

Microbial detection with low molecular weight RNA.

Kourentzi KD, Fox GE, Willson RC.

Department of Chemical Engineering, University of Houston, 4800 Calhoun Avenue, Houston, TX 77204-4004, USA.

The need to monitor microorganisms in the environment has increased interest in assays based on hybridization probes that target nucleic acids (e.g., rRNA). We report the development of liquid-phase assays for specific bacterial 5S rRNA sequences or similarly sized artificial RNAs (aRNAs) using molecular beacon technology. These beacons fluoresce only in the presence of specific target sequences, rendering as much as a 27-fold fluorescence enhancement. The assays can be used with both crude cell lysates and purified total RNA preparations. Minimal sample preparation (e.g., heating to promote leakage from cells) is sufficient to detect many Gram-negative bacteria. Using this approach it was possible to detect an aRNA-labeled *Escherichia coli* strain in the presence of a large background of an otherwise identical *E. coli* strain. Finally, by using a longer wavelength carboxytetramethylrhodamine beacon it was possible to reduce the fraction of the signal due to cellular autofluorescence to below 0.5%.

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