CLAIMS

What is claimed is:

1. A genetically modified cell expressing a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant configured for enhanced generation of small RNA (sRNA) from catalytic cutting of double stranded RNA (dsRNA), wherein said RNase III mutant exhibits at least one of the following enhanced characteristics compared to a wild type RNase III:

- enhanced stabilization of dsRNA cutting patterns;
- enhanced catalytic efficiency of dsRNA cutting; and
- enhanced specificity for one or more discrete dsRNA cutting size preferences.

2. The genetically modified cell of claim 1 wherein said genetically modified cell is selected from the group consisting of: a genetically modified prokaryotic cell, and a genetically modified eukaryotic cell.

3. The genetically modified cell of claim 2 wherein said genetically modified prokaryotic cell comprises a genetically modified bacteria.

4. The genetically modified cell of claim 3 wherein said genetically modified bacteria comprises a genetically modified bacteria that is symbiotic and/or endosymbiotic with a target host.

5. The genetically modified cell of claim 4 wherein said target host is selected from the group consisting of: a plant host, and an animal host.

6. The genetically modified cell of claim 5 wherein said RNase III mutant comprises at least one of the following:

- an E38A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38, or a homologous RNase III mutant thereof;
- an E65A RNase III mutant , wherein a glutamic acid is replaced with an alanine at residue 65, or a homologous RNase III mutant thereof;

- an RNase III E38A-E65A mutant, wherein a glutamic acid is replaced with an alanine at residue 38, and a glutamic acid is replaced with an alanine at residue 65, or a homologous RNase III mutant thereof; and
- an E38A-R107A-R108A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38 and an arginine is replaced with an alanine at residue 107, and an arginine is replaced with an alanine at residue 108, or a homologous RNase III mutant thereof.

7. The genetically modified cell of claim 1 wherein said heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant comprises at least one of the following:

- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 3;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NOs. 9, and 13;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 13;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E65A RNase III mutant according to SEQ ID NO. 5;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-E65A RNase III mutant polynucleotide sequence encoding a the amino acid sequence according to SEQ ID NO. 17;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 7;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 11; and
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 15.

8. The genetically modified cell of claim 1 wherein said heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant comprises at least one of the following:

- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 4;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 10;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 14;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E65A RNase III mutant polypeptide according to SEQ ID NO. 6;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-E65A RNase III mutant polypeptide according to SEQ ID NO. 17;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 8;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 12; and
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 16.

9. The genetically modified cell of claims 6, 7, and 8 wherein the E38A-R107A-R108A RNase III mutant exhibits discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt).

10. The genetically modified cell of claim 1 wherein said RNase III mutant was derived from an RNase III selected from the group consisting of: an RNase III from an *Enterobacteriaceae,* an RNase III from an *E. coli,*an RNase III from an *Enterobacter,* an RNase III from an *Bacillaceae,* an RNase III from a *Bacillus,* an RNase III from a *B. subtilis,* an RNase III from a *B. cereus* ,an RNase III from an *S. enterica,* an RNase III from a *P. aeruginosa*, an RNase III from a *C. burnetii*, an RNase III from a *R. capsulatus*, an RNase III from am *S. coelicolor*, an RNase III from a *C. jejuni*, an RNase III from an *H. pylori,* an RNase III from an *S. aureus*; and an RNase III from an *L. lactis*.

11. The genetically modified cell of claims 6, 7, and 8 wherein said genetically modified bacteria further co-expresses a heterologous polynucleotide sequence operably linked to a promoter sequence encoding a dsRNA.

12. The genetically modified cell of claim 11 wherein said co-expressed dsRNA comprises a dsRNA directed to a an essential pathogen gene.

13. The genetically modified cell of claim 12 wherein said essential pathogen gene comprises an essential viral pathogen gene.

14. The genetically modified cell of claim 6 wherein said genetically modified bacteria is introduced to a target host and wherein said sRNA initiates an RNA interference (RNAi) response pathway in a target host.

15. The genetically modified bacteria of claim 14 wherein said genetically modified bacteria is introduced to a target host comprises genetically modified bacteria is applied to a target host plant topically.

16. The genetically modified cell of claim 15 wherein said genetically modified bacteria is introduced to a target host comprises genetically modified bacteria is introduced to a target host animal through a feed.

17. The genetically modified cell of claim 6 wherein said genetically modified bacteria is grown in an fermenter.

18. The genetically modified cell of claim 17 wherein said sRNAs produced by said genetically modified bacteria are isolated.

