CLAIMS

1. Use of at least one targeting RNA molecule and a Cas protein for binding, cleaving, marking or modifying a double stranded target polynucleotide comprising a target nucleic acid sequence, wherein:

the double stranded target polynucleotide comprises a target nucleic acid strand, comprising said target nucleic acid sequence, and a non-target nucleic acid strand, comprising a protospacer nucleic acid sequence complementary to the target nucleic acid sequence;

the Cas protein has an amino acid sequence of SEQ ID NO: 1 or a sequence of at least 77% identity therewith;

the at least one targeting RNA molecule recognizes the target sequence;

the non-target nucleic acid strand further comprises a protospacer adjacent motif (PAM) sequence directly adjacent to the 3' end of the protospacer nucleic acid sequence , wherein the PAM sequence comprises 5'-NNNNCNN-3' .

2. A use as claimed in claim 1, wherein the binding, cleaving, marking or modifying occurs at a temperature between 20°C and 100°C, at a temperature between 30°C and 80°C, at a temperature between 37°C and 78°C, preferably at a temperature above 55°C; more preferably at a temperature between 55°C and 80°C; even more preferably at a temperature between 55°C and 65°C or 60°C and 65°C.

3. A use as claimed in claim 1 or claim 2, wherein the polynucleotide comprising the target nucleic acid sequence is cleaved by the Cas protein, preferably wherein said cleavage is DNA cleavage.

4. A use as claimed in any of claims 1 to 3 wherein the target nucleic acid strand comprising the target sequence is double stranded DNA and said use results in a double stranded break in the polynucleotide comprising the target nucleic acid sequence.

5. A use as claimed in claim 1 or claim 2 wherein the polynucleotide comprising the target nucleic acid sequence is double stranded DNA, the Cas protein lacks the

ability to cut the double stranded DNA and said use results in gene silencing of the polynucleotide.

6. A use as claimed in any preceding claim wherein the PAM sequence comprises 5'-NNNNCNNA-3', 5'-CNNNCNN-3', 5'-NNNCCNN-3', 5'-NNCNCNN-3', 5'-NNNNCCN-3', and/or 5'-NCNNCNN-3'.

7. A use as claimed in any preceding claim wherein the PAM sequence comprises 5'-CCCCCCNA-3' [SEQ ID NO: 10], preferably wherein the PAM sequence comprises 5'-CCCCCCAA-3' [SEQ ID NO: 11].

8. A use as claimed in any preceding claim, wherein the Cas protein is obtainable from a bacterium, archaeon or virus, preferably from a thermophilic bacterium.

9. A use as claimed in any preceding claim, wherein the Cas protein is obtainable from *Geobacillus* sp., preferably from *Geobacillus thermodenitrificans*.

10. A use as claimed in any preceding claim, wherein the targeting RNA molecule comprises a crRNA and a tracrRNA.

11. A use as claimed in any preceding claim, wherein the length of the at least one targeting RNA molecule is in the range 35 – 200 nucleotide residues.

12. A use as claimed in any preceding claim, wherein the target nucleic acid sequence is from 15 to 32 nucleotide residues in length.

13. A use as claimed in any preceding claim, wherein the Cas protein further comprises at least one functional moiety.

14. A use as claimed in any preceding claim wherein the Cas protein is provided as part of a protein complex comprising at least one further functional or non-functional protein, optionally wherein the at least one further protein further comprises at least one functional moiety.

15. A use as claimed in claim 13 or claim 14, wherein the Cas protein or further protein comprises at least one functional moiety fused or linked to the N-terminus and/or the C-terminus of the Cas protein or protein complex; preferably the C-terminus.

16. A use as claimed in any of claims 13 to 15, wherein the at least one functional moiety is a protein; optionally selected from a helicase, a nuclease, a helicase-nuclease, a DNA methylase, a histone methylase, an acetylase, a phosphatase, a kinase, a transcription (co-)activator, a transcription repressor, a DNA binding protein, a DNA structuring protein, a marker protein, a reporter protein, a fluorescent protein, a ligand binding protein, a signal peptide, a subcellular localisation sequence, an antibody epitope or an affinity purification tag, for example a green fluorescent protein (GFP).

17. A use as claimed in claim 16, wherein the native activity of the Cas9 nuclease is inactivated and the Cas protein is linked to at least one functional moiety.

18. A use as claimed in claim 16 or claim 17, wherein the at least one functional moiety is a nuclease domain; preferably a FokI nuclease domain.

