

Claims

1. A non-naturally occurring tracrRNA nucleic acid molecule, comprising a portion comprising an anti-repeat region sequence that is designed to specifically hybridize to a preselected sensed RNA sequence through forming a complex that mimics the natural crRNA:tracrRNA duplex.
2. A complex comprising the non-naturally occurring tracrRNA nucleic acid molecule according to claim 1, at least one tracrRNA-dependent CRISPR nuclease enzyme, and at least one sensed RNA.
3. The non-naturally occurring tracrRNA nucleic acid molecule according to claim 2, further bound to a target DNA nucleic acid molecule comprising a sequence based or designed based on the at least one sensed RNA sequence.
4. An *in vitro* method for detecting at least one sensed RNA in a cell, tissue, and/or sample, said method comprising:
 - a) contacting said sample with at least one non-naturally occurring tracrRNA comprising a portion that specifically hybridizes with a first portion of said sensed RNA, wherein a second portion of said sensed RNA specifically hybridizes with at least one target nucleic acid; wherein said at least one non-naturally occurring tracrRNA is hybridized or is able to be hybridized with said sensed RNA in the presence of at least one tracrRNA-dependent CRISPR nuclease enzyme, optionally further comprising the presence of at least one RNA-cleaving enzyme, such as RNase III;
 - b) detecting binding of said at least one tracrRNA-dependent CRISPR nuclease enzyme to said at least one target nucleic acid;
 - c) wherein binding detects said at least one sensed RNA in said cell, tissue and/or sample, andwherein preferably said nuclease is inactivated in its cleaving and/or nicking activity.
5. An *in vitro* method for detecting at least one sensed RNA in a cell and/or sample, said method comprising:

- a) contacting said sample with at least one non-naturally occurring tracrRNA comprising a portion that specifically hybridizes with a first portion of said sensed RNA, wherein a second portion of said sensed RNA specifically hybridizes with at least one target nucleic acid; wherein said at least one non-naturally occurring tracrRNA is hybridized or is able to be hybridized with said sensed RNA in the presence of at least one tracrRNA-dependent CRISPR nuclease enzyme, optionally further comprising the presence of at least one RNA-cleaving enzyme, such as RNase III;
- b) detecting cleavage of said at least one target nucleic acid in said sample by said nuclease enzyme;
- c) wherein detecting said cleavage of at least one target nucleic acid detects said at least one sensed RNA in said cell, tissue and/or sample.

6. The method according to claim 4 or 5, wherein detecting said bound and/or cleaved at least one target nucleic acid comprises detecting a change in the signal of a suitable label, such as a dye, a fluorophore, or electrical conductivity, and/or detecting the said cleaved at least one target nucleic acid fragment itself.

7. An *in vitro* method for detecting at least one sensed RNA in a cell, tissue and/or sample, said method comprising:

- a) contacting said sample with at least one non-naturally occurring tracrRNA comprising a portion that specifically hybridizes with a first portion of said sensed RNA, wherein a second portion of said sensed RNA specifically hybridizes with at least one target nucleic acid; wherein said at least one non-naturally occurring tracrRNA is hybridized or is able to be hybridized with said sensed RNA in the presence of at least one tracrRNA-dependent CRISPR nuclease enzyme, optionally further comprising the presence of at least one RNA-cleaving enzyme, such as RNase III; and
- b) nicking or cleaving of said at least one target nucleic acid in said sample by said nuclease,
- c) detectably editing said at least one target nucleic acid comprising, for example, non-homologous end joining (NHEJ) repair, microhomology-mediated end joining (MMEJ), homologous-directed repair (HDR), base editing, prime editing or RNA editing, and
- d) suitably detecting said editing of said at least one target nucleic acid comprising, for example, at least one method selected from detecting the presence and/or length of indels

or gene edits, the activation/repression of a gene or other genetic element as encoded by said at least one target nucleic acid, and/or the detection of said prime or base editing;
 e) wherein detecting said editing of said at least one target nucleic acid detects said at least one sensed RNA in said cell, tissue and/or sample.

8. An *in vitro* method for recording transcription of at least one target DNA in a cell, tissue and/or sample, said method comprising:

- a) contacting said sample, cell or tissue with at least one non-naturally occurring tracrRNA comprising a portion that specifically hybridizes with a first portion of a sensed RNA, wherein a second portion of said sensed RNA specifically hybridizes with said at least one target DNA; wherein said at least one non-naturally occurring tracrRNA is hybridized or is able to be hybridized with said sensed RNA in the presence of at least one tracrRNA-dependent CRISPR nuclease enzyme, optionally further comprising the presence of at least one RNA-cleaving enzyme, such as RNase III; and
- b) nicking or cleaving of said at least one target nucleic acid in said sample by said nuclease,
- c) detectably editing said at least one target DNA comprising, for example, non-homologous end joining (NHEJ) repair, microhomology-mediated end joining (MMEJ), homologous-directed repair (HDR), a detectable marker, a detectable modification, base editing, prime editing or RNA editing, and
- d) optionally, suitably detecting said editing of said at least one target DNA comprising, for example, at least one method selected from detecting the presence and/or length of indels or gene edits, the activation/repression of a gene or other genetic element as encoded by said at least one target nucleic acid, and/or the detection of said prime or base editing;
- e) wherein detecting said editing of said at least one target DNA records transcription of said at least one sensed RNA in said cell, tissue and/or sample.

