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 89% 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'.

In another aspect, the present disclosure refers to 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 89% 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; wherein the Cas protein and targeting RNA molecule enable binding, cleaving, marking or modifying of the target polynucleotide in the cell.

In yet another aspect, the present disclosure refers to an *in vitro* 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 89% identity therewith; and

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

In yet another aspect, the present disclosure refers to the *in vitro* use of at least one targeting RNA molecule and a Cas protein to form a nucleoprotein complex 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 89% 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'.

Accordingly, the present disclosure provides an isolated clustered regularly interspaced short palindromic repeat (CRISPR)-associated (Cas) protein or polypeptide comprising;

CLAIMS

1. 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 89% 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'.

2. 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 89% 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; wherein the Cas protein and targeting RNA molecule enable binding, cleaving, marking or modifying of the target polynucleotide in the cell.

3. A transformed cell as claimed in claim 2, wherein the Cas protein is expressed from an expression vector.

4. A nucleoprotein complex as claimed in claim 1, or a transformed cell as claimed in claim 2 or claim 3, wherein the Cas protein has an amino acid sequence of SEQ ID NO: 1 or a sequence of at least 95% identity therewith.

5. A nucleoprotein complex as claimed in claim 1 or claim 4, or a transformed cell as claimed in any of claims 2 to 4, wherein the nucleoprotein complex forms at a temperature between 20°C and 100°C, at a temperature between 30°C and 80°C, or 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.

6. A nucleoprotein complex or transformed cell as claimed in any preceding claim, 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.

7. A transformed cell as claimed in any of claims 3 to 5, wherein (i) the binding, cleaving, marking or modifying is at a temperature between 20°C and 100°C, at a temperature between 30°C and 80°C, or 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; and/or (ii) the target polynucleotide comprising the target sequence is double stranded DNA and said cleaving results in a double stranded break in the target polynucleotide.

8. A transformed cell or nucleoprotein complex as claimed in any of claims 1 to 5, wherein the Cas protein lacks nuclease activity to cut the double stranded DNA, yet there is gene silencing of the target polynucleotide.

9. A transformed cell or a nucleoprotein complex 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'; preferably wherein the PAM sequence comprises 5'-CCCCCCNA-3' [SEQ ID NO:

10], more preferably wherein the PAM sequence comprises 5'-CCCCCCAA-3' [SEQ ID NO: 11].

10. A transformed cell or a nucleoprotein complex as claimed in any preceding claim, wherein the nucleoprotein complex is in a prokaryotic cell; preferably a thermophilic bacterium.

11. A transformed cell or a nucleoprotein complex as claimed in any preceding claim, wherein the targeting RNA molecule comprises a crRNA and a tracrRNA.

12. A transformed cell or a nucleoprotein complex 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; and/or wherein the target nucleic acid sequence is from 15 to 32 nucleotide residues in length.

13. A transformed cell or a nucleoprotein complex as claimed in any preceding claim, wherein the Cas protein further comprises at least one functional moiety; preferably 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).

14. An *in vitro* 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 89% identity therewith; and

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

15. *In vitro* use of at least one targeting RNA molecule and a Cas protein to form a nucleoprotein complex 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 89% 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'.

16. An *in vitro* method as claimed in claim 14 or an *in vitro* use as claimed in claim 15, wherein the Cas protein has an amino acid sequence of SEQ ID NO: 1 or a sequence of at least 95% identity therewith.

17. An *in vitro* method as claimed in claim 14 or claim 16, or an *in vitro* use as claimed in claim 15 or claim 16, wherein the nucleoprotein complex forms at a temperature between 20°C and 100°C, at a temperature between 30°C and 80°C, or 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.

18. An *in vitro* method as claimed in any of claims 14, 16 or 17, or an *in vitro* use as claimed in any of claims 15 to 17, 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'; preferably wherein the PAM sequence comprises 5'-CCCCCCNA-3' [SEQ ID NO: 10], more preferably wherein the PAM sequence comprises 5'-CCCCCCAA-3' [SEQ ID NO: 11].

19. An *in vitro* method as claimed in any of claims 14, 16, 17 or 18, or an *in vitro* use as claimed in any of claims 15 to 18, wherein the Cas protein further comprises at least one functional moiety; preferably 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).