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1. Effect of polylysine on adherance of DNA to solid plastic matrices.

Polystyrene plates, Falcon 1012 integrid, were immersed in a solution of poly-L-lysine (PPL) (Mr. = 70,000), 10 μg ml⁻¹ in ~~ph~~ 0.1M phosphate buffered saline, pH 7.2 for 1 hr at 37°. After washing ~~to~~ six times with distilled water, aliquots of biotinylated-DNA denatured with NaOH and either treated ~~with~~ subsequently with ammonium acetate or not, were applied to the plates in decreasing concentrations. Identically treated DNA samples were applied in a similar manner to similar non-coated polystyrene plates. As the DNA applied was biotinylated (b-DNA) the presence of ~~such~~ retained bound ~~these~~ macromolecules was determined by sequential addition of goat anti-biotin antibody and rabbit anti-goat antibody complexed with alkaline phosphatase. The amount bound was determined using an insoluble phosphatase substrate detection reagent.

It was observed that b-DNA applied to non-coated polystyrene remained bound and was detectable. However, B-DNA applied to PPL-polystyrene coated plates ~~d~~ bound initially; but upon application the enzyme developing reagent (pH 9.6), the ~~PPL bDNA~~ PPL-bDNA-Ab-AbE complex was released from the solid matrix. This phenomenon was most likely a consequence of reduction of primary electrostatic bonding of PPL to polystyrene at elevated pH.

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2. Detection of b-DNA bound to non-coated polystyrene matrices by the soluble enzyme immunosorbent assay (ELISA)

Biotinylated DNA (b-DNA) was denatured as described above and aliquoted onto Dynatech Immulon II Removeable™ Wells. Samples were allowed to dry onto plastic surface at 37°. The amount of bound b-DNA was determined by sequential addition of goat anti-biotin antibody and rabbit anti-goat antibody complexed to alkaline phosphatase followed by development with para-nitrophenyl phosphate. Enzymic activity was monitored at 405 nm utilizing the automatic Dynatech MicroELISA™ Scanner.

This procedure enabled these investigators to quantitate the amount of bound DNA and circumspectively, the degree of biotinylation.

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3. To ascertain whether bound DNA can serve as a template for hybridization of biotinylated DNA probes.

Several aliquots of denatured adeno-2-DNA were bound to polystyrene plates as described in section two. After blocking with Denhart's- formamide blocking buffer, ~~several~~ different biotinylated probes were added. These were: (i) adeno-2-DNA and, (ii) lambda DNA.; a third ~~contain~~ ~~no~~ hybridization mixture contained no probe. The extent of hybridization was determined by means of the antibody-enzyme reaction (ELISA) as described in section 2.

It was observed that only the homologous, adeno-2-probe hybridized to the immobilized template DNA. This technique demonstrated the feasability of quantitating in vitro hybridization.

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4. To determine the capacity of polystyrene to adsorb DNA.

With the knowledge of the variability of polystyrene binding capacities it was decided after preliminary experiments, that, to date, coating of these plates with duodecadiamine (DDDA) resulted in an uniform binding coefficient.

Radio-labelled, non-biotinylated DNA (2000 ng – 5 ng) denatured as described above was ~~add~~ added to DDDA coated polystyrene ~~plates~~ micro-wells. Samples were not allowed to dry. After incubation at 37° for 0.5, 1.0, 2.0, 3.0, 4.0 and 18 h, samples were ~~counted~~ washed and counted. Binding of DNA was maximal after 2h of incubation; however, only 50% of the original amount bound regardless of the concentration. This indicates that there is an equilibrium between bound and unbound DNA.

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