Nonenzymatic DNA ligation in Escherichia coli cells

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ABSTRACT

We report on a new fluorescence reporting strategy in which dabsyl, a well-known quencher, activates a hydroxyl group in a probe to convert it to a leaving group. When a nucleophilic phosphorothioate probe binds adjacent to a dabsyl quenched probe, autoligation occurs, releasing the quencher, and lighting up the probes. These self-ligating DNA probes were used for sequence-specific detection of 16S rRNA in E. coli cells. Strong fluorescence was observed only when the phosphorothioate and quenched dabsyl probes bind side-by-side on a 16S rRNA target. The results demonstrate the use of QUAL probes to detect specific RNA sequences in bacterial cells without enzymes and without washing steps.

INTRODUCTION

To date, a number of FRET-based strategies for detection of nucleic acids has been reported. Perhaps, the most well-known approach is that of “molecular beacons”, which consist of hairpin-forming DNAs labeled in the stem with fluorophore and quencher. Binding to a complementary sequence results in opening of the hairpin and moving of the quencher away from the fluorophore. Beacons can be used in solution or solid-supported approaches, however, molecular beacons have been confronted with difficulties with nonspecific signals in the cell perhaps due to binding of molecular beacons to any DNA-binding protein. In a recent cellular study, molecular beacons showed no advantage over standard linear probes.

Figure 1. Structure of the dabsyl-thymidine nucleotide and mechanism of dabsyl displacement, showing how autoligation of the two DNA strands leads to loss of quenching.

Herein, we report on new reporter probes, QUAL probes, that “light up” when a nucleophilic phosphorothioate probe binds adjacent to a dabsyl quenched probe. Such probes display high selectivity for even single nucleotide differences, and because quenching is efficient they require no washing away of unbound probes. We demonstrate
their use for direct detection of RNA sequences in bacterial cells.6

RESULTS AND DISCUSSION
Preliminary experiments established that these QUAL probes could be used to detect DNAs at a single nucleotide resolution in solution and on solid-phase.

To demonstrate the nucleic acid detection in cells, we constructed QUAL probes targeted to ribosomal RNAs in *E. coli* K12 strain MG1655. Cells were fixed with paraformaldehyde and were incubated with the QUAL probes. Products were imaged by fluorescence microscopy without washing unbound probes from the specimen. We found a distinct fluorescence signal when the both phosphorothioate and quenched probes were complementary to adjacent site of target rRNA. On the other hand, almost no signal was observed when incubated with the probes which are complementary to rRNA but at spatially separated sites (Figure 2). These results allow us to conclude that the use of a "QUAL probe" enables facile detection of RNA in bacterial cells.

**Figure 2.** Self-ligation on 16S rRNA in fixed *E. coli* cells. *E. coli* cells were incubated with dabsyl/thioate probes which bind (a) side-by-side or (b) at spatially separated sites (553 nt apart).

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REFERENCES