19. The genetically modified cell of claim 17 wherein said isolated sRNAs produced by said genetically modified bacteria are introduced to a target host and wherein said sRNA initiates an RNAi response pathway in said target host.

20. A composition comprising: an E38A-R107A-R108A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38 and an arginine is replaced with an alanine at residue 107, and an arginine is replaced with an alanine at residue 108, or a homologous RNase III mutant thereof.

21. The composition of claim 20 wherein said E38A-R107A-R108A RNase III mutant exhibits:

- a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
- enhanced stabilization of dsRNA cutting patterns; and
- enhanced catalytic efficiency of dsRNA cutting.

22. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 7.

23. The composition of claim 22 wherein said E38A-R107A-R108A RNase III mutant exhibits:

- a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
- enhanced stabilization of dsRNA cutting patterns; and
- enhanced catalytic efficiency of dsRNA cutting.

24. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 11.

25. The composition of claim 24 wherein said E38A-R107A-R108A RNase III mutant exhibits:

- a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
- enhanced stabilization of dsRNA cutting patterns; and
- enhanced catalytic efficiency of dsRNA cutting.

26. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 15.

27. The composition of claim 26 wherein said E38A-R107A-R108A RNase III mutant exhibits:

- a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
- enhanced stabilization of dsRNA cutting patterns; and
- enhanced catalytic efficiency of dsRNA cutting.

28. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 8.

29. The composition of claim 28 wherein said E38A-R107A-R108A RNase III mutant exhibits:

- a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
- enhanced stabilization of dsRNA cutting patterns; and
- enhanced catalytic efficiency of dsRNA cutting.

30. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 12.

31. The composition of claim 30 wherein said E38A-R107A-R108A RNase III mutant exhibits:

- a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
- enhanced stabilization of dsRNA cutting patterns; and
- enhanced catalytic efficiency of dsRNA cutting.

32. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 16.

33. The composition of claim 32 wherein said E38A-R107A-R108A RNase III mutant exhibits:

- a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
- enhanced stabilization of dsRNA cutting patterns; and

– enhanced catalytic efficiency of dsRNA cutting.

34. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 8.

35. The composition of claim 34 wherein said E38A-R107A-R108A RNase III mutant exhibits:

– a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
– enhanced stabilization of dsRNA cutting patterns; and
– enhanced catalytic efficiency of dsRNA cutting.

36. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 12.

37. The composition of claim 36 wherein said E38A-R107A-R108A RNase III mutant exhibits:

– a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
– enhanced stabilization of dsRNA cutting patterns; and
– enhanced catalytic efficiency of dsRNA cutting.

37. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 16.

38. A composition comprising: an E38A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38, or a homologous RNase III mutant thereof.

39. A composition comprising: an E65A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 65, or a homologous RNase III mutant thereof.

40. A composition comprising: an RNase III E38A-E65A mutant, wherein a glutamic acid is replaced with an alanine at residue 38 and a glutamic acid is replaced with an alanine at residue 65, or a homologous RNase III mutant thereof.

41. A composition comprising: an RNase III E58A mutant, wherein a glutamic acid is replaced with an alanine at residue 58, or a homologous RNase III mutant thereof.

42. A composition comprising: an RNase III E59A mutant, wherein an aspartic acid is replaced with an alanine at residue 59, or a homologous RNase III mutant thereof.

43. A composition comprising: an RNase III Q153P mutant, wherein a glutamine is replaced with a proline at residue, or a homologous RNase III mutant thereof.

44. A composition comprising: an RNase III D115E mutant, wherein a glutamic acid is replaced with a lysine at residue, or a homologous RNase III mutant thereof.

45. A composition comprising: an RNase III E115K mutant, wherein a glutamic acid is replaced with a lysine at residue, or a homologous RNase III mutant thereof.

46. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 3.

47. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NOs. 9 and 13.

48. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 13.

49. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E65A RNase III mutant according to SEQ ID NO. 5.

50. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-E65A RNase III mutant polynucleotide sequence encoding the amino acid sequence according to SEQ ID NO. 17.

51. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III E58A mutant according to SEQ ID NO. 24.

52. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III E59A mutant according to SEQ ID NO. 26.

53. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 4.

54. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 10.

55. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 14.

56. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E65A RNase III mutant polypeptide according to SEQ ID NO. 6.

57. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-E65A RNase III mutant polypeptide according to SEQ ID NO. 17.

58. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III E58A mutant polypeptide according to SEQ ID NO. 25.