19. A use as claimed in any of claims 16 to 18, wherein the at least one functional moiety is a marker protein.

20. A method of binding, cleaving, marking or modifying a double stranded target polynucleotide, wherein the double stranded target polynucleotide comprises a target nucleic acid strand comprising a target nucleic acid sequence, and a non-target nucleic acid strand comprising a protospacer nucleic acid sequence complementary to the target nucleic acid sequence, said method comprising:

a. designing at least one targeting RNA molecule, wherein the targeting RNA molecule recognizes the target sequence in the target strand, and the non-target strand further comprises a protospacer adjacent motif (PAM) sequence directly adjacent the 3' end of the protospacer sequence, wherein the PAM sequence comprises 5'-NNNNCNN-3';

b. forming a ribonucleoprotein complex comprising the targeting RNA molecule and a Cas protein, wherein the isolated Cas protein has an amino acid sequence of SEQ ID NO: 1 or a sequence of at least 77% identity therewith; and

c. the ribonucleoprotein complex binding, cleaving, marking or modifying the target polynucleotide.

21. A method as claimed in claim 20, wherein the binding, cleaving, marking or modifying occurs at a temperature between 20°C and 100°C, at a temperature between 30°C and 80°C, at a temperature between 37°C and 78°C, preferably at a temperature above 55°C; more preferably at a temperature between 55°C and 80°C; even more preferably at a temperature between 55°C and 65°C or 60°C and 65°C.

22. A method as claimed in claim 20 or claim 21, wherein the double stranded target polynucleotide comprising the target nucleic acid sequence is cleaved by the Cas protein, preferably wherein said cleavage is DNA cleavage.

23. A method as claimed in any of claims 20 to 22 wherein the target polynucleotide is double stranded DNA and said use results in a double stranded break in the polynucleotide.

24. A method as claimed in claim 20 or claim 21 wherein the target polynucleotide comprising the target nucleic acid sequence is double stranded DNA, the Cas protein lacks the ability to cut the double stranded DNA and said method results in gene silencing of the target polynucleotide.

25. A method as claimed in any of claims 20 to 24 wherein the PAM sequence comprises 5'-NNNNCNNA-3', 5'-CNNNCNN-3', 5'-NNNCCNN-3', 5'-NNCNCNN-3', 5'-NNNNCCN-3', and/or 5'-NCNNCNN-3'.

26. A method as claimed in any of claims 20 to 25 wherein the PAM sequence comprises 5'-CCCCCCNA-3' [SEQ ID NO: 10], preferably wherein the PAM sequence comprises 5'-CCCCCCAA-3' [SEQ ID NO: 11].

27. A method as claimed in any of claims 20 to 26, wherein the Cas protein is obtainable from a bacterium, archaeon or virus, preferably from a thermophilic bacterium.

28. A method as claimed in any of claims 20 to 27, wherein the Cas protein is obtainable from *Geobacillus* sp., preferably from *Geobacillus thermodenitrificans*.

29. A method as claimed in any of claims 20 to 28, wherein the targeting RNA molecule comprises a crRNA and a tracrRNA.

30. A method as claimed in any of claims 20 to 29, wherein the length of the at least one targeting RNA molecule is in the range 35 – 200 nucleotide residues.

31. A method as claimed in any of claims 20 to 30, wherein the target nucleic acid sequence is from 15 to 32 nucleotide residues in length.

32. A method as claimed in any of claims 20 to 31, wherein the Cas protein further comprises at least one functional moiety.

33. A method as claimed in any of claims 20 to 32, wherein the Cas protein is provided as part of a protein complex comprising at least one further functional or non-functional protein, optionally wherein the at least one further protein further comprises at least one functional moiety.

34. A method as claimed in claim 32 or 33, wherein the Cas protein or further protein comprises at least one functional moiety fused or linked to the N-terminus and/or the C-terminus of the Cas protein or protein complex; preferably the C-terminus.

35. A method as claimed in any of claims 32 to 34, wherein the at least one functional moiety is a protein; optionally selected from a helicase, a nuclease, a helicase-nuclease, a DNA methylase, a histone methylase, an acetylase, a phosphatase, a kinase, a transcription (co-)activator, a transcription repressor, a DNA binding protein, a DNA structuring protein, a marker protein, a reporter protein, a fluorescent protein, a ligand binding protein, a signal peptide, a subcellular localisation sequence, an antibody epitope or an affinity purification tag, for example a green fluorescent protein (GFP).

36. A method as claimed in claim 35, wherein the native activity of the Cas9 nuclease is inactivated and the Cas protein is linked to at least one functional moiety.

