

CLAIMS

1. A method for depleting off-target RNA molecules from a nucleic acid sample comprising:
 - a) contacting a nucleic acid sample comprising at least one target RNA or DNA sequence and at least one off-target RNA molecule with a probe set comprising at least ~~two~~ 40 DNA probes complementary to discontinuous sequences along the full length of the at least one off-target RNA molecule, thereby hybridizing the at least 40 DNA probes to the off-target RNA molecules to form DNA:RNA hybrids, wherein
 - i) each of the at least 40 DNA probes is 40 to 60 bases in length, and
 - ii) each DNA:RNA hybrid is from 5 to 40 ~~at least 5 bases apart, or at least 10 bases~~ apart, along a given off-target RNA molecule sequence from any other DNA:RNA hybrid; and
 - b) contacting the DNA:RNA hybrids with a ribonuclease that degrades the RNA from the DNA:RNA hybrids, thereby degrading the off-target RNA molecules in the nucleic acid sample to form a degraded mixture.
2. The method of claim 1, comprising: c) optionally degrading any remaining DNA probes by contacting the degraded mixture with a DNA digesting enzyme, optionally wherein the DNA digesting enzyme is DNase I, to form a DNA degraded mixture; and d) separating the degraded RNA from the degraded mixture or the DNA degraded mixture.
3. The method of claim 1 or claim 2, wherein the contacting with the probe set comprises treating the nucleic acid sample with a destabilizer.
4. The method of claim 3, wherein with the destabilizer is heat or a nucleic acid destabilizing chemical, optionally wherein the nucleic acid destabilizing chemical is betaine, DMSO, formamide, glycerol, ~~or a derivative thereof, or a mixture thereof.~~
5. The method of claim 4, wherein the nucleic acid destabilizing chemical is formamide, optionally wherein the formamide is present during the contacting with the probe set at a concentration of from ~~about~~ 10 to 45% by volume.

6. The method of claim 4, wherein treating the sample with heat comprises applying heat above the melting temperature of the at least one DNA:RNA hybrid.
7. The method of any one of claims 1 to 6, wherein the ribonuclease is RNase H or Hybridase.
8. The method of any one of claims 1 to 7, wherein the nucleic acid sample is from a human or a non-human primate.
9. The method of any one of claims 1 to 7, wherein the nucleic acid sample is from a rat or a mouse.
10. The method of any one of claims 1 to 8, wherein the nucleic acid sample comprises nucleic acids of non-human origin.
11. The method of claim 10, wherein the nucleic acids of non-human origin are from non-human eukaryotes, bacteria, viruses, plants, soil, or a mixture thereof.
12. The method of any one of claims 1 to 11, wherein the off-target RNA is rRNA, mRNA, tRNA, or a mixture thereof.
13. The method of claim 12, wherein the off-target RNA is rRNA and globin mRNA.
14. The method of any one of claims 1 to 12, wherein the probe set comprises at least two DNA probes that hybridize to at least one off-target RNA molecule selected from 28S, 23S, 18S, 5.8S, 5S, 16S, 12S, HBA-A1, HBA-A2, HBB, HBB-B1, HBB-B2, HBG1, and HBG2.
15. The method of claim 14, wherein the probe set comprises at least two DNA probes that hybridize to two or more off-target RNA molecules selected from 28S, 18S, 5.8S, 5S, 16S, and 12S from humans.

16. The method of any one of claims 1 to 15, wherein the probe set comprises at least two DNA probes that hybridize to one or more off-target RNA molecules selected from HBA-A1, HBA-A2, HBB, HBG1, and HBG2 from hemoglobin, and 23S, 16S, and 5S from Gram positive or Gram negative bacteria.

17. The method of any one of claims 1 to 16, wherein the probe set comprises at least two DNA probes that hybridize to one or more off-target RNA molecules from an Archaea species.

18. The method of any one of claims 1 to 17, wherein the probe set comprises at least two DNA probes that hybridize to one or more off-target RNA molecules from rat and/or mouse, optionally selected from rat 16S, rat 28S, mouse 16S, and mouse 28S, and combinations thereof.

19. The method of any one of claims 1 to 18, wherein probes to a particular off-target RNA molecule are complementary to ~~about~~ 80 to 85% of the sequence of the off-target RNA molecule, with gaps of at least 5, or at least 10 bases between each probe hybridization site.

