Fast and Sensitive Analysis of DNA Hybridization in a PDMS Micro-fluidic Channel Using FRET

예권해, 이상엽, 박성희[*], 이기태[*], 이병철[*], 정영욱[*], 주재범[**]

한국원자력연구소, 한양대학교, [*]한국원자력연구소, [**]한양대학교

yeakh@korea.com

Hybridization analysis of DNA plays an important role in the detection of genetic diseases and gene expression profiling. Today, one of the most popular approaches to DNA hybridization analysis is the use of a microarray chip where probe DNA sequences are immobilized on a solid phase surface and incubated with a mixture of the unknown target DNA.[1] The fluorescence is caused by an interaction between a target analyte and an immobilized probe element, and provides valuable information on the presence of a target DNA.[2] Although microarray technology provides a cost-effective method for microscale bioassays, it has several drawbacks such as immobilization schemes and a relatively long DNA hybridization time. To resolve these problems, a quick and accurate DNA analysis technique using microfluidic devices has been developed.[3] This microfluidic analysis method does not use an immobilization procedure; instead, it uses a simple syringe pumping system. The laminar flow along the channel can be easily controlled by the channel structure and flow speed. By injecting target and probe DNA solutions, it is possible to detect the sequence-specific hybridization of both the probe and target DNA, and the simple operation enables a highly accurate DNA analysis to be performed. Microfluidic devices also overcome the slow hybridization problem caused by the diffusion-limited kinetics on a microarray chip, since the hybridization occurs in solution. As a result, the hybridization time is greatly reduced to less than a few seconds if a properly designed channel to obtain optimized mixing

performance is utilized. However, a new detection method in a microfluidic device is required for the identification of the change in fluorescence on hybridization, since nonhybridizing fluorescence oligonucleotides cannot be washed out inside the channel. Therefore, we used fluorescence resonance energy transfer (FRET) for this purpose, where two types of DNA oligonucleotides, which share complimentary base sequences, were prepared. Each DNA oligonucleotide was labelled with a different fluorescence dye at the 5’ − or 3’ − terminus. Here, one oligonucleotide is the fluorescence donor and the other is the acceptor. When the two fluorescent oligonucleotides hybridize to form adjacent sequences in the microfluidic channel, the distance between two fluorophores on the new hybrid becomes close enough for FRET to occur. FRET is caused by an interaction between the donor and acceptor fluorescence dyes located within a distance of < 8 nm from each other. and FRET results from a quenching of the donor fluorescence and an enhancement of the acceptor fluorescence intensity. By monitoring the change in fluorescence intensity between the donor and acceptor DNA oligonucleotides, it is possible to accurately detect their hybridization process.

DNA hybridization analysis using microfluidic technology overcomes many of the drawbacks of microarray chips, such as the long hybridization time and inconvenient immobilization procedures. In a microfluidic device, however, a new detection method is required, since nonhybridizing fluorescence oligonucleotides cannot be washed out inside the channel. In our work, detection using FRET successfully resolved this problem. Compared to the previously reported work on DNA analysis using microfluidic devices, both the detection sensitivity and the quantitative measurement capability have been greatly improved. We expect this analytical technique using FRET to be successfully applied to DNA analysis in a microfluidic channel.

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