37. A method as claimed in claim 35 or claim 36, wherein the at least one functional moiety is a nuclease domain; preferably a FokI nuclease domain.

38. A method as claimed in any of claims 35 to 37, wherein the at least one functional moiety is a marker protein.

39. A use as claimed in claim 16 or method as claimed in claim 35, wherein the double stranded target polynucleotide is dsDNA, the at least one functional moiety is a nuclease or a helicase-nuclease, and the modification is a single-stranded or a double-stranded break at a desired locus.

40. A use as claimed in claim 16 or method as claimed in claim 35, wherein the double stranded target polynucleotide is dsDNA and the functional moiety is selected from a DNA modifying enzyme (e.g. a methylase or acetylase), a transcription activator or a transcription repressor and the binding, cleaving, marking or modifying results in modification of gene expression.

41. A use as claimed in claim 16 or method as claimed in claim 35, wherein said binding, cleaving, marking or modifying occurs *in vivo*.

42. A use as claimed in any of claims 1 to 4, 6 to 19 or 39, or a method as claimed in any of claims 20 to 23, 25 to 39 wherein the binding, cleaving, marking or modifying results in modifying or deleting and/or inserting a desired nucleotide sequence at a desired location, and/or wherein the binding, cleaving, marking or modifying results in silencing gene expression at a desired locus.

43. A transformed cell, having a double stranded target polynucleotide comprising a target nucleic acid sequence, wherein the double stranded target polynucleotide comprises a target nucleic acid strand, comprising said target nucleic acid sequence, and a non-target nucleic acid strand, comprising a protospacer nucleic acid sequence complementary to the target nucleic acid sequence, said cell comprising:

a clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) protein having an amino acid sequence of SEQ ID NO: 1 or a sequence of at least 77% identity therewith;

at least one targeting RNA molecule which recognizes the target nucleic acid sequence in the target nucleic acid strand, wherein the non-target strand further comprises a protospacer adjacent motif (PAM) sequence directly adjacent the 3' end of the protospacer sequence, wherein the PAM sequence comprises 5'-NNNNCNN-3'; and

an expression vector comprising a nucleic acid encoding at least one of said Cas protein and said targeting RNA molecule.

44. A transformed cell as claimed in claim 43, wherein the Cas protein and targeting RNA molecule enable binding, cleaving, marking or modifying of the target polynucleotide in the cell and the binding, cleaving, marking or modifying occurs at a temperature between 20°C and 100°C, at a temperature between 30°C and 80°C, at a temperature between 37°C and 78°C, preferably at a temperature above 55°C; more preferably at a temperature between 55°C and 80°C; even more preferably at a temperature between 55°C and 65°C or 60°C and 65°C.

45. A transformed cell as claimed in claim 43 or claim 44, wherein the target nucleic acid strand comprising the target nucleic acid sequence is cleaved by the Cas protein, preferably wherein said cleavage is DNA cleavage.

46. A transformed cell as claimed in any of claims 43 to 45 wherein the target polynucleotide comprising the target sequence is double stranded DNA and said binding, cleaving, marking or modifying results in a double stranded break in the target polynucleotide.

47. A transformed cell as claimed in claim 43 or claim 44 wherein the target polynucleotide comprising the target nucleic acid sequence is double stranded DNA, the Cas protein lacks the ability to cut the double stranded DNA and said binding, cleaving, marking or modifying results in gene silencing of the target polynucleotide.

48. A transformed cell as claimed in any of claims 43 to 47 wherein the PAM sequence comprises 5'-NNNNCNNA-3', 5'-CNNNCNN-3', 5'-NNNCCNN-3', 5'-NNCNCNN-3', 5'-NNNNCCN-3', and/or 5'-NCNNCNN-3'.

49. A transformed cell as claimed in any of claims 43 to 48 wherein the PAM sequence comprises 5'-CCCCCCNA-3' [SEQ ID NO: 10], preferably wherein the PAM sequence comprises 5'-CCCCCCAA-3' [SEQ ID NO: 11].

50. A transformed cell as claimed in any of claims 43 to 49, wherein the Cas protein is obtainable from a bacterium, archaeon or virus, preferably from a thermophilic bacterium.

51. A transformed cell as claimed in any of claims 43 to 50, wherein the Cas protein is obtainable from *Geobacillus* sp., preferably from *Geobacillus thermodenitrificans*.

