miRNA Detection by Ligation and Amplification of Complementary DNA oligos Using SplintR® ligase

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Abstract

Ligation of two adjacent DNA oligonucleotides splinted by RNA has historically been difficult to achieve. We have discovered that SplintR® ligase (Chlorella virus DNA ligase) is much more efficient in this ligation than T4 DNA ligase, which was traditionally used for this application. When these ligers were compared using the same RNA-DNA substrate, the SplintR® enzyme achieved complete ligation with 10X less ligase and was 15X faster than T4 DNA ligase [1].

We have taken advantage of this efficient RNA splinted ligation and developed an extremely sensitive and specific miRNA detection protocol. In this method, two DNA oligos, splinted by a miRNA, were ligated and amplified by qPCR in presence of a dual labeled DNA probe. Using this method, we can detect miR-122 from less than 1 ng of total liver RNA. Further more we found that efficient ligation can be achieved with only a 4-6 base pair overlap between one of the DNA oligos and the miRNA splint. The SplintR® ligase can discriminate a single nucleotide mismatch between DNA oligos and miRNA splint. We designed DNA oligos that were specific for individual members of the mammalian let-7 family and were able to detect specific let-7 isoforms. The efficient and specific ligation of RNA-DNA hybrids by the SplintR® ligase should allow it to be used in a wide range of RNA detection methods for both cellular and viral RNAs.


miRNA Detection by Splint Ligation

Small overlap required for RNA splint ligation

High sensitivity - miR-122 detection in rat liver RNA

Comparison of SplintR vs TaqMan for miRNA detection

Summary

- Chlorella virus DNA ligase, SplintR® ligase, is much more efficient than either T4 DNA ligase or T4 RNA ligase 2 in ligation of DNA oligos hybridized to a miRNA splint.
- SplintR® ligase requires only a 4-6 base DNA:RNA overlap for ligation.
- The SplintR® method coupled with qPCR can detect miRNAs in biological samples in the sub-femtomolar amounts.
- The SplintR® ligase method is about 10 fold more sensitive than the TaqMan method, that uses cDNA synthesis of the miRNA.
- Multiplex detection of miRNAs can be achieved by the simultaneous ligation of 48 pairs of miRNA-specific DNA oligos followed by NextGen DNA sequencing.
- Single base differences in members of the let-7 family can be detected by SplintR ligation.

Reference