단일세포 형광측정기술과 형광공명에너지전달기술을 이용한 특정유전자보유세포 검색기술 개발

Detection of Cells Containing Specific Genes by Single Cell Fluorescence Assay with PNA FRET Probes

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Gene or DNA with specific sequence can be recognized by optical detection utilizing fluorescently labeled nucleic acids such as DNA, RNA and PNA (Peptide Nucleic Acid)[1]. When the FRET (Fluorescence Resonance Energy Transfer)[2] technique is used for DNA detection, hybridization of probes with target DNA results in the change of fluorescence spectrum. Thus it is possible to detect target genes in buffer solutions or live cells by using the FRET technique.

An analytical method to detect cells containing specific genes with FRET technique using PNA probes has been developed. Four different 12-mer PNA FRET pairs were synthesized based on the target gene sequence information and fulfilling the requirements for PNA probe design. These pairs were used for target DNA detection in buffer solution. The target was herbicide resistance gene encoding EPSPS (5-enolpyruvyl shikimate-3-phosphate synthase) which originated from Roundup Ready® soybean[3]. FITC and TAMRA were used as the donor and acceptor, respectively. Probes were designed to show efficient binding with EPSPS gene. Various conditions for DNA detection such as annealing temperature, salt concentration, annealing time, donor/acceptor concentration ratio, and the relative concentration of target gene and probes were optimized to give highest detection efficiency for the target DNA with EPSPS sequence.

A laser scanning confocal microscope (LSCM) system having a single molecule detection sensitivity based on an inverted microscope (Axiovert-25CFL, Carl Zeiss) was used for FRET fluorescence measurement. The excitation laser beam was 441.6 nm from a He-Cd laser (45 LRS 801-230, Melles Griot). Fluorescence from the sample was collected by a microscope objective lens and focused through a 150 mm focal length lens onto an input slit of 150 mm focal length spectrograph (300 grooves/mm grating) equipped with a CCD detector (Spec-10:100B/TE, Roper Scientific Inc.) at the exit hole. Scattered light around the excitation wavelength was removed by inserting a holographic notch filter (441.6 nm SuperNotch-Plus^{TM}, Kaiser Optics) between the objective lens and the focusing lens. Double stranded DNA with EPSPS sequence of GM soybean was synthesized by PCR (polymerase chain reaction)[4]. Appropriate sequence of PNA probes were designed and pre-screened to have highly binding efficiencies by PCR amplification of target DNA with primers of the same base sequence with probes. Detection efficiencies of target DNA could be compared by observing the fluorescence intensity ratio of acceptor and donor. As FRET occurs between donor and acceptors, the fluorescence intensity of acceptor increases while that of donor decreases due to energy transfer between two molecules. Thus the fluorescence intensity ratio of

acceptor relative to donor will increase as FRET occurs. The fluorescence intensity ratios of four different probes were tested. Then the most efficient FRET pair of PNA probe was selected for further experiment.

In vitro detection efficiencies were optimized by changing the following parameters: annealing temperature, annealing time, salt concentration, donor/acceptor concentration ratio, and the relative concentration of target gene and probes. FRET efficiency of the four different probe pairs aiming different target regions was also compared. The highest detection efficiency was obtained with samples treated at annealing temperature of 25 °C for at least 24 hrs in the presence of 100 μM NaCl, and donor/acceptor/target DNA concentration ratio of 1:10:3.

Target cell detection was then performed through observation of the cell fluorescence using two different lasers after incorporating the probes into cells. Resulting from FRET effect, fluorescence intensity of the target cells was *ca.* 12 times higher than the cells without target genes.

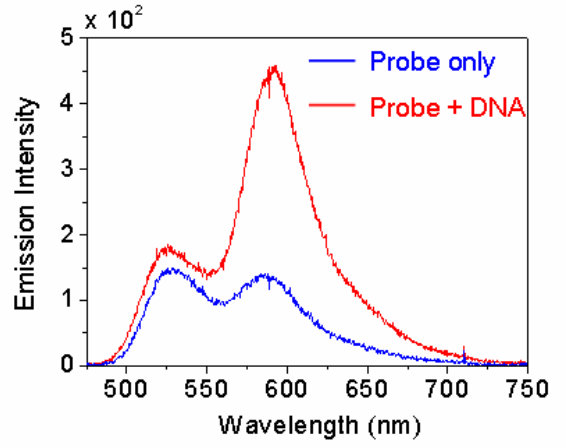

Figure 1. Comparison of PNA FRET probe fluorescence spectra in solution with and without target DNA

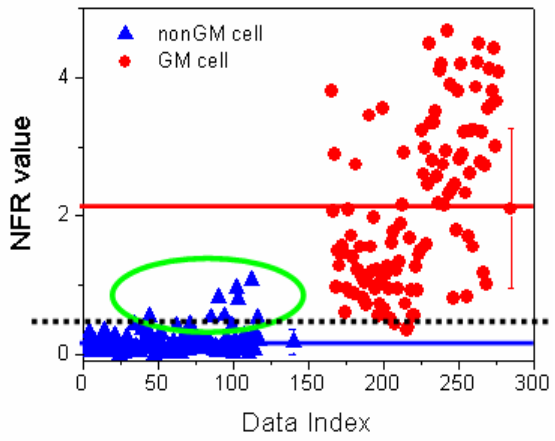

Figure 2. Comparison of FRET ratio in GM and nonGM cells. (Average ratio differs in 12 times for GM and non GM cells.)

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