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Improved detection by time-resolved fluorometry of specific DNA immobilized in microtiter wells with europium/metal-chelator labelled DNA probes

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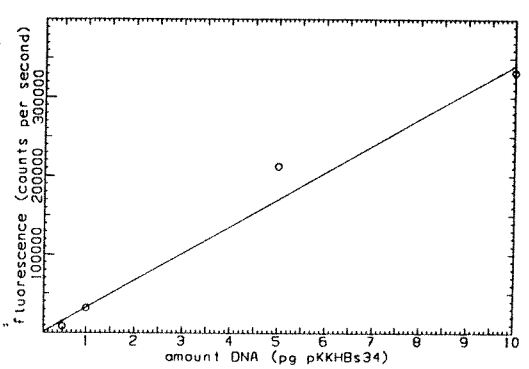
Recently, a cloned DNA probe labelled with metal-chelators (diethylentriamine pentaacetate, DTPA) was used in a dot-blot hybridization assay for the non-radioactive detection of down to 1 attomol target DNA by europium - time-resolved fluorometry (1). We now report modifications of the procedure which led to a more than tenfold increase in sensitivity.

A DTPA-labelled, HBV-specific plasmid DNA probe (pKKHBs34) was prepared as described (1). The hybridization and detection procedure was changed in three points: i) Polystyrene microtiter wells (Titertek 1x12 Well Microstrips, Flow Laboratories) instead of nitrocellulose filters were used as the solid support. Target DNA was immobilized by UV-light (2). ii) A lower Eu^{3+}-concentration (10µM instead of 100µM Eu-EDTA) was used for the chelation of the hybrid-bound DTPA ligands after the hybridization reaction. iii) Washing conditions after the chelation step were changed to 10µM EDTA, 0.1 M Tris-HCl (pH 7.8) to remove unspecifically bound Eu^{3+} more efficiently. The samples in the microtiter strips were directly measured in an "Arcus 1230" time-resolved fluorometer (LKB-Wallac) after releasing the europium ions into an enhancement solution (0.1 M acetate-phthalate buffer, pH 3.2, 0.1% Triton X-100, 15 µM 2-naphtoyltrifluoroacetone, 50 µM tri-n-octylphosphinoxid).

0.5 pg (0.1 attomol) of target plasmid DNA were clearly detectable (8131 cps against 2135 cps background without DNA). Heterologous DNAs gave only blank signals (1µg calf thymus DNA 2354 cps, 1µg pBR322 DNA 2844 cps). The dose-response curve was linear and proportional (see figure) while in the filter-assays a tenfold increase in fluorescence required a 1000fold increase in DNA (1).



The sensitivity of the non-radioactive hybridization assay and the use of microtiter strips as solid support is useful for automation, especially in sandwich hybridization procedures where crude samples may be evaluated (3).

REFERENCES: (1) Oser A., Roth W.K., a. Valet G. (1988) NAR **16**, 1181-1196. (2) Nagata Y., Yokota H., Kosuda O., Yokoo K., Takemura K., a. Kikuchi T. (1985) FEBS L. **183**, 379-382. (3) Ranki M., Palva A., Virtanen M., Laaksonen M., a. Söderlund H. (1983) Gene **21**, 77-85.