicating transgenic particle-based Live birth Optimization of cell reporter MRSA assay - Growth medium formulation

Growth medium preparations were optimized for NRTP-based live-cell reporter MRSA assays. To produce luminescence in an NRTP-based MRSA assay, the medium should be balanced against Staphylococcus aureus growth and should have a sufficient amount of cation and excipients to favor transduction of NRTP. The TSBmod medium used in the analysis before this study was found to have a precipitation problem affecting the stability of the medium. The growth medium formulation required stability of the final formulation for one year at room temperature.

METHODS / PROCEDURES: Cell proliferation for MRSA analysis

(1) Ten distinctive strains of MRSA for subset analysis and one unique strain of MSSA were tested in MRSA analysis.

(2) overnight cultures were initiated in deep 96-well plates at a 1:50 dilution in TSB from frozen 1-fold spent stock and incubated at 37 ° C in an orbital shaker for> 15 hours. MRSA / MSSA (8 μl) in TSB (392 μl).

(3) The next day, day and culture at 1:50 dilution from overnight cultures was started in TSB in 96 well deep well plates (392 μl TSB + 8 ul cells) and incubated at 37 ° C. in an orbital shaker for 4 hours.

(4) The cells were centrifuged for 5 minutes at 1800 g force and 10 < 0 > C, and the spent medium was aspirated without destroying the pellet.

(5) The spun cells were washed in 50 mM Tris-HCl pH 7.2, centrifuged, and the buffer was aspirated without destroying the pellet and resuspended in 400 μl RPMI. RPMI is used to reduce the diversity of cellular metabolic status and mimic the low metabolism found in clinical samples.

(6) The plates were covered with a porous seal and incubated on a bench for 48 hours.

(7) OD was read by transferring 200 [mu] l of RPMI culture to a shallow well OD plate, and blank wells with RPMI brew were used to subtract the blank OD.

(8) Cells were normalized to OD 0.1 at 100 μl.

(9) Another dilution ratio for obtaining an OD of 0.006 was 1:10 in RPMI.

Assay basal media were prepared to be tested as shown in Table 2, and a representative set of media variants in manufacture for MRSA analysis is presented in Table 3. Table 3:

Basic medium for development of growth media

ingredient	TSB	B2	BSS-2	Remarks
The enzyme digest of soybean wheat (g)	3	0	3	
The enzyme digest of casein (g)	17	10	10	
Enzyme extract (g)	N / A	25	25	
Sodium chloride (g)	5	25	25	Adjust to pH 7.2 with 10 N NaOH. High pressure heating or filtration sterilization
Potassium Phosphate (g)	2.5	One	0	
Alpha-D Glucose (g)	2.5	5	5	
Volume (liters)	One	One	One	

Basal medium deformation for development of growth medium preparation

Additives for salt concentration / deformation

Basal medium (30 ml)	Mod number	$CaCl_2$ (mM)	$MgCl_2$ (mM)	BGP (mM)	Tris-HCl pH 7.0 (mM)	EDTA (mM)	HEPES (mM)
B2	M53	5.0	2.0	0.0	50.0	10.0	0.0
BSS-2	M50	10.0	2.0	60.0	50.0	10.0	0.0
BSS-2	M54	6.7	3.3	60.0	50.0	0.0	0.0
BSS-2	M55	5.0	5.0	60.0	50.0	0.0	0.0
BSS-2	M56	6.7	3.3	60.0	0.0	0.0	10.0
BSS-2	M57	5.0	5.0	60.0	0.0	0.0	10.0
TSB	M1 (original)	5.0	10.0	60.0	0.0	0.0	0.0
TSB	M58	5.0	10.0	60.0	0.0	11.1	0.0

NRTP media reagents were prepared by adding NRTP and cypoxitin to each of the media preparations according to Table 4 below:

MRSA Assay Growth Medium / Transfection Reagent Reagent Combination

30 ml medium	Final concentration
Hypoxicin	5 ug / ml
GW24 cell lysate	30X

MRSA analysis was performed according to the following steps:

(1) Assay plate setup: Add 2.0 [mu] l of the respective dilutions (approximately equivalent to 20,000 and 2,000 CFU / mL, respectively) of the phage culture reagent and 2.0 [mu] l of RPMI as the blank in bacterial 0.05 OD and 0.005 OD in RPMI.

(2) Assay plate Incubation: The assay plate is incubated at 37 [deg.] C for 4 hours at about 100 rpm in an orbital shaker.

(3) Luminescent system (Molecular Devices SpectraMax L) Preparation: Wash the reagent lines with 70% ethanol and DI water in turn, and firstly with substrate reagent. The software is set up with Fast Kinetic and after 10 baseline points, 50 μL of substrate reagent is injected at 250 μL / sec and read at 40 points every 0.25 sec.

(4) Perform assay: Equilibrate plates for 5 minutes at room temperature and then test each bacterial dilution plate.

analysis:

(1) Determine the cutoff by averaging the blank RLUs for all replicas and time points and adding the three standard deviations.

(2) Use SoftMaxPro to determine the maximum RLU for each sample.

(3) The case where the maximum RLU exceeds the cutoff RLU is determined, and the sample data when the maximum RLU exceeds the cutoff RLU is used for comparison of the performance of the medium.

(4) Normalize all maximal RLU values for strains analyzed at specific dilution ratios to the maximum RLU in TBS M1 (medium used until development begins).

(5) Average of normalized RLU values for all strains of MRSA against the specific medium and its variants.

(6) The average of the two dilution plates is obtained, which results in a single numerical value representing an increase in performance based on the RLU of a particular medium for 10 different MRSA strains in the two cell dilutions ultimately tested.

NRTP -base Live birth Results of cell reporter MRSA analysis

Determination of Cutoff RLU : The mean and standard deviation of the RLUs were calculated at every time point 25 for each blank replica (4). The cutoff was calculated for each plate as mean blank RLU + three standard deviations.

Determination of Relative Improvement: The maximum RLU was output from SoftMaxPro for each sample (blank, MSSA and MRSA for all dilution ratios) and compared to the cutoff RLU. The maximum RLU value when the sample had two data points exceeding the cutoff for the phage concentration was used for the analysis.

Values were normalized by dividing a particular maximum RLU by the maximum RLU of the control condition (TSB M1 - strain in the original medium, dilution ratio analyzed). The obtained ratios were averaged for 10 MRSA in each medium condition and each dilution ratio, and are shown in Table 5. The average of the two dilution ratios is also shown in the table.

Results of MRSA analysis from various growth medium preparations

badge		Plate 1	Plate 2	The average of the two dilutions
B2	M53	1.89	1.88	1.89
BSS-2	M50	1.37	1.47	1.42
BSS-2	M54	1.50	1.76	1.63
BSS-2	M55	1.82	2.90	2.36
BSS-2	M56	2.38	6.00	4.19
BSS-2	M57	2.00	3.92	2.96
TSB	M1	1.00	1.00	1.00
TSB	M58	1.18	0.96	1.07

conclusion

BSS2-M56 showed the best performance on average in the various media tested. HEPES buffer-based media operated better than Tris-HCl buffered media. HEPES is known to be a biologically favorable buffer system, unlike Tris-HCl. B2-based bass / broch has better performance than TSB based bros.

A variety of other reagents and agents can be used to obtain the agent and are known to those skilled in the art. Other suitable agents have also been developed through similar experiments described above. Examples of other suitable formulations are included in Tables 6, 7 and 8 below.

BSC medium preparation

BSC component	amount
Enzyme digest of casein	14.5g
Yeast extract	35.5 g
Sodium chloride	35.5 g
Alpha-D glucose	7g
Total volume	1 L

BSC medium transformation

BSC-M64	
Chemical name	Final (analysis) concentration
BGP (mM)	60.0
HEPES (mM)	10.0
LiCl (mM)	84.0
BSC	Up to 1 L

Transformation of the transformed particle medium

Transformed particle preparation (PM4)	
chemical substance	Final (analysis) concentration
$CaCl_2$ (M)	0.00667
$MgCl_2$ (M)	0.00335
HEPES (M)	0.01000
GW24 cell lysate stock	0.01250
Sodium azide (%)	0.0006
water	Up to 1mL

Non-replicating transgenic particle-based Live birth Optimization of cell reporter MRSA assay - substrate reagent preparation

In order to generate luminescence in the MRSA assay, the substrate reagent contains an aldehyde as a substrate for luciferase. The initially developed aliphatic aldehyde preparation (4.2 mM tridecanol in TSB) was not stable and formed an inhomogeneous emulsion rather than a solution. This example outlines the development of a substrate reagent formulation that is stable at room temperature or 2-8 < 0 > C for 6 months.

This example discloses steps taken to develop a final formulation from a substrate reagent.

Method / Process

All screening and stability experiments were performed using the LuxAB-expressing plasmid. A " model system consisting of Aureus strain RN4220. Typical manufacturing and test methods were as follows.

(1) overnight culture: 2 mL TSB + 1 uL Model system from 10 mg / mL tetracycline + TSA plate 1 colony of bacteria is shaken at 37 ° C overnight at 225 rpm.

(2) Day cultures: TSB + 5 ug / mL Tetracycline is diluted 1:50 or 1: 100 in overnight culture and shaken at 37 ° C for 1.5-2 hours at 225 rpm.

(3) Work-culture normalization: Measure 1 mL of the work-culture in Nanodrop with a cuvette at 600 nm and make a blank of TSB + 5 ug / mL tetracycline. Dilute to 0.1 OD with TSB + 5 ug / mL tetracycline.

(4) Dilution of the culture for the test: 0.1 OD culture was diluted with TSB + 5 ug / mL tetracycline at 1: 200, 1: 2000 and 1: 20000 dilution ratios equivalent to approximately 100,000, 10000 and 1000 CFU / do.

(5) Bacterial plate: 200 ul of each dilution and blank (TSB + 5 ug / ml tetracycline without bacteria) is added to Greiner Bio-one white assay plate in 3 replicates for each substrate being tested.

(6) Luminescent system (SpectraMax L) Preparation: Wash the reagent line with 70% ethanol and DI water in order, then first stimulate the substrate. The software is set up with Fast Kinetic so that after 10 baseline points, the substrate is scanned at 50 uL at 250 uL / sec and every 0.25 sec at 40 points.

(7) Perform the assay: Wash each substrate and test each preparation of the substrate reagent with the first stimulated SpectraMax L. All substrate reagents are brought to room temperature prior to testing.

All validation experiments were performed using MRSA analysis to ensure similar results in the actual analysis as the model system used to screen the new formulation.

(1) Preparation of culture: Ten MRSA low-performance strains and one MSSA strain are grown in TSB to log-phage in a 2 mL deep well block. The cells are spun and washed with 1x PBS and resuspended in RPMI medium.

(2) Bacterial normalization: 200 uL RPMI culture and RPMI blank are measured on a Greiner Bio-one clear plate at VersaMax at 600 nm. The blank OD is subtracted from each strain. Each strain is normalized to 0.05 OD in RPMI medium.

(3) Bacterial dilution: Dilute 0.05 OD culture to 1: 10 in RPMI medium to 0.005 OD.

(4) Preparation of Phage Medium Reagent: Phage, cephexin, and sodium pyruvate are added to BSS-M56 containing:

a. (5 < RTI ID = 0.0 > pg / mL) <

b. GW24 cell lysate stock (0.03X)

c. Sodium pyruvate (0.025M)

(5) Assay plate set-up = Add 2 uL of RPMI as a blank or 2 uL of each bacterial dilution (equivalent to approximately 20000 or 2000 CFU / mL at 0.05 OD and 0.005 OD RPMI) to both replicates.