52. A transformed cell as claimed in any of claims 43 to 51 wherein the cell is a prokaryotic cell.

53. A transformed cell as claimed in any of claims 43 to 51 wherein the cell is a eukaryotic cell.

54. A transformed cell as claimed in any of claims 43 to 53, wherein the targeting RNA molecule comprises a crRNA and a tracrRNA.

55. A transformed cell as claimed in any of claims 43 to 54, wherein the length of the at least one targeting RNA molecule is in the range 35 – 200 nucleotide residues.

56. A transformed cell as claimed in any of claims 43 to 55, wherein the target nucleic acid sequence is from 15 to 32 nucleotide residues in length.

57. A transformed cell as claimed in any of claims 43 to 56, wherein the Cas protein further comprises at least one functional moiety.

58. A transformed cell as claimed in any of claims 43 to 57, wherein the Cas protein is provided as part of a protein complex comprising at least one further functional or non-functional protein, optionally wherein the at least one further protein further comprises at least one functional moiety.

59. A transformed cell as claimed in claim 57 or 58, wherein the Cas protein or further protein comprises at least one functional moiety fused or linked to the N-terminus and/or the C-terminus of the Cas protein or protein complex; preferably the N-terminus.

60. A transformed cell as claimed in any of claims 57 to 59, wherein the at least one functional moiety is a protein; optionally selected from a helicase, a nuclease, a helicase-nuclease, a DNA methylase, a histone methylase, an acetylase, a phosphatase, a kinase, a transcription (co-)activator, a transcription repressor, a DNA binding protein, a DNA structuring protein, a marker protein, a reporter protein, a fluorescent protein, a ligand binding protein, a signal peptide, a subcellular localisation

sequence, an antibody epitope or an affinity purification tag, for example a green fluorescent protein (GFP).

61. A transformed cell as claimed in claim 60, wherein the native activity of the Cas9 nuclease is inactivated and the Cas protein is linked to at least one functional moiety.

62. A transformed cell as claimed in any of claims 57 to 61, wherein the at least one functional moiety is a nuclease domain; preferably a FokI nuclease domain.

63. A transformed cell as claimed in any of claims 57 to 61, wherein the at least one functional moiety is a marker protein.

64. A transformed cell as claimed in any of claims 57 to 62, wherein the double stranded target polynucleotide is dsDNA, the at least one functional moiety is a nuclease or a helicase-nuclease, and the modification is a single-stranded or a double-stranded break at a desired locus.

65. A transformed cell as claimed in any of claims 57 to 61 or method as claimed in claim 35, wherein the double stranded target polynucleotide is dsDNA and the functional moiety is selected from a DNA modifying enzyme (e.g. a methylase or acetylase), a transcription activator or a transcription repressor and the binding, cleaving, marking or modifying results in modification of gene expression.

66. A transformed cell as claimed in any of claims 57 to 62 wherein the Cas protein is expressed from an expression vector.

67. A transformed cell as claimed in any of claims 43 to 66 wherein the binding, cleaving, marking or modifying results in modifying or deleting and/or inserting a desired nucleotide sequence at a desired location, and/or wherein the binding, cleaving, marking or modifying results in silencing gene expression at a desired locus.

68. A nucleoprotein complex comprising a Cas protein, at least one targeting RNA molecule which recognises a target nucleic acid sequence in a double stranded target polynucleotide, and the target polynucleotide, wherein

the Cas protein has an amino acid sequence of SEQ ID NO: 1 or a sequence of at least 77% identity therewith;

the double stranded target polynucleotide comprises a target nucleic acid strand, comprising said target nucleic acid sequence, and a non-target nucleic acid strand, comprising a protospacer nucleic acid sequence complementary to the target nucleic acid sequence and a protospacer adjacent motif (PAM) sequence directly adjacent the 3' end of the protospacer sequence, wherein the PAM sequence comprises 5'-NNNNCNN-3'.

69. A nucleoprotein complex as claimed in claim 68, wherein the nucleoprotein complex occurs at a temperature between 20°C and 100°C, at a temperature between 30°C and 80°C, at a temperature between 37°C and 78°C, preferably at a temperature above 55°C; more preferably at a temperature between 55°C and 80°C; even more preferably at a temperature between 55°C and 65°C or 60°C and 65°C.

70. A nucleoprotein complex as claimed in claim 68 or claim 69, wherein the double stranded target polynucleotide comprising the target nucleic acid sequence is cleaved by the Cas protein, preferably wherein said cleavage is DNA cleavage.

71. A nucleoprotein complex as claimed in any of claims 68 to 70 wherein the target polynucleotide comprising the target sequence is double stranded DNA and said binding, cleaving, marking or modifying results in a double stranded break in the target polynucleotide.