59. A composition comprising: a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III E59A mutant polypeptide according to SEQ ID NO. 27.

60. A method of generating sRNA comprising the steps of:

- genetically modifying a cell to express one or more heterologous polynucleotide sequences operably linked to at least one promotor encoding:
  - an RNase III mutant configured to generate small RNA (sRNA) from catalytic cutting of double stranded RNA (dsRNA);
  - a dsRNA directed to an essential pathogen gene;
- growing the genetically modified cell in a culture;
- catalytic cutting the dsRNA by the RNase III mutant to form a population of sRNAs to generate a plurality of sRNA of a discrete size; and
- isolating said genetically modified cell or isolating said sRNAs of a discrete size.

61. The method of claim 60 wherein said genetically modified cell comprises a genetically modified cell is selected from the group consisting of: a genetically modified prokaryotic cell, and a genetically modified eukaryotic cell.

62. The method of claim 61 wherein said genetically modified prokaryotic cell comprises a genetically modified bacteria.

63. The method of claim 62 wherein said genetically modified bacteria comprises a genetically modified bacteria that is symbiotic and/or endosymbiotic with a target host.

64. The method of claim 62 wherein said step of growing the genetically modified cell in a culture comprises the step of growing the genetically modified cell in a fermenter.

65. The method of claim 60 wherein said RNase III mutant comprises at least one of the following:

- an E38A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38, or a homologous RNase III mutant thereof;
- an E65A RNase III mutant , wherein a glutamic acid is replaced with an alanine at residue 65, or a homologous RNase III mutant thereof;
- an RNase III E38A-E65A mutant, wherein a glutamic acid is replaced with an alanine at residue 38, and a glutamic acid is replaced with an alanine at residue 65, or a homologous RNase III mutant thereof; and
- an E38A-R107A-R108A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38 and an arginine is replaced with an alanine at residue 107, and an arginine is replaced with an alanine at residue 108, or a homologous RNase III mutant thereof.

66. The method of claim 60 wherein said heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant comprises at least one of the following:

- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 3;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 9 and 13;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 13;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E65A RNase III mutant according to SEQ ID NO. 5;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-E65A RNase III mutant polynucleotide sequence encoding the amino acid sequence according to SEQ ID NO. 17;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 7;

- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 11; and
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 15.

67. The method of claim 60 wherein said heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant comprises at least one of the following:

- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 4;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 10;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 14;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E65A RNase III mutant polypeptide according to SEQ ID NO. 6;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-E65A RNase III mutant polypeptide according to SEQ ID NO. 17;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 8;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 12; and
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 16.

68. The method of claims 65, 66, and 67 wherein said E38A-R107A-R108A RNase III mutant exhibits discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt).

69. The method of claim 60 wherein said RNase III mutant configured to generate small RNA (sRNA) from catalytic cutting of double stranded RNA (dsRNA) exhibits at least one of the following enhanced characteristics compared to a wild type RNase III:

- enhanced stabilization of dsRNA cutting patterns;

- enhanced catalytic efficiency of dsRNA cutting; and
- enhanced specificity for one or more discrete dsRNA cutting size preferences.

70. The method of claim 62 wherein said RNase III mutant was derived from an RNase III selected from the group consisting of: an RNase III from an *Enterobacteriaceae*, an RNase III from an *E. coli,* an RNase III from an *Enterobacter,* an RNase III from an *Bacillaceae,* an RNase III from a *Bacillus,* an RNase III from a *B. subtilis,* an RNase III from a *B. cereus,* an RNase III from an *S. enterica,*, an RNase III from a *P. aeruginosa*, an RNase III from a *C. burnetii*, an RNase III from a *R. capsulatus*, an RNase III from am *S. coelicolor*, an RNase III from a *C. jejuni*, an RNase III from an *H. pylori,* an RNase III from an *S. aureus*; and an RNase III from an *L. lactis*.

80. The method of claim 60 wherein said isolated cells are introduced to a target host and wherein said sRNA initiates an RNAi response pathway in said target host.

81. The method of claim 60 wherein said isolated sRNAs are introduced to a target host and wherein said sRNA initiates an RNAi response pathway in said target host.