(6) Assay plate Incubation = Incubate the assay plate at 37 ° C at approximately 100 rpm for 4 hours in an orbital shaker.

(7) Preparation of luminometer (SpectraMax L): Wash the reagent line with 70% ethanol and DI water in order, and then stimulate the substrate first. The software is set up with Fast Kinetic and after 10 baseline points, the substrate is scanned at 50 uL at 250 uL / sec and read at 40 points every 0.25 sec.

(8) Perform the assay: Wash each substrate and test each preparation of the substrate reagent with the first stimulated SpectraMax L.

We designed an experiment for the development of substrate reagent preparations leading to the following improvements:

(1) Addition of surfactant (Tween 20, Triton X-100, NP-40, Brij-35, SNS etc.), addition of solvent (ethanol, methanol, DMSO etc.), and addition of non-volatile oil Improved solubility

(2) Addition of stabilizer (triethanolamine, cyclodextrin, etc.), addition of antioxidants (vitamin E, vitamin E acetate, vitamin E PEG 1000, oxirase etc.), adjustment of tridecanol (addition of surfactant, Together with the antioxidant in the final solution, etc.), storage of tridecanol and substrate reagents under nitrogen to reduce aldehyde oxidation, and reduction of the possibility of microbial contamination by addition of preservatives such as ProClin and sterile filtration of substrate reagents Improved stability through

(3) Improvement of analytical performance by adjusting the pH of formulation and buffer system

(4) Overall performance improvement through aldehyde crystals with the highest RLU output (testing of aldehydes of 6-14 carbons in multiple formulations to determine if improvements were observed in solubility, stability, and analytical performance)

(5) Improvement of overall performance by addition of a foam blocker to reduce foam formation during addition of reagents to the sample during reagent preparation and analysis

Analysis and results

We plotted the kinetic response for each sample and fitted the lines to an average at each reading point of the three replicates. Typically, results were obtained at a 1: 2000 dilution of 0.1 OD model system bacteria, which was equivalent to approximately 10,000 CFU / mL or 2,000 CFU / assay.

The normalized maximum RLU for the reference substrate reagent was analyzed in a stability experiment. At each stability time point, the maximum RLU for each sample was normalized to the baseline substrate maximum RLU. The normalized maximum RLU was plotted against the time point and a linear regression of 95% CI was plotted.

conclusion

Table 9 summarizes the key variables that are adjusted from the reference formulation to produce the final substrate reagent formulation.

Summary of the results of reagent formulation development

Variations on substrate reagents	Reason
4.2 mM tridecanol + TSB	Original substrate reagent
Remove TSB	Reduced pollution potential
Addition of 1% Tween 20	Improved solubility
adjusted to pH 3, adjusted to 79.45% 0.1 M citric acid-19.55% 0.2 M sodium phosphate di-basic buffer	Improved analytical performance
Add tridecanol directly to concentrated surfactants	Improved stability

Add substrate filtration, use 0.2 um PES membrane	Improved stability
0.05% ProClin 300 addition	Improved stability
Triethanolamine addition	Improved stability
Change from 1% Tween 20 to 0.5% Triton X-100	Improved stability and improved solubility
79.45% 0.1 M citric acid - 19.55% 0.2 M sodium phosphate Change to 82% 0.1 M citric acid -18% 0.1 M sodium citrate buffer in Daibactic buffer, pH 3 maintained	Improved analytical performance, reduced precipitation potential due to removal of phosphate buffer
Add 100 ppm foam inhibitor Y30	Improved analytical performance
Addition of 0.5% vitamin E acetate	Improved stability, reduced precipitation
Changed the main tridecanol production from Alfa Aesar to Sigma / OmegaChem	Improved analytical performance
Change 0.5% vitamin E acetate to 1-2% vitamin E PEG 1000	Improving analytical performance, improving solubility, Improved stability

Two substrate reagent formulations were prepared to be stored at two different temperatures, one stored at 2-8 ° C and one stored at 18-24 ° C.

A final substrate reagent preparation stored at 2-8 ° C. Formulation: 0.5% Triton X-100 + 4.2mM Tridecanol + 0.5% Vitamin E acetate + 100ppm Foam blocker Y30 + 0.5% Triethanolamine + 82% 0.1M Citric acid + 18% 0.1M sodium citrate pH3 + 0.05% ProClin 300. This formulation did not precipitate at 2-8 ° C after one month and was able to detect MRSA strains exactly as at day zero.

A final substrate reagent preparation stored at 18-24 ° C. Formulation: 0.5% Triton X-100 + 6.3 mM Tricanoate + 100 ppm Foam blocker Y30 + 0.5% triethanolamine + 82% 0.1M citric acid + 18% 0.1M sodium citrate pH3 + 2% a- tocopherol- Nate + 0.05% ProClin 300. This formulation did not precipitate after one month at 18-24 ° C and was able to detect MRSA strains exactly as at day zero.

A variety of other reagents and agents may be used to obtain the formulation as is known to those skilled in the art.

Non-replicating transgenic particle-based Live birth Performance of cell reporter MRSA assay

The performance of the optimized NRTP MRSA assay was tested, which includes analyzing the detection limit of the assay and analyzing the cross-reactivity and microbial interference of the assay when infected with a non-target organism.

A) Detection limit analysis

The detection limit of the NRTP assay was assessed by determining the lowest amount of MRSA cells representing the various strains capable of producing relative light units (RLU) signals above the threshold determined from blank samples. MRSA strains included MRSA strains with SCCmec types I, II and IV, and mecA mutant mecC, which failed to detect in conventional FDA-approved MRSA PCR assays.

The following materials were used for clinical performance studies:

Growth medium Reagent: BSS-M56

Substrate Reagent: A final substrate reagent preparation, stored at 18-24 < RTI ID = 0.0 >

Transfection Particle Reagent: BSS-M56 base with 10 ug / mL (i. E., 2x concentration) of hypoxicin and 2x concentration of the above-described transduced particle reagent

LoD Research Protocol:

Overnight cultures: For each MRSA strain and MSSA negative control strain, 2 mL TSB was inoculated with strain colonies already grown on TSA plates. The overnight MRSA culture contained 5 ug / mL of hypoxicin. All samples were incubated overnight at 37 [deg.] C in a shaking incubator.

Work-in-culture: 20 uL of each overnight culture was transferred to a new culture tube containing 2 mL growth media reagent. The inoculum was then incubated at 37 DEG C while shaking for approximately 1 hour and 45 minutes until OD (600 nm) reached 0.1.

Serial dilution:

a) 1000 uL of each sample was dispensed into a 2 mL deepwell 96 well plate.

b) Next, the remaining column (B-H) was filled with 900 uL of growth medium reagent.

c) Next, a 10-fold serial dilution was made through a process such as taking 100 uL in column A and mixing in column B, whereby column H contained a sample of column A material as a 10^{-7} dilution.

Coefficient of bacterial load: 5 uL of each well in row E was spotted onto the TSA plate, which was then tilted so that a spot of liquid spread on the plate (to facilitate colony counting later) (Column E is diluted 10^{-4} in column A Water). Next, the plate was incubated at 37 占 폠 overnight.

Prepare for analysis:

a) Wells of a white 96 well assay plate were filled with 100 uL of 2x transduced particle reagent.

b) Next, wells of 96 well assay plates containing the transfected particle reagents were filled using F and G columns (i.e., 10^{-5} and 10^{-6} -fold dilutions, respectively, in column A) ≪ / RTI > in duplicate.

c) Next, the plate was sealed with an air-permeable seal and incubated at 37 占 폠 for 4 hours while lightly shaking.

After 4 hours, the plate was taken out of the incubator and immediately luminescence was measured on a SpectraMax L injected with 50 µl substrate reagent and luminescence was measured for 1 hour.

analysis:

The emission data from each sample was plotted against RLU versus time. The cutoffs calculated from all time points of the blank sample using the blank samples were determined using the following equation: (Average Blank RLU + 3 * SD Blank RLU)

Next, an average peak RLU after substrate scanning was obtained for each sample, and the highest dilution sample that produced the RLU value above the blank sample cutoff was determined.

The highest dilution colony forming unit (CFU) count that produced the RLU value above the blank sample cutoff was determined from the count study and this CFU count was determined as LoD in this study.

result:

LoD for all MRSA samples tested was found to be less than 10 CFU. Table 11 summarizes the results of the lowest LoD obtained in this study.

[Table 11]: LoD Lowest LoD's result

SCCmec 타입	LoD (CFU)
I	3
II	2
IV	3
mecC	1

All MRSA strains tested resulted in less than 10 CFUs detected in the NRTP assay over the cutoffs calculated from blank samples. The MSSA did not generate an RLU value beyond the blank sample cutoff.

The RLU value is expressed as the mean RLU value for the four replicates tested for each sample and the highest dilution ratio at which the RLU value beyond the blank sample cutoff was plotted as a standard deviation plotted. The horizontal axis is set to the blank sample cutoff, and the CFU count for the sample that generated each RLU data point is superimposed on the data. All MRSA samples generated RLU values beyond the cutoff, but MSSA did not.

Study of cross-reactivity and microbial interference

Cross-reactivity and microbial interference studies. The purpose of this study was to test a group of bacterial strains known to be present within the host range of bacteriophage φ80α in MRSA assays, usually confronted with clinical samples, and the cross-reactivity or interference of these strains with the phage or substrate used .

Existing experiments using clinical samples resulted in false positive results due to the presence of Enterococci faecalis and Staphylococcus epidermidis as shown from the presence of blue and white colonies when plated on BBL ™ CHROMagar ™ stuffer aureus plates I brought it. In addition, Listeria monocytogenes and Listeria monocytogenes can also be within the infectious or permeable host range of Phage 80 [alpha], which can also contribute to cross-reactivity in MRSA assays. The study examined Enterococci faecalis, Staphylococcus epidermidis, Listeria monocytogenes and Listeria monocytogenes for cross-reactivity / interference in viable MRSA assays. Each strain was tested at high cell numbers, such as 10^6 , 10^7 or 10^8 cells. To address the potential self-luminescence of the strain, the test was performed without the addition of GW24 cell lysate.

Experiment 1 was carried out using various strains (MSSA-S121, NRS # 9-Staphylococcus hemolyticus, NRS # 6-staphylococcus epidermidis, ATCC 12228-staphylococcus epidermidis, ATCC 15305- ATCC 29212-Enterococcus faecalis, ATCC 60193-Candida albicans, ATCC 12453-Proteus mirabilis) were tested for luminescence at high cell numbers under normal analytical conditions.

Experiment 2: To quantify the background luminescence, a subset of the strains emitted in experiment 1 were reanalyzed at various concentrations in the presence of various antibiotics.

Experiment 3: This. Paecalis and S32 (MRSA) were tested with GW24 cell lysates and various substrate formulations developed as described above without incubation.

Experiment 4: ATCC 33090-Listeria innocua and ATCC 19111-Listeria monocytogenes were tested for background signal and non-specific luminescence, Pae Kallis and S. Were re-tested with various substrate formulations developed as described above with epidermidis.

Experiment 5: This. Paecalis was re-examined with the final substrate formulation developed as described above.