20. The method of any one of claims 1 to 11, wherein the DNA probes comprise:

- (a) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 333 sequences selected from SEQ ID NOs: 1-333; or
- (b) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 400 or more, or 428 sequences selected from SEQ ID NOs: 1-428; or
- (c) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 377 sequences selected from SEQ ID NOs: 1-377; or
- (d) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 384 sequences selected from SEQ ID NOs: 1-333 and SEQ ID NOs: 378-428; or
- (e) two or more, or five or more, or 10 or more, or 25 or more, or 44 sequences selected from SEQ ID NOs: 334-377; or

(f) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 51 sequences selected from SEQ ID NOs: 378-428;
or a combination thereof.

21. A composition comprising a probe set comprising at least ~~two~~ 40 DNA probes complementary to discontinuous sequences at ~~least 5, or at least 10, bases apart~~ along the full length of at least one off-target RNA molecule in a nucleic acid sample, wherein each of the at least 40 DNA probes is 40 to 60 bases in length; and a ribonuclease capable of degrading RNA in an DNA:RNA hybrid.

22. The composition of claim ~~19~~ 21, wherein the ribonuclease is RNase H.

23. The composition of claim 21 or claim 22, wherein each DNA probe is hybridized at least 10 bases apart along the full length of the at least one off-target RNA molecule from any other DNA probe in the probe set.

24. The composition of any one of claims 21 to 23, wherein the composition comprises a destabilizing chemical.

25. The composition of claim 24, wherein the destabilizing chemical is formamide.

26. The composition of any one of claims 21 to 25, wherein the off-target RNA is rRNA, mRNA, tRNA, or a mixture thereof.

27. The composition of claim 26, wherein the off-target RNA is rRNA and globin mRNA.

28. The composition of any one of claims 21 to 27, wherein the probe set comprises at least two DNA probes that hybridize to at least one off-target RNA molecule selected from 28S, 23S, 18S, 5.8S, 5S, 16S, 12S, HBA-A1, HBA-A2, HBB, HBG1, and HBG2.

29. The composition of claim 28, wherein the probe set comprises at least two DNA probes that hybridize to two or more off-target RNA molecules selected from 28S, 18S, 5.8S, 5S, 16S, and 12S from humans.

30. The composition of any one of claims 21 to 29, wherein the probe set comprises at least two DNA probes that hybridize to one or more off-target RNA molecules selected from HBA-A1, HBA-A2, HBB, HBG1, and HBG2 from hemoglobin, and 23S, 16S, and 5S from Gram positive or Gram negative bacteria.

31. The composition of any one of claims 21 to 30, wherein the probe set comprises at least two DNA probes complementary to one or more rRNA molecules from an Archaea species.

32. The composition of claim 21 to 31, wherein the probe set comprises DNA probes that hybridize to one or more off-target RNA molecules from rat and/or mouse, optionally selected from rat 16S, rat 28S, mouse 16S, and mouse 28S, and combinations thereof.

33. The composition of any one of claims 21 to 32, wherein the DNA probes comprise:

- (a) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 333 sequences selected from SEQ ID NOs: 1-333; or
- (b) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 400 or more, or 428 sequences selected from SEQ ID NOs: 1-428; or
- (c) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 377 sequences selected from SEQ ID NOs: 1-377; or
- (d) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 384 sequences selected from SEQ ID NOs: 1-333 and SEQ ID NOs: 378-428; or
- (e) two or more, or five or more, or 10 or more, or 25 or more, or 44 sequences selected from SEQ ID NOs: 334-377; or

(f) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 51 sequences selected from SEQ ID NOs: 378-428;
or a combination thereof.

34. A kit comprising a probe set comprising at least ~~two~~ 40 DNA probes complementary to discontinuous sequences that are from 5 to 40 at least 5, or at least 10, bases apart along the full length of at least one off-target RNA molecule in a nucleic acid sample and a ribonuclease capable of degrading RNA in an DNA:RNA hybrid, wherein each of the at least 40 DNA probes is 40 to 60 bases in length.

35. The kit of claim 34, comprising a buffer and nucleic acid purification medium, and optionally comprising a destabilizing chemical.

36. The kit of any one of claims 34 to 35, wherein the off-target RNA is rRNA, mRNA, tRNA, or a mixture thereof.

37. The kit of claim 36, wherein the off-target RNA is rRNA and globin mRNA.

38. The kit of any one of claims 34 to 37, wherein the probe set comprises at least two DNA probes that hybridize to at least one off-target RNA molecule selected from 28S, 23S, 18S, 5.8S, 5S, 16S, 12S, HBA-A1, HBA-A2, HBB, HBG1, and HBG2.