82. The method of claim 60 wherein said essential pathogen gene is an essential viral pathogen gene.

83. A method of initiating a DICER independent RNA interference (RNAi) response pathway in a target host comprising the steps of:

- genetically modifying a cell that lacks a DICER enzyme to express one or more heterologous polynucleotide sequences operably linked to at least one promotor encoding:
  - an RNase III mutant configured to catalytic cut double stranded RNA (dsRNA) in the absence of a DICER enzyme; and
  - a dsRNA directed to an essential pathogen gene;
- introducing the genetically modified cell to a target host;
- catalytic cutting the expressed dsRNA by the RNase III mutant to form a population of small RNAs (sRNAs) capable of initiating a DICER-independent RNAi response pathway; and

- allowing said sRNAs to diffuse from the cell to the target host and initiate a DICER independent RNAi response pathway directed to said essential in said target host and
- downregulating said essential pathogen gene through said a DICER-independent RNAi response pathway.

84. The method of claim 83 wherein said genetically modified cell comprises a genetically modified cell is selected from the group consisting of: a genetically modified prokaryotic cell, and a genetically modified eukaryotic cell.

85. The method of claim 84 wherein said genetically modified prokaryotic cell comprises a genetically modified bacteria.

86. The method of claim 85 wherein said genetically modified bacteria comprises a genetically modified bacteria that is symbiotic and/or endosymbiotic with said target host.

87. The method of claim 85 wherein said target host is selected from the group consisting of: a plant target host, and an animal target host.

88. The method of claim 83 wherein said RNase III mutant comprises at least one of the following:

- an E38A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38, or a homologous RNase III mutant thereof;
- an E65A RNase III mutant , wherein a glutamic acid is replaced with an alanine at residue 65, or a homologous RNase III mutant thereof;
- an RNase III E38A-E65A mutant, wherein a glutamic acid is replaced with an alanine at residue 38, and a glutamic acid is replaced with an alanine at residue 65, or a homologous RNase III mutant thereof; and
- an E38A-R107A-R108A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38 and an arginine is replaced with an alanine at residue 107, and an arginine is replaced with an alanine at residue 108, or a homologous RNase III mutant thereof.

89. The method of claim 83 wherein said heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant comprises at least one of the following:

- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 3;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 9 and 13;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant according SEQ ID NO. 13;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E65A RNase III mutant according to SEQ ID NO. 5;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-E65A RNase III mutant polynucleotide sequence encoding the amino acid sequence according to SEQ ID NO. 17; and
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 7;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 11; and
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 15.

90. The method of claim 84 wherein said heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant comprises at least one of the following:

- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 4;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 10;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A RNase III mutant polypeptide according to SEQ ID NO. 14;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E65A RNase III mutant polypeptide according to SEQ ID NO. 6;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-E65A RNase III mutant polypeptide according to SEQ ID NO. 17;

- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 8;
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 12; and
- a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 16.

91. The method of claims 88, 89, and 90 wherein said E38A-R107A-R108A RNase III mutant exhibits discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt).

92. The method of claim 83 wherein said RNase III mutant configured to generate small RNA (sRNA) from catalytic cutting of double stranded RNA (dsRNA) exhibits at least one of the following enhanced characteristics compared to a wild type RNase III:

- enhanced stabilization of dsRNA cutting patterns;
- enhanced catalytic efficiency of dsRNA cutting; and
- enhanced specificity for one or more discrete dsRNA cutting size preferences.

93. The method of claim 85 wherein said RNase III mutant was derived from an RNase III selected from the group consisting of: an RNase III from an *Enterobacteriaceae,* an RNase III from an *E. coli,* an RNase III from an *Enterobacter,* an RNase III from an *Bacillaceae,* an RNase III from a *Bacillus,* an RNase III from a *B. subtilis,* an RNase III from a *B. cereus* ,an RNase III from an *S. enterica,* an RNase III from a *P. aeruginosa*, an RNase III from a *C. burnetii*, an RNase III from a *R. capsulatus*, an RNase III from am *S. coelicolor,* an RNase III from a *C. jejuni*, an RNase III from an *H. pylori,* an RNase III from an *S. aureus*; and an RNase III from an *L. lactis*.

94. The method of claim 83 wherein said essential pathogen gene is an essential viral pathogen gene.

CLAIMS

1. A genetically modified cell expressing a heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant from *E. coli* or *Enterobacter* adapted for enhanced generation of small RNA (sRNA) from catalytic cutting of double stranded RNA (dsRNA), wherein said RNase III mutant comprises:

- an E38A-R107A-R108A RNase III mutant, wherein a glutamic acid is replaced with an alanine at residue 38, and an arginine is replaced with an alanine at residue 107, and an arginine is replaced with an alanine at residue 108, or a homologous RNase III mutant thereof, wherein the homologous RNase III's wild-type sequence includes the conserved residues E38, R107 and R108.