The substrate reagent formulations tested in this study are summarized in Table 10.

Substrate reagent preparation

Experiment	temperament	Explanation
One	Original temperament	1% Tween 20 + 4.2 mM tridecanol, pH 3.0
2	Original temperament	
3	Substrate 1	6.3 mM tridecanol + 0.5% vitamin E acetate, pH 3.0
	Substrate 2	20 mM furnace + 0.5% vitamin E acetate, pH 3.0
	Substrate 3	8.4 mM tridecanol + 0.5% vitamin E acetate, pH 3.0
	Substrate 4	6.3 mM tridecanol + 1% a-tocopherol-PEG 1000 succinate, pH 3.0
4	Original temperament	1% Tween 20 + 4.2 mM tridecanol, pH 3.0
	Substrate 5	0.5% Triton + 4.2 mM tridecanol (Sigma) + 0.5% vitamin E acetate, pH 3.0
5	Substrate 6	6.3mM tridecanol + 2% VitE PEG, pH 3.0

Method / Process

The following steps were performed for MRSA analysis.

A) Strain grown for experiments 1-5

On the day before the analysis, overnight cultures were initiated in dip 96-well plates at a 1:50 dilution ratio of TSB from frozen 1-fold used stock and incubated at 37 ° C in an orbital shaker for> 15 hours. Bacteria (8uL) in TSB (392uL).

The absorbance of the culture was measured at Versamax. TSB was set as blank in the template in SoftmaxPro. The optical density (OD) was measured at 600 nm.

On the day of analysis, cells were resuspended to OD 0.5 and analyzed. BSS-M56 was prepared for experiments 1-5.

B) Transfection particle media reagents were prepared for all experiments 1, 2, 4 and 5 (in Experiment 3, no transfection particle reagents were used): 15 ug / mL of hypoxicin + 30x from GW 24 cells Liquor stock

C) Sample preparation: Various dilutions were prepared from the overnight culture of the strain. All strains were diluted BSS M56.

D) MRSA analysis was performed in Experiments 1-5.

The medium was loaded on the assay plate with or without phlegm and 5 μg / ml of hypoxicin. 2.5 ul cells were added. The assay plate was incubated for 4 hours at a rate set at approximately 100 rpm in a track shaker at 37 캯 with the plate lid covered.

The assay plates were then measured in SpectraMax L according to the following standard assay parameters.

Fast Kinetic Glow:

Reading at 20-hour points at 0.5 second intervals. Substrates were injected at 50 ul / well at 250 ul / sec with an M syringe, including 5 baseline readings. The incubation temperature was not set and was read at room temperature.

SpectraMax L was firstly stimulated with substrate reagents before performing the assay.

The results were analyzed as follows:

A) The cutoff was determined by averaging the blank RLUs for all replicas and time points and adding the three standard deviations.

B) We used SoftMaxPro to determine the maximum RLU for each sample.

C) Determine if the maximum RLU has exceeded the cutoff RLU, and if so, use the sample data for analysis.

Summary of results

Experiment 1: Various strains were tested for cross reactivity and interference using original substrate formulations. Among the tested NRS # 9-S. Haemolyticus, NRS # 6-S. Epidermidis < / RTI > Paekalis tested false positives in MRSA analysis.

Experiment 2: Among the three strains tested, NRS # 9 and NRS # Paecalis tested for MRSA positivity in all tested hypoxic conditions. All three strains (NRS # 9, E. faecalis, NRS # 6) tested positive when the transduced particle reagent was used for analysis, indicating that non-specific luminescence was not dependent on the transduction particle reagent, And substrate reagent-dependent. At all concentrations tested, Carb (carbenyl) was effective in removing false positives.

Experiment 3: This. Paekalis provided a positive signal without a transduction particle reagent. The MRSA strain S32 also provided a positive signal without the transduced particle reagent. This result showed substrate reagents that caused background luminescence. Substrate 4 was effective in removing background signals in the assay.

Experiment 4: Strain ATCC 33090-Listeria monocytogenes, ATCC 19111-Listeria monocytogenes were tested for luminescence with the transfection particle reagent and substrate reagent, and the Listeria species can be within the host range of the bacteriophage used in the MRSA assay. Luminescence is el. The presence of the transfected particle reagent from InocuA and the absence of the original substrate material were observed, indicating that the luminescence was potentially due to a non-specific reaction with the substrate. Substrate 5 was effective in removing luminescence from listeria. It was not effective in Paekalis.

Experiment 5: With this substrate 6, I retested Pae Kalis. Analysis in two independent experiments performed on two different days with a high load of 0.5 OD cells provided negative results.

conclusion

Cross-reactivity studies have demonstrated background luminescence from several bacterial species in high loading. The light output did not require specific substrate formulations with the transfection particle reagent and the phosphate ion contributed to the non-specific signal. Because light output from cross-reactive species has not been observed from the use of the transduction particle reagent, when the < RTI ID = 0.0 ># 80a < / RTI > infiltrates the cross-reactive species, the light output is amplified by the Slu operatively linked to the bacterial luciferase gene. An active lack of the Aureus PclpB promoter and / or S in these species. It is blocked by the active lack of Aureus pT181 replication origin.

Substituting sodium phosphate diabetic buffer with sodium citrate and citric acid in the formulation, The background luminescence was removed from all cross-reactive species tested except for paealis. The substrate 6 to which the tocopherol-PEG 1000 succinate component was added, The remaining non-specific signals were removed from the paealis.

Non-replicating transgenic particle-based Live birth Clinical performance of cell reporter MRSA assay - results for direct reputation on CHROMAgar MRSA II

MRSA screening assays with φ80α-based luxAB expression non-replicable transduction particles (NRTP) have been developed. This assay involves adding NRTP to a clinical sample suspected of containing MRSA, incubating the sample for 4 hours at 37 ° C, and then analyzing the incubated sample by injecting aldehyde into the sample while measuring luminescence from the photopattern tube Respectively. To determine the sensitivity and specificity of the assay, we compared the results of commercially available chromogenic media designed for the detection of SA on the basis of the assay results. Both NRTP-based assays were expected to correlate well with culture-based criteria, as both require the presence of viable MRSA cells and depend on the expression of the MRSA phenotype. The results showed good correlation with the standard.

The aim of this study was to determine the performance of NRTP-based MRSA assays for CHROMAgar MRSA II from testing of the remaining co swab samples collected for MRSA screening.

range:

Unidentified co-swab samples collected from patients for clinical supervision of MRSA by clinical instruments were analyzed by NRTP-based MRSA assay, CHROMAgar MRSA II, CHROMAgar SA and blood agar TSA, directly in the presence of MRSA, . To calculate the sensitivity and specificity of NRTP-based MRSA assays for CHROMAgar MRSA II, the results of NRTP-based MRSA assays were compared with those of CHROMAgar MRSA II assays.

The following materials were used for clinical performance studies:

Growth medium Reagent: BSS-M56

Substrate Reagent: The final substrate reagent preparation to be stored at 18-24 < RTI ID = 0.0 >

Transfection Particle Reagent: BSS-M56 base with 10 ug / mL (i. E., 2x concentration) of hypoxicin and 2x concentration of the above-described transduced particle reagent

Method / Process

Clinical Sample: A sample transport tube containing liquid Amies (220093-BD BBL ™ CultureSwab ™ Liquid Amies) was provided to the clinical instrument to collect the remaining unidentified nose swab collected by the clinical instrument. Prior to placing the co swab into the provided sample transport tube, the clinical instrument was instructed to perform direct culture MRSA screening by streaking on the culture plate using this swab. More specifically, specimens in front of the nostrils were collected using a standard collection swab of the clinical apparatus according to the internal standard procedure of the clinical apparatus. Next, the clinical apparatus performed culture screening directly with this swap. The remaining swaps were then placed in the sample transport tube with the end of the swap immersed in the Amies buffer in the sample transport tube. Next, the sample was kept at room temperature for 2-24 hours before further processing.

Sample Handling: Upon receipt, the samples were placed up in a bio-safety cabinet and kept overnight at room temperature to ensure that the swab was immersed in the sample transport tube Amies buffer. After overnight storage, the samples were treated as follows.

Clinical Sample Preparation:

Using a 1 mL pipette, 300 µL growth medium reagent was added to a 15 mL Falcon tube.

The swab from the remaining coswash was taken from the original transport tube and immersed in the growth medium of the corresponding Falcon tube. Next, the swab was rolled back and forth 4-6 times in the growth medium reagent and the swap contents were eluted with a growth medium reagent in a Falcon tube. Next, the swab was placed back in the original transport tube and stored at 2-8 ° C until study termination, and the eluted clinical sample in the falcon tube was transferred to a 1.5 mL tube and kept at room temperature until further processing.

NRTP MRSA Assay: The following sample was loaded directly onto a white 96 well assay plate.

Clinical sample: 100 µL of eluted material from each clinical sample,

MRSA Positive Control: 98 uL Growth Medium Known MRSA isolate in reagent CAUTION 2 µL of fully mixed 0.1 OD culture, three times

MSSA Negative control: 98uL Growth medium Known MSSA separations in reagents Caution 2 µL of fully mixed 0.1 OD culture, three times

Blank: Growth medium Reagent 100 µL, three times

100 [mu] L of the transfected particle reagent was added to each sample. Next, the assay plate was placed in an incubator set at 37 DEG C and shaked in an orbital shaker for 4 hours. At the end of 4 hours, the plate was immediately taken out of the incubator and immediately the luminescence was measured in SpectraMax L injected with 50 µL of substrate reagent, and the luminescence was measured for 1 minute.

Clinical sample CFU Bacteria reputation for count:

Each eluted clinical sample was evaluated to determine bacterial colony counts on CHROMAgar MRSA II, CHROMAgar SA, and blood agar (TSA II) through direct and hatched cultures as follows. The organism CFU count was determined by direct plateau. The MRSA CFU count was determined by reputation on CHROMAgar MRSA II. s. The Aureus CFU count was determined by reputation on a CHROMAgar SA plate. The CFU count of any organism for which growth was supported by blood agar TSAdp was determined by reputation on blood agar TSA. If no colonies were created due to loading of the organism below the detection limit of the plate used directly, a portion of the clinical sample eluted in TSB overnight at 37 ° C with shaking was incubated and incubated on CHROMAgar MRSA II Lt; RTI ID = 0.0 > incubation. ≪ / RTI > All plates were incubated at < RTI ID = 0.0 > 37 C < / RTI > for 20-24 hours. After incubation, the CFU counts of any colonies appearing on each plate were recorded.

Analysis: MRSA, S. The presence of total organisms per aureus and eluted clinical sample and the CFU load were calculated based on the CFU counts obtained in CHROMAgar MRSA II, CHROMAgar SA, and blood agar TSA, respectively.

NRTP Analysis : Data from each sample was plotted against RLU versus time.

Cutoff decision: The analysis cutoff was calculated from all time points of the blank sample using the following equation: (mean blank RLU + 3 * SD blank RLU).

MRSA Positive Determination: It was determined whether the RLU of each time point after the substrate injection was above or below the analytical cutoff. If two or more data points after the scan were above the analytical cutoff, this sample was designated "MRSA positive. &Quot;

RESULTS: MRSA positive results of NRTP assays were compared with those of direct plate and incubated cultures on CHROMAgar MRSA II. The following calculations were performed to determine the NTRP assay sensitivity and specificity for CHROMAgar MRSA II.

