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AMINATED SURFACE

TECHNICAL NOTE N. 8

1. DNA binding on secondary amine support

Phosphorylation and labeling of the capture probe

DNA used for carbodiimide-mediated binding is phosphorylated at their 5' end with T_4 polynucleotide kinase and an aliquot is radiolabeled with [γ-^{32}P]-ATP by chromatography on Sephacryl 200 spin column (1 ml) (Pharmacia, Uppsala, Sweden). DNA concentration is then measured by fluorescence using the Hoechst H33258 compound (Labarca and Paigen,1980).
The purity of the fragment can be checked by agarose gel electrophoresis. Radiolabeled DNA is mixed with cold DNA in the ratio of 1:10 in order to reduce the level of radioactivity in the experiments.

DNA binding

Covalent linking of DNA can be obtained by fixation of its 5' end phosphate on the activated amino groups of the plastic (Zamatteo *et al*,1996) (Figure 1). Phosphorylated capture probes for Human Cytomegalovirus detection are denatured for 10 min at 100°C, cooled on ice (10 min), and diluted in ice-cold water (1.54 ng/μl). Ice cold 0.1 M 1-methylimidazole, pH 7.5, is added to obtain a final 1-methylimidazole concentration of 10 mM. The denatured DNA solution is dispensed into the microwells (75 μl/well, 0.7 pmol/well) standing on ice. A fresh solution of 0.04 M 1-ethyl-3(3-dimethylaminopropyl)-carbodiimide (EDC) in 10 mM 1-methylimidazole is added to each well (25 μl/well and they are incubated for 5 h at 50°C.
After incubation, the wells are washed three times with washing buffer (0.4 N NaOH, 0.25% Tween 20 at 50°C) 200 μl/well, then incubated 5 min with washing buffer, and finally washed three times again. Microwells are stored dried at 4°C. After the binding, the wells are cut and the amounts of ^{32}P-labelled DNA bound to the wells were measured by liquid scintillation counting.

2. Peptide Binding on primary amine support

Peptides can be chemically grafted on primary amino group via the free thiol group of a cysteine incorporated in the sequence using heterobinctional reagents such as succinimidyl 4-(N-maleimidomethyl) cycloexane-1-carboxilate (SMCC) (Yashida *et al*,1979: Hashida *et al*,1984) (figure 2) that can be purchased from Pierce Chemical Company (Rokford, USA). This crosslinker consists of an NHS ester and a maleimide group connected with a spacer arm. NHS esters react with primary amine, maleimides react with sulfhydryls.
Grafting can be evaluated by radiochemical assay if a tyrosine incorporated in the sequence is iodinated by oxidizing agent such as chloramine-T (Greenwood *et al*,1963).
Aminated microwells are incubated in a 6.5 10^{-2} mM solution of SMCC in 0.1M phosphate buffer (NHS esters react with primary amines at pH 7-9) during 1 h at room temperature. After 3 washes with phosphate buffer and 3 washes with water, the grafting is achieved by incubating cysteine -containing peptides (6.5 μM) overnight at room temperature, in 0.1 M phosphate buffer (maleimides react with SH groups at pH 6.5-7.5). After 3 washes in phosphate buffer, the wells are cut and the amount of coupled ^{125}I peptide is measured by a gamma irradiation counter.
Other bifunctional reagents such as N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) (Carlsson *et al*, 1978) can also be used.

References

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