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Title: RAPID ANALYSIS OF SPECIFIC DNA SEQUENCES BY FLUORESCENT SINGLE-MOLECULE DETECTION

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Rapid analysis of specific DNA sequences by fluorescent single-molecule detection

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Abstract: We have developed a technique for the detection of specific nucleic acid sequences at the single molecule level of sensitivity. This method is based on the synthesis of a highly fluorescent reporter molecule using the target nucleic acid as a template.

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The detection of specific sequences of DNA or RNA with high sensitivity is of fundamental importance in many fields, including genetic and medical research, clinical chemistry and forensic science, among others. We present here a newly developed technique for the analysis of specific nucleic acids sequences in homogeneous solution based on a polymerase extension reaction. This method consists of synthesizing a highly fluorescent nucleic acid reporter molecule using a sequence of the target as a template. The synthesis of the reporter molecule is accomplished by using a short oligonucleotide primer that is complementary to the target. A suitable polymerase and free nucleotides are added to the sample. One of these oligonucleotides is -at least partially- labeled with a fluorophore. If the target is present in the sample, the primer binds to it, and the polymerase incorporates the labeled and unlabeled nucleotides, reconstructing the target's complementary sequence. The sample is flowed through the capillary cell of a single molecule detector. Fluorescence from the reporter molecule is much stronger than that of the free nucleotide background over the detection time. Detection of the reporter signifies the presence of the target being sought. This method allows for the rapid, direct detection of single-copy targets at femtomolar concentrations without the use of an amplification reaction, such as the polymerase chain reaction.