‘Direct’ Detection and Separation of DNA Using Nanoporous Alumina Membranes.

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In this report we demonstrate that modified nanoporous alumina filters can be used for detection and separation of unmodified DNA (Fig.1).

High density of covalently linked DNA (~4×10^{12} cm^{-2}) inside the nanopores is achieved using glutaraldehyde linker which joins the amino groups of 5’-aminated DNA and the terminal amino group of aminosilane. Figure 2 illustrates a high optical density (OD ~ 1) can be reliably obtained with ss-DNA 21-mers on AAO filters. Higher loadings, OD > 1.6, can be obtained, however are inconvenient for UV measurements due to very high absorbance.

Upon hybridization with a complementary 41-mer, the UV absorbance increases to 1.45 (Fig. 2, curve B), which accounts to *ca*. 50% hybridization efficiency. This high efficiency is corroborated by infrared (IR) spectra. The bound complementary ss-DNA can be eluted by either heating the filter in water, as shown in Fig. 2 (curve C), or by using denaturing solutions.

The AAO filter with covalently immobilized DNA can be also used as an affinity separation filter for target ss-DNA, as illustrated in Fig3. A solution is simply passed through the filter and the bound target ss-DNA is then eluted in the purified form by denaturing the hybrid at an elevated temperature or by using a denaturing solution. We demonstrate this on a filter with 200 nm pores, on which 4 nmol of ss-DNA was immobilized (OD = 1.0 for the 21-mer used). When excess (6 nmoles) of the complementary ss-DNA (also a 21-mer) in 0.1M NaCl was slowly passed through that filter at room temperature, 2 nmol of the target ss-DNA was captured The captured DNA was eluted with >90% efficiency by 9 M urea solution, as observed by UV absorption spectra changes of the filter and the solutions.

Thus alumina nanoporous filters can be successfully employed for covalent DNA immobilization using aminosilanes and glutaraldehyde linker. The high surface density of DNA (~4×10^{12} cm^{-2}) and high efficiency of hybridization (*ca*. 50%) in combination with high surface area, make this system very attractive for further development of various applications to detection and separation of nucleic acids.

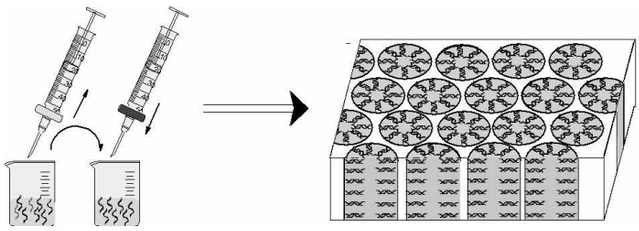

Figure 1.
Sketch of DNA separation procedure using modified alumina membranes.

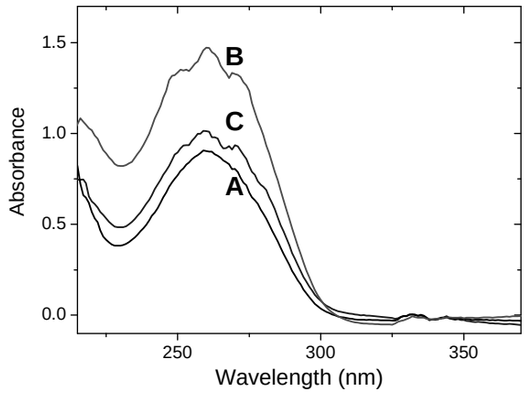

Figure 2. UV absorption spectra of a 200 nm AAO filter (60 μm thick) with: **A** – ss-DNA 21-mer immobilized inside the pores; **B** - same as A after hybridization by a complementary 41-mer; **C** – same as B after denaturing in 70^{o}C water for 1 hour and then cooling.

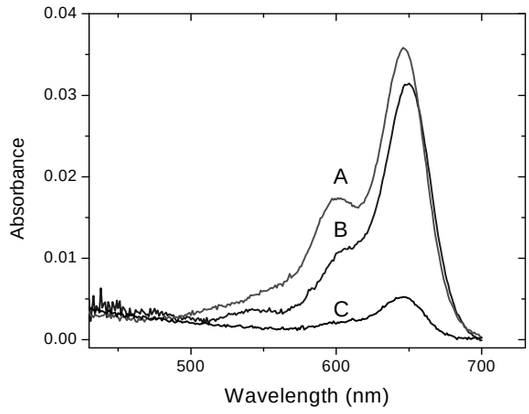

Figure 3. Absorption spectra of 1 mL solution originally with 300 nM of Cy5-tagged ssDNA (21-mer) before (**A**) and after a single pass through the AAO affinity filter (**C**). Solution also contained 6 μM of noncomplementary 21-mer that did not bind. **B** – spectrum of the filter after binding Cy5 tagged complementary ssDNA.