Claims

CLAIMS

1. A method for analyzing a higher-order structure of RNA comprising the steps of:

(a) preparing one or a plurality of RNA probes, each comprising an RNA to be analyzed attached to a barcode sequence,

(b) contacting the RNA probes and an RNA modification reagent,

(c) detecting a position and a frequency of modified nucleotides in a sequence of the RNA probe obtained in step (b),

wherein the RNA modification reagent selectively modifies a single chain region in the RNA probe, and
each of the barcode sequences has is a sequence that forms a structure that has a reduced reactivity with the RNA modification reagent. comprising a plurality of base pairs.

2. The method of claim 1 wherein step (c) comprises:

(c1) synthesizing complementary DNAs using a mixture of the RNA probes obtained in step (b) as a template by a reverse transcriptase,

(c2) determining nucleotide sequences of the complementary DNAs and aligning the nucleotide sequences comprising the barcode sequence, and

(c3) detecting a position and a frequency of mutations occurred in the aligned nucleotide sequences.

3. The method of claim 1 or 2, wherein the barcode sequence does not form a base pair, in a case where the RNA modification reagent selectively modifies a bound nucleotide in the RNA probe.

4. The method of claim 1 or 2, wherein the barcode sequence forms a structure comprising a plurality of base pairs in a case where the RNA modification reagent selectively modifies an unbound nucleotide in the RNA probe.

5. The method of claim 4, wherein the structure comprising a plurality of base pairs is a complementary double-stranded structure, a triple chain structure or a quadruple chain structure.

6. 4. The method of claim 41 or 52, wherein the plurality of base pairs are present in a stem region of a stem-loop structure or a pseudo-knot structure.

7. 5. The method of any one of claims 4 to 6claim 1 or 2, wherein the structure comprising a plurality of base pairs is a stem-loop structure comprising one or more bulge and/or an internal loop in the stem region.

8. 6. The method of any one of claims 41 to 75, wherein the structure comprising a plurality of base pairs is obtained from an RNA structure deposited in PDB (Protein Data Bank) or a derivative thereof.).

9. 7. The method of any one of claims 1 to 86, wherein the RNA to be analyzed comprises at least one RNA structural motif.

10. An8. A composition comprising an RNA probe for use in a method of any one of claims 1 to 7, wherein the RNA probe comprising an RNA to be analyzed attached to a barcode sequence that forms a structure comprising a plurality of base pairs.

11. 9. The RNA probecomposition of claim 108, wherein the structure comprising a plurality of base pairs is a complementary double-stranded structure, a triple chain structure or a quadruple chain structure.

12. 10. The RNA probecomposition of claim 10 or 118, wherein the plurality of base pairs are present in a stem region of a stem-loop structure or a pseudo-knot structure.

11. The composition of claim 8, wherein the structure comprising a plurality of base pairs is a stem-loop structure comprising one or more bulge and/or an internal loop in the stem region.

12. The composition of claim 8, wherein the structure comprising a plurality of base pairs is obtained from an RNA structure deposited in PDB (Protein Data Bank).

13. An RNA probe library comprising a plurality of RNA probes,compositions of any one of claims 8 to 12, wherein an RNA probe comprised in each of whichthe compositions comprises an RNA to be analyzed attached tothat has a barcodedifferent sequence that forms a structure comprising a plurality of base pairs..

14. A group of RNA probe libraries comprising two or more replicates of the RNA probe libraries of claim 13, wherein all the replicated RNA probes further comprise second barcode sequences, and the second barcode sequences are identical within each of the RNA probe libraries, while distinguishable between other RNA probe libraries.