2. The genetically modified cell of claim 1, wherein said genetically modified cell is selected from the group consisting of: a genetically modified prokaryotic cell, and a genetically modified eukaryotic cell.

3. The genetically modified cell of claim 1, wherein said heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant is selected from:

- a heterologous polynucleotide sequence, operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 7, or a fragment thereof;
- a heterologous polynucleotide sequence, operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 11, or a fragment thereof; and
- a heterologous polynucleotide sequence, operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 15, or a fragment thereof.

4. The genetically modified cell of claim 1, wherein said heterologous polynucleotide sequence operably linked to a promoter sequence encoding an RNase III mutant is selected from:

- a heterologous polynucleotide sequence, operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 8, or a fragment thereof;
- a heterologous polynucleotide sequence, operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 12, or a fragment thereof; and
- a heterologous polynucleotide sequence, operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 16, or a fragment thereof.

5. The genetically modified cell of claim 1, wherein the E38A-R107A-R108A RNase III mutant exhibits discrete dsRNA cutting size preferences of 22 and 23 nucleotides (nt).

6. The genetically modified cell of claim 1, wherein said genetically modified cell co-expresses a heterologous polynucleotide sequence operably linked to a promoter sequence encoding a dsRNA.

7. The genetically modified cell of claim 6, wherein said co-expressed dsRNA comprises a dsRNA directed to an essential pathogen gene.

8. The genetically modified cell of claim 7, wherein said essential pathogen gene comprises an essential viral pathogen gene.

9. The genetically modified cell of claim 1, wherein said genetically modified cell is introduced to a target host and cuts said co-expressed dsRNA into sRNA which initiates an RNA interference (RNAi) response pathway in said target host.

10. A composition comprising an E38A-R107A-R108A RNase III mutant from *E. coli* or *Enterobacter*, wherein a glutamic acid is replaced with an alanine at residue 38, and an arginine is replaced with an alanine at residue 107, and an arginine is replaced with an alanine at residue 108.

11. A composition comprising polynucleotide sequence operably linked to a promoter sequence encoding an E38A-R107A-R108A RNase III mutant selected from:

- an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 7, or a fragment thereof;
- an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 11, or a fragment thereof;
- an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 15, or a fragment thereof.

12. A composition comprising an E38A-R107A-R108A RNase III mutant polypeptide mutant selected from:

- an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 12, or a fragment thereof;
- an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 16, or a fragment thereof;
- an E38A-R107A-R108A RNase III mutant polypeptide according to SEQ ID NO. 8, or a fragment thereof.

13. The composition of claim 10, wherein said E38A-R107A-R108A RNase III mutant exhibits at least one of the following enhanced characteristics compared to a wild type RNase III:

- a discrete dsRNA cutting size preferences of 22, and 23 nucleotides (nt);
- enhanced stabilization of dsRNA cutting patterns; and
- enhanced catalytic efficiency of dsRNA cutting.

14. The composition of claim 10, wherein said E38A-R107A-R108A RNase III mutant comprises at least one of the following:

- a polynucleotide sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 7;
- a polynucleotide sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 11;

- a polynucleotide sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 15;
- an amino acid sequence encoding an E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 8;
- an amino acid sequence encoding E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 12; and
- an amino acid sequence encoding E38A-R107A-R108A RNase III mutant according to SEQ ID NO. 16.

15. The composition of claim 10, wherein said E38A-R107A-R108A RNase III mutant is co-expressed in a prokaryotic or eukaryotic cell with a heterologous polynucleotide sequence operably linked to a promoter sequence encoding a dsRNA.

16. The composition of claim 15, wherein said co-expressed dsRNA comprises a dsRNA directed to an essential pathogen gene.

17. The composition of claim 16, wherein said essential pathogen gene comprises an essential viral pathogen gene.

18. The composition of claim 17, wherein the cell comprises a bacteria that is introduced to a target host and wherein the RNase III mutant cuts said co-expressed dsRNA into 22 and 23 nucleotides (nt) sRNA fragments that initiate an RNA interference (RNAi) response pathway in said target host.

19. The composition of claim 18, wherein said RNAi response pathway in said target host is initiated independently of a DICER enzyme.

20. The genetically modified cell of claim 9, wherein said RNAi response pathway in said target host is initiated independently of said DICER enzyme.