9. The method according to any one of claims 4 to 8, wherein several or a multitude of sensed RNAs are detected in one or more samples, tissues and/or cells.

10. The method according to any one of claims 4 to 9, wherein said at least one sensed RNA is environment, species, strain, disease, cell- and/or tissue specific or related to a

condition selected from a viral infection, such as, for example, coronavirus infection, infection with a pathogen, a metabolic disease, cancer, neurodegenerative diseases, ageing, a drug, and biotic or abiotic stress.

11. The method according to any one of claims 4 to 10, wherein said at least one sensed RNA is added to said cell, tissue and/or sample prior to step a), and/or further comprising a step of transcribing DNA into sensed RNA prior to step a).

12. The non-naturally occurring tracrRNA nucleic acid molecule or method according to any one of claims 1 to 11, wherein said at least one tracrRNA-dependent CRISPR nuclease enzyme is selected from type II Cas9 and type V Cas12 nuclease enzymes, such as type II Cas9 nuclease selected from the group consisting of II-A, -B, and -C, and type Cas12 nuclease selected from the group consisting of type V-B, -C, -D, -E, -F, -G, and -K.

13. The non-naturally occurring tracrRNA nucleic acid molecule or method according to any one of claims 1 to 12, wherein said portion of said at least one non-naturally occurring tracrRNA that specifically hybridizes with said first portion of said sensed RNA hybridizes with 10 or more nucleotides, preferably with 11 and more nucleotides, and more preferred with about 12 nucleotides or more, and wherein preferably said first portion of said sensed RNA comprises a protospacer adjacent motif (PAM).

14. The non-naturally occurring tracrRNA nucleic acid molecule or method according to any one of claims 1 to 13, wherein the nucleotide sequence of said at least one portion of said tracrRNA that specifically hybridizes with a first portion of said sensed RNA is produced and/or modified in order to be complementary to at least 80%, preferably complementary to more than 90%, more preferably to more than 95%, and most preferably are 100% complementary to said first portion to said sensed RNA.

15. The non-naturally occurring tracrRNA nucleic acid molecule or method according to any one of claims 1 to 14, wherein more than one non-naturally occurring tracrRNA are generated and used, each specifically hybridizing with a different portion of said sensed RNA.

16. The method according to any one of claims 4 to 15, further comprising detecting the amount of said at least one target nucleic acid and/or said at least one sensed mRNA in said sample, tissue and/or cell, preferably the amount per sample, tissue and/or cell, and preferably further comprising detecting a change in the amount of said at least one target nucleic acid and/or said at least one sensed mRNA in said sample, tissue and/or cell when compared to a control.

17. An *in vitro* method for detecting a medical condition in a mammal, wherein said condition is related to the presence of, expression of and/or mutation(s) in at least one sensed RNA, comprising performing the method according to any one of claims 4 to 16, and detecting said medical condition based on the presence of, expression of and/or mutation(s) in said at least one sensed RNA as detected, wherein preferably said at least one sensed RNA is environment, species, strain, disease, cell- and/or tissue specific or related to a condition selected from a viral infection, such as, for example, coronavirus infection, infection with a pathogen, a metabolic disease, cancer, neurodegenerative diseases, ageing, a drug, and biotic or abiotic stress, wherein optionally said at least one sensed RNA is added to said cell, tissue and/or sample prior to step a), and/or further comprising a step of transcribing DNA into sensed RNA prior to step a).

18. A sensed RNA detection system comprising a) a non-naturally occurring tracrRNA designed to bind to at least one portion of said sensed RNA, said sensed RNA further comprising a portion that specifically hybridizes with a first portion of a target DNA, and b) at least one tracrRNA-dependent CRISPR nuclease enzyme, preferably further comprising c) at least one target nucleic acid molecule comprising a label for detecting cleavage of said target nucleic acid, and optionally further comprising d) at least one RNA cleaving enzyme, such as RNase III enzyme.

19. Use of the non-naturally occurring tracrRNA nucleic acid molecule according to any one of claims 1 to 3 or the sensed RNA detection system according to claim 18 for performing a method according to any one of claims 4 to 17, in particular for detecting a sensed RNA, a viral sensed RNA, a sensed RNA transcribed from a disease marker, or for generating an expression profile for one or more sensed RNAs.