72. A nucleoprotein complex as claimed in claim 68 or claim 69 wherein the target polynucleotide comprising the target nucleic acid sequence is double stranded DNA, the Cas protein lacks the ability to cut the double stranded DNA and the presence of said nucleoprotein complex results in gene silencing of the target polynucleotide.

73. A nucleoprotein complex as claimed in any of claims 68 to 72 wherein the PAM sequence comprises 5'-NNNNCNNA-3', 5'-CNNNCNN-3', 5'-NNNCCNN-3', 5'-NNCNCNN-3', 5'-NNNNCCN-3', and/or 5'-NCNNCNN-3'.

74. A nucleoprotein complex as claimed in any of claims 68 to 73 wherein the PAM sequence comprises 5'-CCCCCCNA-3' [SEQ ID NO: 10], preferably wherein the PAM sequence comprises 5'-CCCCCCAA-3' [SEQ ID NO: 11].

75. A nucleoprotein complex as claimed in any of claims 68 to 74, wherein the Cas protein is obtainable from a bacterium, archaeon or virus, preferably from a thermophilic bacterium.

76. A nucleoprotein complex as claimed in any of claims 68 to 75, wherein the Cas protein is obtainable from *Geobacillus* sp., preferably from *Geobacillus thermodenitrificans*.

77. A nucleoprotein complex as claimed in any of claims 68 to 76 wherein the nucleoprotein complex is in a prokaryotic cell.

78. A nucleoprotein complex as claimed in any of claims 68 to 76 wherein the nucleoprotein complex is in a eukaryotic cell.

79. A nucleoprotein complex as claimed in any of claims 68 to 78, wherein the targeting RNA molecule comprises a crRNA and a tracrRNA.

80. A nucleoprotein complex as claimed in any of claims 68 to 79, wherein the length of the at least one targeting RNA molecule is in the range 35 – 200 nucleotide residues.

81. A nucleoprotein complex as claimed in any of claims 68 to 80, wherein the target nucleic acid sequence is from 15 to 32 nucleotide residues in length.

82. A nucleoprotein complex as claimed in any of claims 68 to 81, wherein the Cas protein further comprises at least one functional moiety.

83. A nucleoprotein complex as claimed in any of claims 68 to 82, wherein the Cas protein is provided as part of a protein complex comprising at least one further functional or non-functional protein, optionally wherein the at least one further protein further comprises at least one functional moiety.

84. A nucleoprotein complex as claimed in claim 82 or 83, wherein the Cas protein or further protein comprises at least one functional moiety fused or linked to the N-terminus and/or the C-terminus of the Cas protein or protein complex; preferably the C-terminus.

85. A nucleoprotein complex as claimed in any of claims 82 to 84, wherein the at least one functional moiety is a protein; optionally selected from a helicase, a

nuclease, a helicase-nuclease, a DNA methylase, a histone methylase, an acetylase, a phosphatase, a kinase, a transcription (co-)activator, a transcription repressor, a DNA binding protein, a DNA structuring protein, a marker protein, a reporter protein, a fluorescent protein, a ligand binding protein, a signal peptide, a subcellular localisation sequence, an antibody epitope or an affinity purification tag, for example a green fluorescent protein (GFP).

86. A nucleoprotein complex as claimed in claim 85, wherein the native activity of the Cas9 nuclease is inactivated and the Cas protein is linked to at least one functional moiety.

87. A nucleoprotein complex as claimed in any of claims 82 to 86, wherein the at least one functional moiety is a nuclease domain; preferably a FokI nuclease domain.

88. A nucleoprotein complex as claimed in any of claims 82 to 86, wherein the at least one functional moiety is a marker protein.

89. A nucleoprotein complex as claimed in any of claims 82 to 87, wherein the nucleic acid is dsDNA, the at least one functional moiety is a nuclease or a helicase-nuclease, and the target polynucleotide has a single-stranded or a double-stranded break at a desired locus.

90. A nucleoprotein complex as claimed in any of claims 82 to 86, wherein the nucleic acid is dsDNA and the functional moiety is selected from a DNA modifying enzyme (*e.g.* a methylase or acetylase), a transcription activator or a transcription repressor and the nucleoprotein complex formation results in modification of gene expression.

91. A nucleoprotein complex as claimed in any of claims 68 to 90 wherein the nucleoprotein formation results in modifying or deleting and/or inserting a desired nucleotide sequence at a desired location, and/or wherein the nucleoprotein complex formation results in silencing gene expression at a desired locus.