- True positive (TP)

o samples that produced MRSA positive results for NRTP analysis and CHROMAgar MRSA II

- True Speech (TN)

o NRTP analysis and samples that generated MRSA negative results for CHROMAgar MRSA II

- False positive (FP)

o samples that produced MRSA positive results in NRTP analysis and MRSA negative results in CHROMAgar MRSA II

- False speech (FN)

o MRNA negative results from NRTP analysis and samples with MRSA positive results from CHROMAgar MRSA II

- Sensitivity = TP / (TP + FN)

- specificity = TN / (TN + FP)

CHROMAgar Results for direct reputation above MRSA II

Table 11 shows the results obtained by comparing NRTP assays for direct plates on CHROMAgar MRSA II.

NRTP analysis results vs. direct reputation results on CHROMAgar MRSA II

Total sample	CHROMAgar MRSA II positivity	CHROMAgar MRSA II voice	NRTP analysis positivity	NRTP analysis voice	Oh yeah positivity	Oh yeah voice	lie positivity	lie voice
69	7	62	12	57	7	57	5	0

Based on the above data, the sensitivity and specificity of the assay for the direct plate on CHROMAgar MRSA II were calculated as follows:

Sensitivity = 100%

Specificity = 92%

Non-replicating transgenic particle-based Live birth Clinical performance of cell reporter MRSA assay - incubated cultures CHROMAgar Results for reputation on MRSA II

Based on the results for the plate directly from CHROMAgar MRSA II, all clinical samples were retested for incubated cultures and subsequently rewarded on CHROMAgar MRSA II. The rationale for follow-up testing was based on the possibility that false positive results compared to direct plating were indeed detectable by NRTP analysis but could have been true for direct reputation. A portion of the remaining eluted swap samples was retested through NRTP analysis as described above. Other portions of the remaining eluted swap samples were tested through hatch cultures and subsequently ranked on CHROMAgar MRSA II. The incubated culture test consisted of adding 100 µL of the remaining eluted swab material to 2 mL of TSB and incubating at 37 ° C. with shaking for a period of 18-24 hours. The resulting culture

was then streaked on CHROMAgar MRSA II to confirm the presence of MRSA in the culture. Table 12 summarizes data from the direct plate and post hatching plate analyzes, which only show samples that produced MRSA positive results in NRTP analysis or CHROMAgar MRSA II.

Results of NRTP assay versus direct plate and incubated cultures for CHROMAgar MRSA II

Sample #	NRTP analysis	directly CHROMagar MRSA II	Hatching + NRTP analysis	Hatching + CHROMagar MRSA II
One	+	+	+	+
2	+	+	+	+
3	+	+	+	+
4	+	+	+	+
5	+	+	+	+
6	+	+	+	+
7	+	+	+	+
8	+	-	+	+
9	+	-	+	+
10	+	-	+	+
11	+	-	+	+
12	+	-	+	-

Only NRTP assays or samples that generated MRSA positive results in CHROMAgar MRSA II are shown.

Table 13 shows the results obtained by comparing incubated cultures with NRTP assays for clinical samples reputed on CHROMAgar MRSA II.

As a result of NRTP analysis, the culture supernatant was evaluated on CHROMAgar MRSA II

Total sample	CHROMAgar MRSA II positive	CHROMAgar MRSA II voice	NRTP assay	NRTP analysis voice	Oh yeah positivity	Oh yeah voice	False positive	A false voice
69	11	58	12	57	11	57	One	0

Based on this data, the sensitivity and specificity of the assay for hatching cultures reputed to CHROMAgar MRSA II was calculated as follows:

- Sensitivity = 100%

- specificity = 98.3%

Example 8: For antibacterial susceptibility test NRTP - Based analysis - Correlation of minimum suppression concentration and emission output

As another example, sulfoxide-resistant S. To determine the minimum inhibitory concentration of the cypercitine needed to inhibit the growth of Aureus, Analysis of susceptibility to aureus-secocin was developed. Hypoxic tolerance S. Unlike the above-described MRSA sulfoximine resistance assay which distinguishes from aureus to be sensitive to the cytotoxin, the MRSA < RTI ID = 0.0 > hypoxicin < / RTI > The development of an assay to determine the minimum amount of cypercitin that is necessary to inhibit the growth of Aureus.

The following materials were used for clinical performance studies:

Growth medium Reagent: BSS-M56

Substrate Reagent: The final substrate reagent preparation stored at 18-24 < 0 > C as described in Example 7

Transfection Particle Reagent: BSS-M56 base with 10 ug / mL (i. E., 2x concentration) of hypoxicin and 2x concentration MIC In the study protocol, the transduced particle reagent

Nascent incubation: For each of the MRSA strains (NRS35 and S7) and the MSSA negative control strain (MSSA121), 2 mL of TSB was inoculated with the strain colonies already grown on TSA plates. The overnight MRSA culture contained 5 ug / mL of hypoxicin. All samples were incubated overnight in a shaking incubator at < RTI ID = 0.0 > 37 C. < / RTI >

Work-culture: 20 uL of each overnight culture was transferred to a new culture tube containing 2 mL of growth medium reagent. Next, the inoculum was incubated at 37 until OD (600 nm) reached 0.1 while shaking the inoculum for approximately 1 hour and 45 minutes.

Through reputation MIC decision:

a) Each day and culture was streaked onto TSA plates containing 4, 8, 16, 32, 64, and 128 ug / ml of cypoxitin.

b) Plates were incubated at 37 ° C for 18 hours to determine growth.

NRTP Prepare for analysis:

a) Wells of a white 96 well assay plate were filled with 100 uL of 2x transduced particle reagent.

b) For each of the days and cultures, 5 wells were filled with 100 uL of day and culture.

c) For each of the days and cultures, the cypoxitin was added to each well to give a concentration of cypoxithine in the wells of 4, 8, 16, 32, 64, and 128 ug / mL.

d) Next, the plate was sealed with an air-permeable seal and incubated at 37 DEG C for 4 hours while lightly shaking at 50 rpm.

At the end of the 4 hour period, immediately after the plate was removed from the incubator, 50 μL of substrate reagent was measured for luminescence on the scanned SpectraMax L and measured for luminescence for 1 minute.

analysis:

The maximum luminescence values were plotted after substrate reagent addition from each sample. The MSSA sample RLU values were used to determine the calculated cutoff according to the following equation: (average MSSA RLU + 3 * SD MSSA RLU).

result:

Figure 23 shows the results of S [beta] in the hypoxicin at 4, 8, 16, 32, 64, and 128 [mu] g / Lt; RTI ID = 0.0 > aureus < / RTI > Figure 24 shows the RLU values obtained by NRTP analysis in the presence of 4, 8, 16, 32, 64, and 128 ug / mL of hypoxicin. In FIG. 24, the x-axis is set to the MSSA RLU cutoff value.

As can be seen in FIG. 23, MRSA NRS25 showed a MIC of 128 ug / mL secoxithine, and MRSA S7 showed a MIC of 64 ug / mL secoxithine. Correspondingly, MRSA NRS25 displayed perceptible luminescence above the MSSA RLU cutoff up to a concentration of up to 64 ug / mL of hypoxicin of cytotoxic, and MRSA S7 showed luminescence in excess of MSSA RLU up to a concentration of up to 32 ug / mL of cytoxytin .

Based on the above data, NRTP analysis demonstrates that the obtained RLU values are correlated with the MIC results and thus the NRTP assay can be used to develop an antimicrobial susceptibility assay.

Example 9: Transcript Reporter Analysis: Mrsa's mecA gene On the transcript Activated by Luxab Translation RBS -block Cis - Mechanism of steric structure change by inhibition

As described above, the reporter transcript may be designed such that translation of the reporter gene sequence is blocked by cis-suppression of the ribosome-binding site (RBS) of the reporter gene.

The following manner was used to design the reporter transcript of the present invention.

1) RNA secondary structure was calculated using a secondary structure program such as Mfold (http://mfold.rna.albany.edu/?q=mfold/RNA-Folding-Form).

2) Intermolecular RNA interactions were calculated using a software program such as RNA-RNA Interaction Prediction using Integer Programming (RactIP) (http://rna.naist.jp/ractip/).

3) Visualization of RNA The RNA secondary structure was visualized using an applet (VARNA) (http://varna.lri.fr/).

Figure 25 shows the secondary structure of a mecA transcript generated based on the lowest energy steric structure computed by MFold and visualized as VARNA. End loop 23 (T23) contains the YUNR sequence UUGG consisting of bases 1,487-1,490 of the mecA transcript sequence. Analysis of the secondary structure of the mecA gene transcript was suitable for designing a cis-suppressed luxAB reporter that could be repressed through interaction between reporter and ssRNA regions.

As shown in detail in Fig. 26, the terminal loop 23 (T23) of the mecA transcript contains the YUNR consensus sequence. The pYrimidine-Uracil-Nucleotide-puRine (YUNR) consensus sequence has been identified as an important target for intermolecular RNA complexes in nature. The cis-suppression sequence was designed to form a stem-loop structure with the RBS of the reporter sequence, whereby the cis-suppression sequence blocks the binding of the RNA polymerase to the RBS of the reporter sequence. The reporter sequence was exposed at the junction of the cis-suppressing stem-loop loop with the T23 of the mecA transcript.

27, a cis-suppression sequence 2701 was added to the 5 'end of the luxAB gene and designed to form a stem-loop structure that blocks the RBS sequence ("AAGGAA") 2702 of the luxA gene . The cis-inhibitory stem-loop structure is luxA RBS ("AAGGAA") based on the lowest energy stereo structure of luxAB transcripts containing cis-suppression sequences at the 5 ' end of luxAB transcripts calculated by MFold and visualized with VARNA It was predicted to block the sequence.

The first 61 nucleotides of the cis-inhibited luxAB gene are shown in Figure 7, which is up to the start codon AUG of the luxA gene. The RBS sequence "AAGGAA" includes bases 47-52. This end loop of the reporter transcript was designed to interact (bind) with the end loop 23 (T23) of the mecA transcript containing the YUNR sequence.

The end loop of the cis-suppression sequence was designed to interact with the T23 of the mecA transcript, thereby enabling the loop from the cis-suppressing trunk-loop structure of the cis-suppressed luxAB transcript and mecA transcript and the T23 of the mecA transcript Hybridization through action causes exposure of RBS in the luxA gene. Figure 28 shows the predicted intermolecular interactions between the mecA T23 sequence and the cis-suppression sequence on the luxAB transcript calculated by RactIP and visualized by VARNA. Line represents the base pairing between the mecA transcript and the cis-suppressed luxAB transcript. The interaction between the two sequences results in the exposure of the luxA RBS sequence AAGGAA and consequent suppression of the luxAB reporter.

Example 10: Transcript Reporter Analysis: mecA - luxAB Method for detecting a target transcript or gene using a reporter system

As another example, a method for detecting a target mecA gene using the mecA-luxAB reporter system is provided. Here, mecA is the target transcript and luxAB is the reporter molecule.

