
Dual enzyme electrochemical coding for detecting DNA hybridization

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Enzyme-based hybridization assays for the simultaneous electrochemical measurements of two DNA targets are described. Two encoding enzymes, alkaline phosphatase and β -galactosidase, are used to differentiate the signals of two DNA targets in connection to chronopotentiometric measurements of their electroactive phenol and α -naphthol products. These products yield well-defined and resolved peaks at +0.31 V (α -naphthol) and +0.63 V (phenol) at the graphite working electrode (*vs.* Ag/AgCl reference). The position and size of these peaks reflect the identity and level of the corresponding target. The dual target detection capability is coupled to the amplification feature of enzyme tags (to yield fM detection limits) and with an efficient magnetic removal of non-hybridized nucleic acids. Proper attention is given to the choice of the substrates (for attaining well resolved peaks), to the activity of the enzymes (for obtaining similar sensitivities), and to the selection of the enzymes (for minimizing cross interferences). The new bioassay is illustrated for the simultaneous detection of two DNA sequences related to the BCRA1 breast-cancer gene in a single sample in connection to magnetic beads bearing the corresponding oligonucleotide probes. Prospects for electrochemical coding of multiple DNA targets are discussed.