Analytical Letters 2005 vol.38 N15, pages 2493-2507

DNA hybridization on membrane-modified carbon electrodes

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Abstract

The DNA-modified membrane electrode was prepared by casting a mixture of nitrocellulose (NC) with target DNA (tDNA) in organic solvent on glassy carbon electrode (GCE). Unlabeled polymerase chain reaction (PCR)-amplified human genomic sequence (628 bp) or synthetic oligodeoxynucleotides (ODNs) were used as tDNAs, creating a recognition layer. Biotinylated ODNs were used as hybridization probes to recognize specific nucleotide sequences. The hybridization events were detected via an enzyme-linked electrochemical assay involving binding of streptavidin-coupled alkaline phosphatase (SALP) to the biotin labels of the probe bound to tDNA. After the probe hybridization and SALP binding, the electrode was immersed into an electroinactive enzyme substrate (1-naphthyl phosphate). The alkaline phosphatase converted the inactive substrate into electroactive 1-naphthol that penetrated through the NC membrane to the GCE surface and was subsequently detected using an anodic voltammetric signal. The optimized method offered a good discrimination between complementary and nonspecific DNAs and yielded well-defined responses for both single-copy and repetitive tDNA sequences. In contrast to previously published methods using electrodes with mechanically attached membranes, the previously mentioned electrode is easily amenable to parallel DNA analysis. Copyright © Taylor & Francis, Inc.

http://dx.doi.org/10.1080/00032710500369679

Keywords

Alkaline phosphatase, DNA hybridization, Electrochemical DNA sensor, Glassy carbon electrode, Nitrocellulose membrane