1. Structure of reporter structure

A vector containing a reporter structure that encodes luxAB contains the reporter structure. Kola Iwasu. Can be constructed through standard molecular biology techniques by integrating into a shuttle vector that can all multiply in aurios. This vector is a vector illustration. In E. coli, functional replication origin and this. May contain a selectable marker expressed in E. coli, which is transformed into a vector and grown under selective conditions. Which is suitable for growing E. coli cells. In addition, Functional origin of replication in Aureus and S. It may contain a selectable marker expressed in Aureus, which is transformed into a vector and grown under selective conditions. Which is suitable for growing E. coli cells. Proliferation of the vector for performing in vitro manipulation and verifying the manipulation, Can be achieved through suitable laboratory cloning strains of E. coli, Can be introduced into the Aureus strain.

The reporter structure was first used for the transfer of the structure and for the production of the reporter transcript. Can be introduced into Aureus cells.

2. Cis - Suppressor Reporter Transpositional Configuration

A method for constructing a cis-inhibitory reporter transcript capable of binding to a mecA-target transcript is provided. Reporter transcripts can be constructed using standard molecular biology techniques. The luxA and luxB genes act as reporter genes, which can be derived from Vibrio hybenii. These genes lack a transcriptional promoter, each containing its own ribosome binding site (RBS). When both luxA and luxB genes are translated in cells, luxA and luxB proteins are complexed to form the active luciferase enzyme (luxAB) (Farinha, MA and AM Kropinski, Construction of broad-host-range plasmid vectors for easy J. Bacteriol., 1990. 172 (6): p. 3496-3499).

The cis-suppressing sequence may be located upstream of the luxAB gene and downstream of the promoter, and includes a sequence complementary to luxA RBS. The linker sequence can separate the complementarity region of the cis-suppression sequence and the luxA sequence. After vector transcription, a complementary region of the cis-inhibitory sequence and the luxA RBS sequence complexes to block the docking of the ribosomes to produce a stem-loop that blocks translation.

The stem-loop of the reporter transcript is designed to form an open complex by destabilization when interacting with the naturally occurring mecA transcript sequence (endogenous to the cell). To activate translation of the luxA gene sequence, the natural mecA transcript acts as a trans-activating RNA that binds the cis-suppressing reporter transcript and opens an inhibitory stem-loop that isolates the RBS of the luxA gene. If RBS is not sequestered by cis-suppression sequences, translation of luxA may proceed. Transcription of the reporter construct is achieved by operably linking the reporter sequence to a constitutive promoter upstream of the cis-suppression sequence.

An example of a target mecA gene sequence is shown in Fig. This sequence is the mecA locus DNA sequence (Staphylococcus aureus subsp. Aureus SA40, complete genomic GenBank: CP003604.1; SEQ ID NO: 15), a reporter sequence and a reporter Can be used to create structures. A transcription start position 2902, an RBS 2903, a coding region (gray portion 2904) and a transcription termination sequence 2905 are shown.

Figure 30 shows an exemplary mecA transcript sequence that can be used to design a reporter transcript (SEQ ID NO: 16), in accordance with embodiments of the present invention. RBS 3001 and coding sequence 3002 are shown for mecA.

Figure 31 is an example of a luxAB locus DNA sequence that can be used to design a reporter transcript according to embodiments of the present invention. The luxAB locus DNA sequence was obtained from the Vibrio peas cherry genes luxA and luxB for luciferase alpha and beta subunits (GenBank: X06758.1) (SEQ ID NO: 17). A

position 1010, a transfer start position 3102, an RBS 3103 for luxA, a luxA coding region 3104 (gray shade) and an RBS 3105 for luxB, and a luxB coding sequence 3106 (gray Shade) is shown.

32 is an example of a luxAB transcript sequence that can be used to design a reporter transcript (SEQ ID NO: 18). RBS 3201 for luxA, luxA coding sequence 3202 (gray shades), RBS 3203 for luxB, and luxB coding sequence 3204 (gray shades) are shown.

33 is an example of a luxAB cis-suppressed transcript sequence that can be used in a reporter transcript (SEQ ID NO: 19). (Gray shading) for luxA, RBS 3304 for luxB, and luxB coding sequence 3305 (gray shading) for luxB, cS-suppression sequence 3301 (dotted box), RBS 3302 for luxA, luxA coding sequence 3303 Lt; / RTI >

3. Reporter The whole body using mecA Target Transpositional How to detect presence or absence

Examples are provided for detecting the presence or absence of a mecA target transcript in a cell using the reporter transcript of the present invention. Figure 34 shows an example of a cell containing a vector 3400 encoding the reporter transcript 1410, wherein the cell 3401 has no endogenous mecA transcript (e. G., The genome of the cell contains the mecA gene I never do that). In this case, the cis-inhibition sequence 3420 binds to the RBS 3430 of the luxAB gene. In some embodiments, the cis-suppression sequence 3420 may be associated with the RBS of the luxA gene, the RBS of the luxB gene, or some or all of both. This binding blocks and prevents translation of the luxAB gene, so that no reporter molecule (e.g., luciferase) is produced in the cell. Thus, no signal was detected, indicating the absence of the mecA gene in the cell.

As another example, the cell comprises an endogenous mecA transcript (e. G., The genome of the cell contains the mecA gene). 35 shows vector 3400 introduced into cell 3401. Fig. Vector 3400 encodes a reporter transcript 3410 comprising a cys suppression sequence 3420 and a reporter sequence (luxA and luxB genes). When the mecA transcript 3510 present in the cell binds to the cis-suppression sequence 3420, the inhibitory hairpin loop is opened and the lux A gene RBS 3430 is exposed. In this case, translation of the reporter sequences (luxA and luxB) proceeds to form the luxAB enzyme 3520. [The luxAB enzyme 3520 produces a detectable emission signal 3530. In this manner, transcript reporter vector 3400 reports the presence of endogenous mecA transcript 3510 in cell 3401.

While the present invention has been particularly shown and described with reference to preferred embodiments and various alternative embodiments, it will be understood by those skilled in the art that various changes in form and details may be made therein without departing from the spirit and scope of the invention.

All references, issued patents and patent applications cited within this specification are hereby incorporated by reference in their entirety for all purposes.

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Abbreviated sequence list

SEQ ID NO: 1

Native P1 pac-site

CCACTAAAAAGCATGATCATTGATCACTCTAATGATCAACATGCAGGTGATCACATTGCGGCTGAAATAGCGGAAAAACAAAGAGTTAATGCCGTTGTCAGTGCCGCAGTCGAGAAT
GCGAAGCGCCAAAATAAGCGCATAAATGATCGTTCAGATGATCATGACGTGATCACCCGC

SEQ ID NO: 2

P1 pac-site with silent mutation, lower case indicates mutated base

CCACTAAAAAGCATGATaATaGAcCACTCTAAcGAcCAACATGCAGGgGAgCACATTGCGGCTGAAATAGCGGAAAAgCAgAGgGTgAATGCCGTTGTCAGTGCCGCAGTCGAGAATG
CGAAGCGCCAAAATAAGCGCATAAAcGAcCGTTCAGAcGAcCATGACGTtATtACCCGC

SEQ ID NO: 3

C1 inhibitor-control P53 promoter, promoter P53 antisense, repL gene, and P1A cell lysate replicon containing the in-frame deletion of the kilA gene

CTCGCTAAGACATTGGCTTTATTTAATTTTTTATCCACACCCCCATGTCAAAATGATACCCCCTCCCCCTGTCAGGATGACGTGGCAATAAAGAATAAGAAGTCACAAGTTAAAAAAA
CAAAAAGATCAGTTTCCGGCGGTGCCGGAACAACCAGCCTCAAAAAATTGACTTCATGGATCGCTAAGGCAAAAGCAAAGGCTGACAATCTGCGGTTATCCAAAAAACGCACTCAA
AAACATGAGTTCAAGCAGAAAGTAGAGGCGGCTGCGCGGAAATATGCTTACCTGAAGAACAAGCGTTCGCCTGATATTGGCGGGATATCAAACTTCGATAACCTACCGCATTGCATG
ACGGTAAACGAAGCTCTTAATGCGGTTTTAGCCAAAAATAAAGATAACGAACAATGGGGTATACCGGCAGGATTCAGAGGGTAATGAATTGCTCTAATTATAACCATGCATACTTTCA
ACACCTCTAGTTTGCCATGAGGCAAACTCATAGGTGTCCTGGTAAGAGGACACTGTTGCCAAAACTGGACGCCCCATTATTGCAATTAATAAACAACTAACGGACAATTCTACCTAAC
AATAAGTGGCTTAAAAAAACCCGCCCCGGCGGGTTTTTTTATCTAGAGCTAGCGGATCCGGCGCGCCGGGCCCTTCTGGGCCTCATGGGCCTTCCGCTCACTGCCCGCTTTCCAG

SEQ ID NO: 4

Pblast promoter sequence

CGTCAGGTGGCACTTTTCGGGAAATGTGCGCGGAACCCCTATTTGTTTATTTTCTAAATACATTCAAATATGTATCCGCTCATGAGACAATAACCCTGATAAATGCTTCAATAATATTG
AAAAGGAAGAGT

SEQ ID NO: 5

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

TACTCAACTGAAAAATAATGAGGTCATTATTATTGGAGAAATTCCTGCTCGATGTATTCAAGATTATCGCAATGATTTAACTTTTTTTACAAATGGGCTTAGTGTTTGTTTAGCAGAGC
TAAAAGGATATCAGGTTACCACTGGCGAACCTGTTTGCCAGACCCGTCGTCTAAATAGTCGGATAGATAAAGTAAGATATATGTTCAATAAAATAACTTAGTGCGTTTTATGTTGTTAT
ATAAATATGGTTTCTTATTAAATAAGATGAAATATTCTTTAATATAGATTTGAATTAAAGTGGAAAGGAGGAGATTGTTATTATAAACTACAAGTGGATATTGTGTCCTATTTGTGGAAA
TAAAACAAGACTACGAATACGAGTGGATACTATACTTAAAAATTTCCCTTTATACAGCCCCAAATGTAAGAACGAAACTTTAATTAATGTTCAAAAAATGAATATAATAACAATCAAAG
AGCCAGACGCCAAGACGCAGAGCCGATAATTTGAGAAATGAAACTCTCATCTTATCGGCTCTTTTTGTTTATCTGAATTTTACTGACTAGCCTTCAATATTTCC

SEQ ID NO: 6

P1 pacA gene, the lower case represents the deleted pac-site sequence

Gt;

SEQ ID NO: 7

The native P1 pacA gene

Gt;

SEQ ID NO: 8

The terS gene, the lower case represents the deleted sequence

ATGAACGAAAAACAAAAGAGATTCGCAGATGAATATATAATGAATGGATGTAATGGTAAAAAAGCAGCAATTTCAGCAggttatagtaagaaaacagcagagtctttagcaagtcgattgttaagaaatg
ttaatgtttcggaatatattaaagaacgattagaacagatacaagaagagcgtttaatgagcattacagaagctttagcgttatctgcttctattgctagaggagaacctcaagaggcttacagtaagaaatatgaccatttaaacgatgaagt
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TACTAAAAGTTCATGGTGCGTATATCGACAAAAAAGAAATTACTCAGAAGAATATTGAGATTAATATTGGTGAGTACGATGACGAAAGTTAA

SEQ ID NO: 9

The sequence containing the native terS gene

AATTGGCAGTAAAGTGGCAGTTTTTGATACCTAAAATGAGATATTATGATAGTGTAGGATATTGACTATCTTACTGCGTTTCCCTTATCGCAATTAGGAATAAAGGATCTATGTGGGTT
GGCTGATTATAGCCAATCCTTTTTTAATTTTAAAAAGCGTATAGCGCGAGAGTTGGTGGTAAATGAAATGAACGAAAAACAAAAGAGATTCGCAGATGAATATATAATGAATGGATGT
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GAGATTAATATTGGTGAGTACGATGACGAAAGTTAAATTAAACTTTAACAAACCATCTAATGTTTTCAACAG

