

Claims

1. A method for analyzing an RNA molecule having a cleavage site for a catalytic nucleic acid molecule, the method comprising the steps of:
 - a) providing an RNA molecule having a cleavage site for a catalytic nucleic acid molecule,
 - b) cleaving the RNA molecule with the catalytic nucleic acid molecule into a 5' terminal RNA fragment and at least one 3' RNA fragment by contacting the RNA molecule with the catalytic nucleic acid molecule under conditions allowing the cleavage of the RNA molecule,
and
 - c) determining a physical property of the RNA molecule by analyzing the 5' terminal RNA fragment,
wherein step c) comprises determining a structural feature selected from the group consisting of (i) the presence or absence of a cap structure at the 5' terminus of the RNA molecule having a cleavage site for the catalytic nucleic acid molecule, (ii) the orientation of the cap of the cap structure at the 5' terminus of the RNA molecule having a cleavage site for the catalytic nucleic acid molecule and (iii) the presence of a modified cap structure at the 5' terminus of the RNA molecule having a cleavage site for the catalytic nucleic acid molecule.
2. A method for analyzing a population of RNA molecules, wherein the population comprises at least one RNA molecule that has a cleavage site for a catalytic nucleic acid molecule, the method comprising the steps of:
 - a) providing a sample containing the population of RNA molecules,
 - b) cleaving the at least one RNA molecule having a cleavage site for the catalytic nucleic acid molecule with the catalytic nucleic acid molecule into a 5' terminal RNA fragment and at least one 3' RNA fragment by contacting the sample with the

catalytic nucleic acid molecule under conditions allowing the cleavage of the RNA molecule,

- c) determining a physical property of the at least one RNA molecule having a cleavage site by analyzing the at least one 5' terminal RNA fragment obtained in step b),
 wherein step c) comprises determining a structural feature selected from the group consisting of (i) the presence or absence of a cap structure at the 5' terminus of the RNA molecule having a cleavage site for the catalytic nucleic acid molecule, (ii) the orientation of the cap of the cap structure at the 5' terminus of the RNA molecule having a cleavage site for the catalytic nucleic acid molecule and (iii) the presence of a modified cap structure at the 5' terminus of the RNA molecule having a cleavage site for the catalytic nucleic acid molecule,

and

- d) measuring the relative amount of the at least one 5' terminal RNA fragment obtained in step b), thereby determining the relative amount of RNA molecules having said physical properties in the RNA population.
3. The method according to claim 1 or 2, wherein step c) comprises analysis of a structural feature or of a physical parameter of the 5' terminal RNA fragment.
 4. The method according to any one of claims 1 to 3, wherein step c) involves spectroscopic analysis, quantitative mass spectrometry, or sequencing.
 5. The method according to any one of claims 2 to 4, wherein the population comprises at least one capped RNA molecule having a cleavage site for the catalytic nucleic acid molecule and wherein step d) comprises determining the relative amount of the at least one capped RNA molecule in the population.
 6. The method according to claim 5, wherein the population comprises at least one capped RNA molecule having a cleavage site for the catalytic nucleic acid molecule and at least one non-capped RNA molecule having a cleavage site for the catalytic nucleic acid molecule and wherein step c) comprises separating capped 5' terminal RNA fragments and non-capped 5' terminal RNA fragments.

