Smart probe: A novel fluorescence quenching-based oligonucleotide probe carrying a fluorophore and an intercalator

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ABSTRACT
A novel probe (Smart probe) has been developed for nucleic acid detection. The smart probe is an oligodeoxyribonucleotide carrying a fluorophore and an intercalator internally. Fluorescence of the smart probe is quenched by the intercalator in the absence of target sequence. While upon hybridization the probe emits greater fluorescence due to the interference of quenching by intercalation. The smart probe has been shown to recognize a single base mismatch in the double-stranded form without utilizing thermal stability difference of hybrids.

INTRODUCTION
As the Human Genome Project nears completion, the need for rapid and cost-efficient methods to detect specific nucleic acid sequences will become much more important. Recent advances of DNA probe technology have concentrated in homogeneous assays using fluorescence detection (1-3). To improve the previous methods, I have developed a novel probe, termed Smart probe, modifying the method described by Shinozuka et. al. (4). It is expected that some of intercalators interact photochemically with fluorophore for example fluorescence energy transfer or quenching. When intercalator binds to double-stranded DNA, the photochemical interaction between the intercalator and the fluorophore must be weakened and the fluorophore emits fluorescence. This idea is illustrated in Fig. 1.

On the basis of above idea, a couple of probes carrying fluorescein and intercalator were synthesized and its photochemical characters were studied. For practical applications of the smart probe, a single base mismatch detection and a real-time polymerase chain reaction were also studied.

EXPERIMENT
Labeled oligonucleotide syntheses were conducted on a 392 (ABI) synthesizer using a standard phosphoramidite chemistry. Fluorescein was incorporated internally into oligodeoxynucleotides by using Fluorescein Phosphoramidite (Glen Research). Intercalator was introduced into a designated position by the use of Uni-Link AminoModifier (CLONTECH), and the succinimide derivative of intercalator was attached to the primary amino group.
A POLAstar (bMG) was used to measure the fluorescence intensities. Temperature dependent fluorescence measurement and real-time assay of PCR were conducted in a Light Cycler (Roche Molecular Systems).

RESULTS
Four oligodeoxynucleotides containing fluorescein and pyrene were synthesized and their fluorescence intensities were measured with and without complementary oligodeoxynucleotides (Fig. 2). It was
found that free probes showed low fluorescence, which increased almost 5-fold upon hybridization to complementary nucleic acid. This made the smart probes suitable for homogeneous hybridization assay. When pyrene was replaced by coumarin, DABCYL and TAMRA, only coumarin showed similar results to that of pyrene. This result supported the idea that the quenching was inhibited by intercalation.

Fig. 2 Relative fluorescence intensity of the smart probe with and without complementary oligonucleotide. Fluorescence intensity was determined at 538 nm upon excitation at 485 nm. Single-stranded smart probes were EFN1:5’GCAACAGGC(Py)(F)CGACAACG(No.1), EFN2:5’GCAACAGGC(Py)C(F)CGACAACG(No.3), EFN3:5’ACGCACAAA(Py)(F)ACCAAGCA(No.5), EFN4:5’ACGCACAAA(Py)A(F)ACCAAGCA(No.7). Double-stranded smart probes were prepared by the annealing with each complementary oligonucleotide, ds-EFN1 (No.2), ds-EFN2 (No.4), ds-EFN3 (No.6), ds-EFN4 (No.8). (Py):pyrene, (F): fluorescein.

Ideally, the smart probe seemed to distinguish a perfect match from a single base mismatch because the intercalation should be influenced by the double-stranded structure. Typical results of the single base discrimination experiments were shown in Fig. 3. It was apparent that the duplex containing a single base mismatch showed decreased fluorescence comparing to the fully matched duplex. However, the extent of decreased fluorescence was affected significantly by the structure surrounding mismatch. To reveal the ability of the smart probe in real-time PCR assay, PCR was conducted in a Light Cycler in which Light Cycler probe was replaced by a smart probe. As the cases in TaqMan (1), Molecular Beacon (2) and Light Cycler (3), fluorescence intensities of the smart probe were correlated to both the cycle number and copy number of template. The smart probe has proved to be particularly suited for monitoring nucleic acid amplification and opens up new possibilities for the detection of single base mismatch without utilizing thermal stability difference of hybrids.

REFERENCES