SEQ ID NO: 10

SaPibov2 integrase gene

≪

SEQ ID NO: 11

pGWP10001 whole sequence

TATCCAGCTGAACGGTCTGGTTATAGGTACATTGAGCAACTGACTGAAATGCCTCAAAATGTTCTTTACGATGCCATTGGGATATATCAACGGTGGTATATCCAGTGATTTTTTTCTCC
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AAGGT

SEQ ID NO: 12

S. aureus PclpB promoter sequence

GTCTAGTTAATGTGTAACGTAACATTAGCTAGATTTTTTTATTCAAAAAAATATTTACAAATATTAGGAAATTTAAGTGTAAAAGAGTTGATAAATGATTATATTGGGACTATAATATAAT
TAAGGTC

SEQ ID NO: 13

RN10616 genomic sequence locus showing deletion and complementarity of φ80α terS. terS = parenthesized text, elimination = underlined text, complementary = dark text

**AATTGGCAGTAAAGTGGCAGTTTTTGATACCTAAAATGAGATATTATGATAGTGTAGGATATTGACTATCTTACTGCGTTTCCCTTATCGCAATTAGGAATAAAGGATCTATGTGGG
TTGGCTGATTATAGCCAATCCTTTTTTAATTTTAAAAAGCGTATAGCGCGAGAGTTGGTGGTAAATGAA**

**[[ATGAACGAAAAACAAAAGAGATTCGCAGATGAATATATAATGAATGGATGTAATGGTAAAAAAGCAGCAATTTCAGCA
GGTTATAGTAAGAAAACAGCAGAGTCTTTAGCAAGTCGATTGTTAAGAAATGTTAATGTTTCGGAATATATTAAAGAACGATTAGAACAGATACAAGAAGAGCGTTTAATGAGCAT
TACAGAAGCTTTAGCGTTATCTGCTTCTATTGCTAGAGGAGAACCTCAAGAGGCTTACAGTAAGAAATATGACCATTTAAACGATGAAGTGGAAAAAGAGGTTACTTACACAATCA
CACCAACTTTTGAAGAGCGTCAGAGATCTATTGACCACATACTAAAAGTTCATGGTGCGTATATCGACAAAAAAGAAATTACTCAGAAGAATATTGAGATTAATATT
GGTGAGTACGATGACGAAAGTTAA]]**

ATTAAACTTTAACAAACCATCTAATGTTTTCAACAG
GTTGTTTCAAGACTATTGAAGAGTTTGACAACTACACATGGCAAAAGGACAAAGATACAGGTGAATATACCAATGAACCAGTAGATACATACAATCATTGTATCGATTCGTTGCGTTA
TTCAGTGGAACGATTC

SEQ ID NO: 14

pGW80A0001 whole sequence

TATTTAAAGTGCGTTGCTTTTTTCTCATTTATAAGGTTAAATAATTCTCATATATAAGCAAAGTGACA

SEQ ID NO: 15 (mecA locus DNA sequence (from Staphlyococcus aureus subsp. Aureus SA40, whole genome GenBank: CP003604.1)

AGTGAAGCAATCCGTAACGATGGTTGCTTCACTGTTTT

SEQ ID NO: 16 (mecA transcript sequence)

GUAACGAUGGUUGCUUCACUGUUUU

SEQ ID NO: 17 (luxAB locus DNA sequence (Vibrio fischeri gene luxA and luxB-GenBank: X06758.1 for luciferase alpha and beta subunits)

TGTCCGATATTAAAGATGTAAAAGATATTATTGATATGTTGAACCAAAAAATCGAAATGAATTTACCATAATAAAATTAAAGGCAATTTCTATATTAGATTGCCTTTTTAAATTTC

SEQ ID NO: 18 (luxAB transcript sequence)

AUGUAAAAAAUAUAUUAUUGAGAAAUAUGAACCAAAAAAUCGAAAUGAAUUUACCAUAAUAAAAUUAAAGGCAAUUUCUAUAUUAGAUUGCCUUUU

SEQ ID NO: 19 (cis-suppressed luxAB transcript sequence)

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SEQUENCE LISTING <110> GENEWEAVE BIOSCIENCES, INC. <120> NON-REPLICATIVE TRANSDUCTION PARTICLES AND TRANSDUCTION PARTICLE-BASED REPORTER SYSTEMS <130> 28421-25938 / PCT <140> PCT / US2014 / 026536 <141> 2014-03-13 <150> 61 / 939,126 <151> 2014-02-12 <150> 61 / 897,040 <151> 2013-10-29 <150> 61 / 779,177 <151> 2013-03-13 <160> 22 <170> PatentIn version 3.5 <210> 1 <211> 177 <212> DNA <213> Enterobacteria phage P1 <400> 1 tcacattgcg 60 gctgaaatag cggaaaaaca aagagttaat gccgttgtca gtgccgcagt cgagaatgcg 120 aagcgccaaa ataagcgcat aaatgatcgt tcagatgatc atgacgtgat cacccgc 177 <210> 2 <211> 177 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Synthetic 폴리 누리otide <400> 2 ccactaaaaa gcatgataat agaccactct aacgaccaac atgcagggga gcacattgcg 60 gctgaaatag cggaaaagca gagggtgaat gccgttgtca gtgccgcagt cgagaatgcg 120 aagcgccaaa ataagcgcat aaacgaccgt tcagacgacc atgacgttat tacccgc 177 <210> 3 <211> 1727 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Synthetic 폴리 누리otide <400> 3 cactataggg cgaattggcg gaaggccgtc aaggccgcat ttgggcccgg cgcgccggat 60 ccgctagctc tagactggca ggtttctgag cagatcgtcc aacccgatct ggatcgggtc 120 agaaaaattt gctctaataa atttcgtttt ctaagtgcaa agaatcacca tttcgagctg 180 gtgattgaag gttgatgcaa atttggagaa aaaatgcaac aaacattcaa tgcggatatg 240 aatatatcaa accttcatca aaatgtcgat ccttcaacca ctctgcccgt tatttgtggt 300 gttgaaatta cgaccgaccg cgctggccgt tacaacctta atgctctaca cagagcgagc 360 ggactcggtg cccataaagc gccagctcaa tggctaagaa cgctgtcagc taaacagctc 420 atcgaagagc ttgaaaaaga aactatgcag aattgcatag tttcgttcac aagcaatgga 480 agcaggattt ctttcacgac tcgtataacc ggcaaaggtc agcagtggct gatgaagcga 540 ttgcttgatg ctggtgtgct ggtacctgtc gcggcaacgc gctaacagac gtagtaagaa 600 ccaccagcat tgtaatgctg gctaaagtca ctttcctgag ctgtataacg atgagcgatt 660 ttacttttttc tggctatgaa ttggcctgct ttgtaacaca ctccggtcta tcccgtagcg 720 ccgggcatat cctgtcgcaa tgtgcaaatc tcgcggcaac aaccagtgaa tacttcattc 780 acaagcctca ccgcctgatc gcggcagaaa ctggttatag ccaatcaacc gtcgttcgtg 840 cattccgtga agctgtaaac aaaggaattc tgtctgtaga gattgttatc ggcgatcacc 900 gtgaacgtcg cgctaacctg taccggttta caccatcctt tttggccttc gcacaacaag 960 ccaaaaatgc gctgatagaa agcaaattaa agatctcttc agcggcaacc aaggttaaag 1020 ctgttctcgc taagacattg gctttattta atttttatc cacaccccca tgtcaaaatg 1080 atacccccctc cccctgtcag gatgacgtgg caataaagaa taagaagtca caagttaaaa 1140 aaacaaaaag atcagtttcc ggcggtgccg gaacaaccag cctcaaaaaa ttgacttcat 1200 ggatcgctaa ggcaaaagca aaggctgaca atctgcggtt atccaaaaaa cgcactcaaa 1260 aacatgagtt caagcagaaa gtagaggcgg ctgcgcggaa atatgcttac ctgaagaaca 1320 agcgttcgcc tgatattggc gggatatcaa acttcgataa cctaccgcat tgcatgacgg 1380 taaacgaagc tcttaatgcg gttttagcca aaaataaaga taacgaacaa tggggtatac 1440 cggcaggatt cagagggtaa tgaattgctc taattataac catgcatact ttcaacacct 1500 ctagtttgcc atgaggcaaa ctcataggtg tcctggtaag aggacactgt tgccaaaact 1560 ggacgcccca ttattgcaat taataaacaa ctaacggaca attctaccta acaataagtg 1620 gcttaaaaaa acccgccccg gcgggttttt ttatctagag ctagcggatc cggcgcgccg 1680 ggcccttctg ggcctcatgg gccttccgct cactgcccgc tttccag 1727 <210> 4 <211> 131 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Synthetic 폴리 누리otide <400> 4 cgtcaggtgg cacttttcgg gaaatgtgcg cggaacccct atttgtttat tttctaaata 60 cattcaaata tgtatccgct catgagacaa taaccctgat aaatgcttca ataatattga 120 aaaggaagag t 131 <210> 5 <211> 4681 <212> DNA <213> Artificial Sequence <220> <223> Description of Artificial Sequence: Synthetic 폴리 누리otide <220> <221> modified_base ≪ 222 > (301) <223> a, c, t, g, unknown or other <220> <221> modified_base ≪ 222 > (4056) .. (4056) <223> a, c, t, g, unknown or other <400> 5 tttgcggaaa gagttagtaa gttaacagaa gacgagccaa acctaaatgg tttagcagga 60 aacttagata aaaaaatgaa tccagaatta tattcagaac aggaacagca acaagagcaa 120 caaaagaatc aaaaacgaga tagaggtatg cacttataga acatgcattt atgccgagaa 180 aacttattgg ttggaatggg ctatgtgtta gctaacttgt tagcgagttg gttggacttg 240 aattgggatt aatcccaaga

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Patent Citations (128)

