MicroRNA (miRNA) biomarker discovery and RNomics. Chromatography methods are possible alternatives. 

Oligonucleotides were then mixed with 3-hydropicolinic acid matrix and subjected to AXIMA Performance MALDI mass spectrometry (Shimadzu Corporation, Kyoto, Japan) for detection and sequence analysis of miRNA molecules. 

Mature microRNA molecules are collected and identified through ‘fishing’ process (1). 

Results: 

Mature microRNA molecules are collected and identified through ‘fishing’ process (2). 

Validation of target microRNA by qRT-PCR. 

Conclusions: 

- Mature microRNA molecules can be distinguished by MALDI mass spectrometry. 
- ‘Fishing’ for the target microRNA molecules is possible by applying antisense oligo-probe and biotinylated beads. 
- Verified the ‘fished’ molecules as target by miR qRT-PCR. 
- Direct observation of oligonucleotides (e.g. microRNA, siRNA) opens a new field in order to apply these molecules as biomarkers, as well as studying these compounds in forms of pharmaceutical agents. 

References: 

- Pandit, et al. Inhibition and role of let-7d in idiopathic pulmonary fibrosis. 
- 1 Koichi Tanaka Laboratory of Advanced Science and Technology, Shimadzu Corporation 
- 2 World-Leading Drug Discovery Research Center, Graduate School of Pharmaceutical Sciences, Kyoto University 
- Kyoto, Japan 

Materials and Methods: 

- Mature sequence molecules for hsa-miR-16a, hsa-let-7d, hsa-miR-21, as prepared (Sigma-Aldrich Japan, Ishikari, Japan). 0.1 ug of oligonucleotides were either eluted in nuclease-free water or spiked to RNA pool. 
- Mature sequence molecules for hsa-miR-16a, hsa-let-7d, hsa-miR-21 were prepared (Sigma-Aldrich Japan, Ishikari, Japan) for detection and sequence analysis of miRNA molecules. 

Performance MALDI mass spectrometry revealed the spectra of the whole mature molecules of miR-16a, let-7d, miR-21, as well as their sequence from 3’ end up to 10 nucleotides. The spectra were detectable as less as 3.75 picomoles without amplification processes, and the yields were measurable by HPLC through ultra-violet laser absorption ratio of 280/260. 

Conclusions: 

- MALDI mass spectrometry and liquid chromatography demonstrated potential as alternative methods for qualitative and quantitative analysis of mature microRNA. This novel approach has advantage over conventional methods in terms of its capability to directly observe the microRNA molecules without amplification. 

Mature microRNA molecules can be distinguished by MALDI mass spectrometry. 

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