39. The kit of claim 38, wherein the probe set comprises at least two DNA probes that hybridize to two or more off-target RNA molecules selected from 28S, 18S, 5.8S, 5S, 16S, and 12S from humans.

40. The kit of any one of claims 34 to 39, wherein the probe set comprises at least two DNA probes that hybridize to one or more off-target RNA molecules selected from HBA-A1, HBA-A2, HBB, HBG1, and HBG2 from hemoglobin, and 23S, 16S, and 5S from Gram positive or Gram negative bacteria.

41. The kit of any one of claims 34 to 40, wherein the probe set comprises at least two DNA probes complementary to one or more rRNA molecules from an Archaea species.
42. The kit of claim 34 to 41, wherein the probe set comprises DNA probes that hybridize to one or more off-target RNA molecules from rat and/or mouse, optionally selected from rat 16S, rat 28S, mouse 16S, and mouse 28S, and combinations thereof.
43. The kit of any one of claims 34 to 42, wherein the DNA probes comprise:
- (a) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 333 sequences selected from SEQ ID NOs: 1-333; or
 - (b) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 400 or more, or 428 sequences selected from SEQ ID NOs: 1-428; or
 - (c) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 377 sequences selected from SEQ ID NOs: 1-377; or
 - (d) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 100 or more, or 150 or more, or 200 or more, or 250 or more, or 300 or more, or 350 or more, or 384 sequences selected from SEQ ID NOs: 1-333 and SEQ ID NOs: 378-428; or
 - (e) two or more, or five or more, or 10 or more, or 25 or more, or 44 sequences selected from SEQ ID NOs: 334-377; or
 - (f) two or more, or five or more, or 10 or more, or 25 or more, or 50 or more, or 51 sequences selected from SEQ ID NOs: 378-428;
- or a combination thereof.
44. The kit of claim 34, comprising:
- (1) a probe set comprising SEQ ID NOs: 1-333;
 - (2) a ribonuclease, optionally wherein the ribonuclease is RNase H;
 - (3) a DNase; and
 - (4) RNA purification beads;

and optionally further comprising an RNA depletion buffer, a probe depletion buffer, and a probe removal buffer.

45. A method of supplementing a probe set for use in depleting off-target RNA nucleic acid molecules from a nucleic acid sample comprising:

- a) contacting a nucleic acid sample comprising at least one RNA or DNA target sequence and at least one off-target RNA molecule from a first species with a probe set comprising at least ~~two~~ 40 DNA probes complementary to discontinuous sequences along the full length of the at least one off-target RNA molecule from a second species, thereby hybridizing the at least 40 DNA probes to the off-target RNA molecules to form DNA:RNA hybrids, wherein each DNA:RNA hybrid are from 5 to 40~~is at least 5 bases apart, or at least 10 bases apart~~, along a given off-target RNA molecule sequence from any other DNA:RNA hybrid; wherein each of the at least 40 DNA probes is 40 to 60 bases in length;
- b) contacting the DNA:RNA hybrids with a ribonuclease that degrades the RNA from the DNA:RNA hybrids, thereby degrading the off-target RNA molecules in the nucleic acid sample to form a degraded mixture;
- c) separating the degraded RNA from the degraded mixture;
- d) sequencing the remaining RNA from the sample;
- e) evaluating the remaining RNA sequences for the presence of off-target RNA molecules from the first species, thereby determining gap sequence regions; and
- f) supplementing the probe set with additional DNA probes complementary to discontinuous sequences in one or more of the gap sequence regions.

46. The method of claim 45, wherein the gap sequence regions comprise 50 or more base pairs.

47. The method of claim 45 or claim 46, wherein the first species is a non-human species and the second species is human.

48. The method of claim 47, wherein the first species is rat or mouse.

49. The method of claim 47 or claim 48, wherein the composition of any one of claims 21 to 33 is used to supply the ribonuclease and the probe set comprising DNA probes complementary to discontinuous sequences along the full length of the at least one off-target RNA molecule of a human.

50. The method of claim 48 or claim 49, wherein the method is used to identify DNA probes that hybridize to one or more off-target RNA molecules from rat and/or mouse, optionally selected from rat 16S, rat 28S, mouse 16S, and mouse 28S, and combinations thereof.