Publication number	Priority date	Publication date	Assignee	Title
US5736388A *	1994-12-30	1998-04-07	Chada; Sunil	Bacteriophage-mediated gene transfer systems capable of transfecting eukaryotic cells
WO2008131230A1 *	2007-04-18	2008-10-30	Cornell University	Microorganism detection method and apparatus
Family To Family Citations				
NL289281A	1962-02-28			
US3826574A	1973-02-12	1974-07-30	Continental Distributors	Nephelometer
US4057148A	1974-06-25	1977-11-08	G. D. Searle & Co.	Multiple sample support assembly and apparatus for facilitating radioimmunoassays and the like
CA1277931C	1984-06-05	1990-12-18	Shimon Ulitzur	Detection and/or identification of microorganisms in a test sample usingbioluminescence or other exogenous genetically-introduced marker
US4861709A	1985-05-31	1989-08-29	Technicon Research A.G.	Detection and/or identification of microorganisms in a test sample using bioluminescence or other exogenous genetically-introduced

				marker
JP2703893B2	1985-07-05	1998-01-26	ホワイトヘッド・インスティテュ−ト・フォ−・バイオメディカル・リサ−チ	Epithelial cells expressing foreign genetic material
US4980286A	1985-07-05	1990-12-25	Whitehead Institute For Biomedical Research	In vivo introduction and expression of foreign genetic material in epithelial cells
US5139941A	1985-10-31	1992-08-18	University Of Florida Research Foundation, Inc.	AAV transduction vectors
DE3540823C1	1985-11-16	1986-10-02	Berthold Lab Prof R	Photometric measuring station
DE3751516T2	1986-04-30	1996-02-15	Igen Inc	ELECTROCHEMILUMINESCENCE TEST PROCEDURE.
US5221623A	1986-07-22	1993-06-22	Boyce Thompson Institute For Plant Research, Inc.	Use of bacterial luciferase structural genes for cloning and monitoring gene expression in microorganisms and for tagging and identification of genetically engineered organisms
WO1988000617A1	1986-07-22	1988-01-28	Boyce Thompson Institute For Plant Research	Use of bacterial luciferase structural genes for cloning and monitoring gene expression in microorganisms and for tagging and identification of genetically engineered organisms
AT117375T	1987-09-11	1995-02-15	Whitehead Biomedical Inst	TRANSDUCTION-CHANGED FIBROBLASTS AND THEIR USE.
DE3851153T2	1987-12-11	1995-01-05	Whitehead Biomedical Inst	GENETIC MODIFICATION OF ENDOTHELIAL CELLS.
DE68927996T2	1988-02-05	1997-12-04	Whitehead Biomedical Inst	MODIFIED HEPATOCYTES AND THEIR USE
FR2641793B1	1988-12-26	1993-10-01	Setratech	Method of in vivo recombination of dna sequences having basic matching
US5139745A	1990-03-30	1992-08-18	Block Medical, Inc.	Luminometer
GB9017443D0	1990-08-09	1990-09-26	Amersham Int Plc	Reporter bacteria for rapid microbial detection
CA2095256A1	1990-10-31	1992-05-01	Brad Guild	Retroviral vectors useful for gene therapy
US5188455A	1990-11-13	1993-02-23	The Pennsylvania Research Corporation	Apparatus for remote mixing of fluids
US5494646A	1993-04-14	1996-02-27	Seymour; Eugene H.	Sampling device and sample adequacy system
US5086233A	1991-01-22	1992-02-04	Dynatech Corporation	Luminometers with sample container displacement controlled by ramped abutment
US5252479A	1991-11-08	1993-10-12	Research Corporation Technologies, Inc.	Safe vector for gene therapy
US6300061B1	1992-02-07	2001-10-09	Albert Einstein College Of Medicine Of Yeshiva University	Mycobacterial species-specific reporter mycobacteriophages
US5242660A	1992-02-28	1993-09-07	Paul Hsei	Sample preparation device
US5587308A	1992-06-02	1996-12-24	The United States Of America As Represented By The Department Of Health & Human Services	Modified adeno-associated virus vector capable of expression from a novel promoter
JPH0634546A	1992-07-17	1994-02-08	Tosoh Corp	Fluorescene detector
US5478745A	1992-12-04	1995-12-26	University Of Pittsburgh	Recombinant viral vector system
AU687363B2	1993-03-19	1998-02-26	Ciba Corning Diagnostics Corp.	Luminometer
WO1994025572A1 *	1993-04-29	1994-11-10	Albert Einstein College Of Medicine Of Yeshiva University, A Division Of Yeshiva University	Mycobacterial species-specific reporter mycobacteriophages
US5645801A	1993-10-21	1997-07-08	Abbott Laboratories	Device and method for amplifying and detecting target nucleic acids
US6054299A	1994-04-29	2000-04-25	Conrad; Charles A.	Stem-loop cloning vector and method
US5656424A	1995-02-15	1997-08-12	Albert Einstein College Of Medicine, A Division Of Yeshiva University	Identification of mycobacterium tuberculosis complex species
US5476768A	1995-03-10	1995-12-19	Becton, Dickinson And Company	Mycobacteriophage DSGA specific for the mycobacterium tuberculosis complex
DE19517940A1	1995-05-18	1996-11-21	Merck Patent Gmbh	Detection of listeria using recombinant bacteriophages
CA2196793A1	1995-06-07	1996-12-19	Hugh V. Cottingham	Device and method for phage-based antibiotic susceptibility testing
US5814022A	1996-02-06	1998-09-29	Plasmaseal Llc	Method and apparatus for applying tissue sealant
US5939262A	1996-07-03	1999-08-17	Ambion, Inc.	Ribonuclease resistant RNA preparation and utilization
US5677124A	1996-07-03	1997-10-14	Ambion, Inc.	Ribonuclease resistant viral RNA standards

US5905029A	1997-02-19	1999-05-18	Fritz Berthold	Method for rapid hygiene testing
US5989499A	1997-05-02	1999-11-23	Biomerieux, Inc.	Dual chamber disposable reaction vessel for amplification reactions
SE9702005D0	1997-05-28	1997-05-28	Alphahelix Ab	New reaction vessel and method for its use
AU8408998A	1997-07-17	1999-02-10	Synermed International Inc.	Assay for total and direct bilirubin
US6054312A *	1997-08-29	2000-04-25	Selective Genetics, Inc.	Receptor-mediated gene delivery using bacteriophage vectors
US6506559B1	1997-12-23	2003-01-14	Carnegie Institute Of Washington	Genetic inhibition by double-stranded RNA
US6003566A	1998-02-26	1999-12-21	Becton Dickinson And Company	Vial transferset and method
EP3214177A3	1998-04-08	2017-11-22	Commonwealth Scientific and Industrial Research Organisation	Methods and means for obtaining modified phenotypes
AR020078A1	1998-05-26	2002-04-10	Syngenta Participations Ag	METHOD FOR CHANGING THE EXPRESSION OF AN OBJECTIVE GENE IN A PLANT CELL
CA2345936A1	1998-10-09	2000-04-20	Ingene, Inc.	Production of ssdna in a cell
JP2002527061A	1998-10-09	2002-08-27	インジーン・インコーポレイテッド	Enzymatic synthesis of ssDNA
DE19956568A1	1999-01-30	2000-08-17	Roland Kreutzer	Method and medicament for inhibiting the expression of a given gene
EP1031630B1	1999-02-22	2004-10-20	Matsushita Electric Industrial Co., Ltd.	Method for detecting bacteria
EP1169480A4	1999-04-14	2005-02-02	Musc Found For Res Dev	Tissue-specific and pathogen-specific toxic agents and ribozymes
US6818185B1	1999-05-28	2004-11-16	Cepheid	Cartridge for conducting a chemical reaction
US6271034B1	1999-07-08	2001-08-07	Albert Einstein College Of Medicine Of Yeshiva University	One step allelic exchange in mycobacteria using in vitro generated conditional transducing phages
US6737266B1	1999-10-01	2004-05-18	3M Innovative Properties Company	Devices and methods for microorganism detection
US7160511B2	2000-02-18	2007-01-09	Olympus Corporation	Liquid pipetting apparatus and micro array manufacturing apparatus
DE10100586C1	2001-01-09	2002-04-11	Ribopharma Ag	Inhibiting gene expression in cells, useful for e.g. treating tumors, by introducing double-stranded complementary oligoRNA having unpaired terminal bases
US8216797B2	2001-02-07	2012-07-10	Massachusetts Institute Of Technology	Pathogen detection biosensor
GB0108830D0	2001-04-09	2001-05-30	Univ Nottingham	Controlling DNA packaging
US6544729B2	2001-07-20	2003-04-08	University Of Tennessee	Bioluminescent biosensor device
MXPA04002899A	2001-09-27	2005-07-01	Gangen Inc	Lysin-deficient bacteriophages having reduced immunogenicity.
AU2002360272A1	2001-10-10	2003-04-22	Superarray Bioscience Corporation	Detecting targets by unique identifier nucleotide tags
CN1571922A	2001-10-19	2005-01-26	蒙诺根有限公司	Automated system and method for processing multiple liquid-based specimens
US20050118719A1	2001-11-07	2005-06-02	Schmidt Michael G.	Nucleic acid delivery and expression
BRPI0214680B1	2001-12-06	2018-09-18	Biocontrol Systems Inc	instrument for use in monitoring a product, ingredient, environment or process, instrument for detecting light emission from a sample and method for monitoring a sample of a product, ingredient, process or environment
AU2003228505B2	2002-04-12	2009-01-08	Colorado School Of Mines	Method for detecting low concentrations of a target bacterium that uses phages to infect target bacterial cells
US8216780B2	2002-04-12	2012-07-10	Microphage (Tm) Incorporated	Method for enhanced sensitivity in bacteriophage-based diagnostic assays
US20050003346A1	2002-04-12	2005-01-06	Colorado School Of Mines	Apparatus and method for detecting microscopic living organisms using bacteriophage
WO2004005553A1	2002-07-10	2004-01-15	Massachusetts Institute Of Technology	Apparatus and method for isolating a nucleic acid from a sample
SE521478C2	2002-11-14	2003-11-04	Magnetic Biosolutions Sweden A	Pipetting Device
US9534224B2	2002-11-15	2017-01-03	Trustees Of Boston University	Cis/trans riboregulators
US7291482B2 *	2002-12-20	2007-11-06	E.I. Du Pont De Nemours And Company	Mutations affecting plasmid copy number
US7125727B2	2003-01-29	2006-10-24	Protedyne Corporation	Sample handling tool with piezoelectric actuator
FI20030867A	2003-06-10	2004-12-11	Wallac Oy	Optical measuring method and laboratory measuring apparatus

