Förster resonance energy transfer sensor with a quantum dot for detection of specific DNA sequences

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Abstract:

The synthesis, structure and application of a Förster resonance energy transfer (FRET) sensor for the detection of specific DNA sequences are investigated. The sensor is intended for detection of circulation tumor DNA from blood samples. Therefore, we also focused on sample preparation of nucleic acids.

The sensor is based on the hybridization of oligonucleotide modified with quantum dots (QDs) and a fluorescently labeled sample DNA resulting in changes of the laser-induced fluorescence emission due to the FRET effect. The analyzed sample DNA is a nucleotide primer fluorescently labeled at its 3' end by carboxy-*X*-rhodamine (ROX). Upon hybridization of the ssDNA sample with the QD conjugate, the QD and ROX label get close to each other. After irradiation, part of the energy absorbed by QD donor is nonradiatively transferred to the ROX acceptor and a decrease in QD luminescence emission and an increase in ROX fluorescence emission is observed. The structure of the sensor was determined by the dependence of FRET efficiency on the concentration of complementary ROX-ssDNA and confirmed by capillary electrophoresis analysis.

Real DNA samples often include a complex matrix (such as blood and other bodily fluids, or exogenous impurities, e.g., from the scene of crime). Most of the common nucleic acids isolation techniques are based on extractions; however, isotachophoretic focusing has recently attracted some interest for its simplicity and potential for very high enrichment factors and ease of automation. We report on the use of a commercial isotachophoretic instrument for optimization of DNA and preparative fraction collection. The sample of a DNA ladder was injected in 30 µl volume and after ITP

focusing the DNA zone was recovered using an on-column micropreparative collection valve. The DNA content in the collected sample was verified by fluorescence spectrometry and chip capillary electrophoresis with fluorescence detection.

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