Claims

What is claimed is:

1. A variant Csy4 endoribonuclease comprising an amino acid sequence having at least about 95% amino acid sequence identity to the amino acid sequence set forth in ~~Figure 6~~SEQ ID NO: 8, wherein the variant Csy4 endoribonuclease comprises an amino acid substitution at His-29, wherein the variant Csy4 endoribonuclease is enzymatically inactive in the absence of imidazole, and wherein the variant Csy4 endoribonuclease is activatable in the presence of imidazole.

2. The variant Csy4 endoribonuclease of claim 1, wherein the amino acid substitution is a His29 to Ala29 substitution.

3. The variant Csy4 endoribonuclease of claim 1, wherein the variant Csy4 endoribonuclease comprises a moiety that provides a detectable signal.

4. The variant Csy4 endoribonuclease of claim 1, wherein the moiety that provides a detectable signal is a fluorophore, a quantum dot, an enzyme other than the endoribonuclease, or a nanoparticle.

5. The variant Csy4 endoribonuclease of claim 1, wherein the endoribonuclease is immobilized on an insoluble support.

6. The variant Csy4 endoribonuclease of claim 5, wherein the insoluble support is a bead.

7. The variant Csy4 endoribonuclease of claim 1, wherein, in the variant Csy4 endoribonuclease binds an RNA substrate comprising the nucleotide sequence 5'-GUUCACUGCCGUAUAGGCAGCUAAGAAA-3' (SEQ ID NO:1).

8. A nucleic acid comprising a nucleotide sequence encoding the variant Csy4 endoribonuclease of claim 1.

18. The kit of claim 14, wherein the recombinant expression vector comprises, inserted into the multiple cloning site, a nucleotide sequence encoding the target RNA.

19. The kit of claim 13, further comprising imidazole.

20. The kit of claim 13, further comprising one or more wash buffers.

21. The kit of claim 13, further comprising a positive control expression vector.

22. The kit of claim 13, wherein the variant Csy4 endoribonuclease is immobilized on an insoluble support.

23. A method of isolating a target RNA present in a mixed population of nucleic acids, the method comprising:

a) contacting a mixed population of nucleic acids with the variant Csy4 endoribonuclease of claim 1, where the variant Csy4 endoribonuclease is immobilized on an insoluble support, wherein the mixed population of nucleic acids comprises a tagged target RNA comprising a recognition nucleotide sequence that is specifically bound by the immobilized variant Csy4 endoribonuclease, forming a tagged target RNA-immobilized variant Csy4 endoribonuclease complex, wherein said contacting is carried out in a binding solution lacking imidazole;

b) adding imidazole to the binding solution to a final concentration of from about 100 mM to about 500 mM, thereby forming a reactivation solution that enzymatically reactivates the immobilized variant Csy4 endoribonuclease, wherein the reactivated immobilized variant Csy4 endoribonuclease cleaves the target RNA from the tag; and

c) collecting the released target RNA.

24. The method of claim 23, further comprising a wash step carried out after step (a) and before step (b).

~~25. A method of isolating a polypeptide that binds a target RNA, the method comprising:~~

a) contacting an immobilized complex with a liquid solution comprising a polypeptide that binds the target RNA, wherein the immobilized complex comprises the variant Csy4 endoribonuclease and a tagged target RNA comprising a recognition nucleotide sequence that is specifically bound by the variant Csy4 endoribonuclease, wherein said contacting results in binding of the polypeptide to the target RNA, wherein said contacting is carried out in a binding solution lacking imidazole; and

b) eluting the bound polypeptide.

26. A method of regulating production of a target RNA in a eukaryotic cell, the method comprising contacting a genetically modified host cell with an agent that activates an inducible promoter, wherein the genetically modified host cell is genetically modified with a recombinant expression vector comprising a nucleotide sequence encoding an enzymatically active sequence-specific Csy4 endoribonuclease that catalyzes cleavage at a sequence-specific cleavage site in a substrate polyribonucleotide, wherein the enzyme-encoding nucleotide sequence is operably linked to the inducible promoter, wherein, upon activation of the inducible promoter, the enzyme is produced in the cell and cleaves said target RNA from a precursor RNA.

27. The method of claim 26, wherein the target RNA species is a regulatory RNA.

28. The method of claim 26, wherein cleavage of said target RNA from a precursor RNA inactivates the precursor RNA.

29. A method of detecting a specific sequence in a target polyribonucleotide, the method comprising:

a) contacting the target polyribonucleotide with a oligonucleotide probe comprising the specific sequence and an enzymatically active sequence-specific Csy4 endoribonuclease under conditions that favor duplex formation between the oligonucleotide probe and the target polyribonucleotide, wherein the duplex is cleaved by the Csy4 endoribonuclease; and

b) detecting specific binding between the oligonucleotide probe and the target polyribonucleotide, wherein detection of duplex formation between the oligonucleotide probe

and the target polyribonucleotide indicates the presence of the specific sequence in the target polyribonucleotide.

30. The method of claim 29, wherein the oligonucleotide probe is linked to a peptide, wherein the peptide is released upon cleavage of the duplex by the Csy4 endoribonuclease, and where the detection step comprises detection of the released peptide.

31. The method of claim 29, wherein the released peptide is detected by binding to an antibody specific for the peptide.

32. The method of claim 31, wherein the antibody is immobilized.

33. The method of claim 29, wherein the target polyribonucleotide is immobilized on a solid support.

34. The method of claim 29, wherein the target polyribonucleotide is a polyribonucleotide of a pathogen.

35. The method of claim 29, wherein the enzymatically active Csy4 endoribonuclease comprises an amino acid sequence of a Csy4 amino acid sequence depicted in Figure 5.