EP2402089A1	2003-07-31	2012-01-04	Handylab, Inc.	Processing particle-containing samples
US20070020240A1	2003-10-06	2007-01-25	Gangagen, Inc.	Defined dose therapeutic phage
FR2860731B1	2003-10-14	2006-01-21	Maxmat S A	APPARATUS FOR MIXING A CHEMICAL OR BIOCHEMICAL ANALYZER WITH PIPELINE DRIVE OF A PIPETTE
DE102004003434B4	2004-01-21	2006-06-08	Eppendorf Ag	Pipetting device with a displacement device and a drive device detachably connected thereto
US20060257991A1	2004-02-27	2006-11-16	Mcdevitt John T	Integration of fluids and reagents into self-contained cartridges containing particle-based sensor elements and membrane-based sensor elements
US8101431B2	2004-02-27	2012-01-24	Board Of Regents, The University Of Texas System	Integration of fluids and reagents into self-contained cartridges containing sensor elements and reagent delivery systems
AU2005324479A1	2004-04-07	2006-07-20	The Government Of The United States Of America, As Represented By The Secretary, Department Of Health And Human Services	Reporter plasmid phage packaging system for detection of bacteria
US7674621B2	2004-05-21	2010-03-09	The United States Of America As Represented By The Department Of Health And Human Services	Plasmids and phages for homologous recombination and methods of use
WO2006003001A1	2004-07-06	2006-01-12	Mixis France S.A.	Generation of recombinant genes in bacteriophages
US8182804B1	2004-09-13	2012-05-22	Trustees Of Boston University	Engineered enzymatically active bacteriophages and methods of uses thereof
US20060068398A1	2004-09-24	2006-03-30	Cepheid	Universal and target specific reagent beads for nucleic acid amplification
US7244612B2	2004-10-07	2007-07-17	University Of Wyoming	Template reporter bacteriophage platform and multiple bacterial detection assays based thereon
KR100668310B1	2004-11-01	2007-01-12	삼성전자주식회사	A method for the detection of a target cell using a target cell specific virus
EP1849012A2	2005-01-28	2007-10-31	Parker-Hannifin Corporation	Sampling probe, gripper and interface for laboratory sample management systems
US20110097702A1	2005-03-31	2011-04-28	Voorhees Kent J	Methods and compositions for in situ detection of microorganisms on a surface
WO2006105504A1	2005-03-31	2006-10-05	Microphage Incorporated	Apparatus and method for detecting microorganisms using flagged bacteriophage
CA2610793A1	2005-05-31	2007-05-10	Labnow, Inc.	Methods and compositions related to determination and use of white blood cell counts
WO2007035504A1	2005-09-15	2007-03-29	Microphage Incorporated	Method and apparatus for identification of microorganisms using bacteriophage
US20070072174A1	2005-09-28	2007-03-29	Sayler Gary S	Bioreporter for detection of microbes
US7727473B2	2005-10-19	2010-06-01	Progentech Limited	Cassette for sample preparation
US20070178450A1	2006-01-27	2007-08-02	Microphage (Tm) Incorporation	Method and apparatus for determining level of microorganisms using bacteriophage
US20080003564A1	2006-02-14	2008-01-03	Iquum, Inc.	Sample processing
WO2007115378A1	2006-04-11	2007-10-18	Minifab (Australia) Pty Ltd	Microfluidic package housing
US20070292397A1	2006-06-19	2007-12-20	Mcnulty Amy K	Method for the detection and neutralization of bacteria
WO2008049036A2	2006-10-17	2008-04-24	Zymera, Inc.	Enzyme detection system with caged substrates
JP2010508044A	2006-10-31	2010-03-18	マイクロファージ・インコーポレーテッド	Method and apparatus for enhanced bacteriophage-based diagnostic assays by selective inhibition of potentially cross-reactive organisms
US20080153096A1	2006-11-02	2008-06-26	Vectrant Technologies Inc.	Cartridge for conducting diagnostic assays
ITPD20060419A1	2006-11-13	2008-05-14	Federico Nalesso	Device for the maintenance treatment of central venous catheters
US8355132B2	2007-04-06	2013-01-15	Qiagen Gaithersburg, Inc.	Sample adequacy measurement system having a plurality of sample tubes and using turbidity light scattering techniques
US8483174B2	2007-04-20	2013-07-09	Qualcomm Incorporated	Method and apparatus for providing gateway relocation
WO2008136769A1	2007-05-03	2008-11-13	Nanyang Technological University	Online contaminant detection and removal system
AU2008265989B8	2007-06-15	2012-01-12	Microphage Incorporated	Method of detection of microorganisms with enhanced bacteriophage amplification
GB0712844D0	2007-07-02	2007-08-08	Univ Leuven Kath	Colorimetric assay for the visual detection of primary and secondary amines

US8314222B2 *	2007-10-05	2012-11-20	Sapphire Energy, Inc.	System for capturing and modifying large pieces of genomic DNA and constructing organisms with chloroplasts
CA2706451A1	2007-11-28	2009-06-11	Smart Tube, Inc.	Devices, systems and methods for the collection, stimulation, stabilization, and analysis of a biological sample
US8619257B2	2007-12-13	2013-12-31	Kimberley-Clark Worldwide, Inc.	Recombinant bacteriophage for detection of nosocomial infection
US8153119B2	2007-12-18	2012-04-10	Trustees Of Boston University	Engineered enzymatically active bacteriophage and methods for dispersing biofilms
US8329889B2	2008-02-15	2012-12-11	Trustees Of Boston University	In vivo gene sensors
WO2009140375A2	2008-05-14	2009-11-19	Guild Associates, Inc.	Phage-mediated bioluminescent detection of yersinia pestis
CN102099671A	2008-05-20	2011-06-15	大学健康网络	Device and method for fluorescence-based imaging and monitoring
US20120134975A1	2008-08-13	2012-05-31	Searete Llc, A Limited Liability Corporation Of The State Of Delaware	Biological targeting compositions and methods of using the same
RU2523903C2	2008-12-16	2014-07-27	Биомерьё, Инк.	Method of characterising microorganisms on solid and semi-solid media
WO2010075441A1	2008-12-22	2010-07-01	Trustees Of Boston University	Modular nucleic acid-based circuits for counters, binary operations, memory, and logic
EP2398775B1	2009-02-18	2019-04-24	Cornell University	Coupled recognition/detection system for in vivo and in vitro use
US20110183314A1	2010-01-26	2011-07-28	Microphage Incorporated	Bacteriophage-based microorganism diagnostic assay using speed or acceleration of bacteriophage reproduction
TW201217783A	2010-09-15	2012-05-01	Mbio Diagnostics Inc	System and method for detecting multiple molecules in one assay
CN107338189B	2011-05-04	2021-02-02	卢米耐克斯公司	Apparatus and method for integrated sample preparation, reaction and detection
CN103764838B	2011-06-22	2018-07-27	国家医疗保健研究所	For detecting the method that there is the bacterium for generating carbapenem enzyme in sample
US9234227B2	2011-09-26	2016-01-12	Sample6 Technologies, Inc.	Recombinant phage and methods
US9481903B2	2013-03-13	2016-11-01	Roche Molecular Systems, Inc.	Systems and methods for detection of cells using engineered transduction particles
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US9481903B2	2013-03-13	2016-11-01	Roche Molecular Systems, Inc.	Systems and methods for detection of cells using engineered transduction particles
US10227661B2 *	2014-11-21	2019-03-12	GeneWeave Biosciences, Inc.	Sequence-specific detection and phenotype determination
ES2780827T3	2013-03-13	2020-08-27	Geneweave Biosciences Inc	Non-replicative transduction particles and reporter systems based on transduction particles
US9540675B2	2013-10-29	2017-01-10	GeneWeave Biosciences, Inc.	Reagent cartridge and methods for detection of cells
US10351452B2	2014-01-29	2019-07-16	Synphagen Llc	Compositions for in vivo expression of therapeutic sequences in the microbiome
SG11201610430QA	2014-06-13	2017-01-27	Geneweave Biosciences Inc	Growth-independent detection of cells
WO2016033088A1	2014-08-25	2016-03-03	GeneWeave Biosciences, Inc.	Non-replicative transduction particles and transduction particle-based reporter systems
US10351893B2	2015-10-05	2019-07-16	GeneWeave Biosciences, Inc.	Reagent cartridge for detection of cells

US10718003B2	2015-12-31	2020-07-21	Roche Molecular Systems, Inc.	Detecting an analyte in a flash and glow reaction
JP2019509746A	2016-03-28	2019-04-11	ザ・チャールズ・スターク・ドレイパ・ラボラトリー・インコーポレイテッド	Bacteriophage modification method
US20180179600A1	2016-12-20	2018-06-28	Roche Molecular Systems, Inc.	Systems & methods for detecting cells using engineered transduction particles
WO2018213301A1 *	2017-05-15	2018-11-22	New York University	Compositions and methods for non-antibiotic treating of bacterial infections by blocking or disrupting bacterial genes involved in virulence or viability
US11008602B2 *	2017-12-20	2021-05-18	Roche Molecular Systems, Inc.	Non-replicative transduction particles and transduction particle-based reporter systems
US10968491B2	2018-12-05	2021-04-06	GeneWeave Biosciences, Inc.	Growth-independent detection of cells
WO2020136154A1 *	2018-12-27	2020-07-02	F. Hoffmann-La Roche Ag	Non-replicative transduction particles and transduction particle-based reporter systems for detection of acinetobacter baumannii
WO2020136153A1 *	2018-12-29	2020-07-02	F. Hoffmann-La Roche Ag	Use of sideromycins to limit cross-reactivity and improve bacterial identification in antibiotic susceptibility assays
US20200208226A1	2018-12-31	2020-07-02	Roche Molecular Systems, Inc.	Non-replicative transduction particles with one or more non-native tail fibers and transduction particle-based reporter systems
WO2020245123A1	2019-06-06	2020-12-10	F. Hoffmann-La Roche Ag	Use of peptidomimetic antimicrobial peptides to limit cross-reactivity and improve bacterial identification in antibiotic susceptibility assays
WO2021041524A1 *	2019-08-26	2021-03-04	Laboratory Corporation Of America Holdings	Devices and methods for detecting microorganisms using recombinant reproduction-deficient indicator bacteriophage
WO2021136752A1	2019-12-31	2021-07-08	F. Hoffmann-La Roche Ag	Quantitative pcr screening of inducible prophage from bacterial isolates

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JP2020198885A	2020-12-17	Non-replicative transduction particles and transduction particle-based reporter systems
Bryksin et al.	2010	Overlap extension PCR cloning: a simple and reliable way to create recombinant plasmids
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KR20210030973A	2021-03-18	Engineered immunostimulatory bacterial strains and uses thereof
Wielgosz et al.	2015	Generation of a lentiviral vector producer cell clone for human Wiskott-Aldrich syndrome gene therapy
CN110191955A	2019-08-30	The method that exogenous drugs activation is carried out to the signal transduction compound for the chemical substance induction expressed in the cell of engineering in vitro and in vivo
Caposio et al.	2019	Characterization of a live-attenuated HCMV-based vaccine platform
EP3155133B1	2021-04-07	Growth-independent detection of cells
US20200208226A1	2020-07-02	Non-replicative transduction particles with one or more non-native tail fibers and transduction particle-based reporter systems
KR20190053236A	2019-05-17	AAV treatment of Huntington's disease
KR102271675B1	2021-07-02	AAV treatment of Huntington's disease
US20200291384A1	2020-09-17	Humanized cell line
US20200181688A1	2020-06-11	Growth-independent detection of cells

Priority And Related Applications

Priority Applications (7)

Application	Priority date	Filing date	Title
US201361779177P	2013-03-13	2013-03-13	*US Provisional Application*
US61/779,177		2013-03-13	
US201361897040P	2013-10-29	2013-10-29	*US Provisional Application*

US61/897,040		2013-10-29	
US201461939126P	2014-02-12	2014-02-12	*US Provisional Application*
US61/939,126		2014-02-12	
PCT/US2014/026536	2013-03-13	2014-03-13	Non-replicative transduction particles and transduction particle-based reporter systems

Legal Events

Date	Code	Title	Description
2019-10-21	E902	Notification of reason for refusal	
2020-04-25	E701	Decision to grant or registration of patent right	
2020-06-22	GRNT	Written decision to grant	

Concepts

machine-extracted

Download Filter table

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deoxyribonucleic acid		claims,description	106	0.000
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gene expression		claims,description	60	0.000
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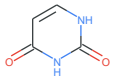
Uracil 	claims,description	14	0.000
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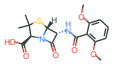

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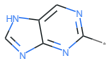
elimination reaction	claims,description	3	0.000
insertion	claims,description	3	0.000
penetrating	claims,description	3	0.000
promoting	claims,description	3	0.000
cell culture	claims,description	2	0.000
gold Au	claims,description	2	0.000
purinyl group 	claims,description	2	0.000
pyrimidinyl group	claims,description	2	0.000
Complement C1 Inhibitor Protein	claims	3	0.000
Complement C1 Inhibitor Protein	claims	3	0.000
aberrant	claims	3	0.000
Viruses	abstract,description	45	0.000
virological	abstract,description	37	0.000

Show all concepts from the description section

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