7. The method according to claim 5 or 6, wherein the amount of the capped and/or the amount of the non-capped 5' terminal RNA fragments are measured in step c) by spectroscopic methods, quantitative mass spectrometry, or sequencing.
8. The method according to any one of claims 2 to 7, wherein the population comprises at least one correctly capped RNA molecule having a cleavage site for the catalytic nucleic acid molecule and wherein step d) comprises determining the relative amount of correctly capped RNA molecules having a cleavage site for the catalytic nucleic acid molecule in the population.
9. The method according to claim 8, wherein the population comprises at least one correctly capped RNA molecule having a cleavage site for the catalytic nucleic acid molecule and at least one reverse-capped RNA molecule having a cleavage site for the catalytic nucleic acid molecule, and wherein step c) comprises separating correctly capped 5' terminal RNA fragments and reverse-capped 5' terminal RNA fragments and/or wherein step d) comprises calculating the ratio of the amount of correctly capped RNA molecules having a cleavage site for the catalytic nucleic acid molecule and the amount of reverse-capped RNA molecules having a cleavage site for the catalytic nucleic acid molecule in the population.
10. A method of determining the capping degree of a population of RNA molecules having a cleavage site for a catalytic nucleic acid molecule, the method comprising the steps of:
 - a) providing a sample containing the population of RNA molecules,
 - b) cleaving the RNA molecules with the catalytic nucleic acid molecule into a 5' terminal RNA fragment and at least one 3' RNA fragment by contacting the sample with the catalytic nucleic acid molecule under conditions allowing the cleavage of the RNA molecules,
 - c) separating the RNA fragments obtained in step b),
 - d) determining a measure for or measuring the amount of the capped and non-capped 5' terminal RNA fragments separated in step c) of said population of RNA molecules, and

- e) comparing said measures of capped and non-capped 5' terminal RNA fragments determined in step d), thereby determining the capping degree of said population of RNA molecules,

wherein the measure determined in step d) is the signal intensity of the capped and non-capped 5' terminal RNA fragments or the amount of the RNA fragments.

11. The method according to claim 10, wherein said measure is determined by spectroscopic methods, quantitative mass spectrometry, or sequencing.
12. The method according to any one of claims 1 to 11, wherein the cleavage site of the catalytic nucleic acid molecule is within the first 50 nucleotides of the 5'-end of the RNA molecules.
13. The method according to any one of claims 1 to 12, wherein the catalytic nucleic acid molecule is a ribozyme, preferably selected from the group consisting of hammerhead ribozymes, hairpin ribozymes, and HDV ribozymes.
14. The method according to any one of claims 2 to 13, wherein the sample containing the population of RNA molecules is generated by *in vitro* transcription in the presence of a cap analog or by *in vitro* transcription and subsequent enzymatic capping, wherein the cap analog is preferably selected from the group consisting of G[5']ppp[5']G, m⁷G[5']ppp[5']G, m₃^{2,2,7}G[5']ppp[5']G, m₂^{7,3'-O}G[5']ppp[5']G (3'-ARCA), m₂^{7,2'-O}GpppG (2'-ARCA), m₂^{7,2'-O}GppspG D1 (β-S-ARCA D1) and m₂^{7,2'-O}GppspG D2 (β-S-ARCA D2).
15. The method according to any one of claims 1 to 14, wherein the RNA fragments are separated in step c) by denaturing gel electrophoresis or liquid chromatography, preferably HPLC, FPLC or RPLC.
16. The method of any of claims 1 to 15, wherein the RNA molecule having a cleavage site for the catalytic nucleic acid molecule is an mRNA molecule.
17. The method according to any one of claims 10 to 16, wherein step c) comprises separating the capped 5' terminal RNA fragments and the non-capped 5' terminal RNA fragments.

18. The method according to any one of claims 10 to 17, wherein the amount of the capped and the amount of the non-capped 5' terminal RNA fragments are measured in step d) by spectroscopic methods, quantitative mass spectrometry, or sequencing.
19. The method of any one of claims 10 to 18, wherein the orientation of the cap in the 5' terminal RNA fragment of a capped RNA molecule is determined.
20. Use of a catalytic nucleic acid molecule for determining the capping degree of a population of RNA molecules, wherein the catalytic acid molecule is used to cleave the RNA molecules of the population into a 5' terminal RNA fragment and at least one 3' RNA fragment with a length useful for the determination of the capping degree, wherein the 5' terminal RNA fragment has a length of from 1 to 30 nucleotides.
21. The use according to claim 20, wherein the method for determining the capping degree further comprises at least one of the steps as defined in any of claims 10 to 19.