A Modification To Polypropylene To Reduce Adsorption of DNA When Used As A Storage Medium For Prolonged Periods Of Time.

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Introduction

DNA containers often use labware made of polypropylene to store samples for prolonged periods of time, which may total many years. Adsorption of DNA to the containing vessel decreases its sample concentration having a negative impact on quantitative measurements.

Polypropylene is a hydrophobic polymer and will therefore inhibit adsorption of the very hydrophilic DNA molecule. However, it has been observed that DNA can adsorb to polypropylene and also that there is a large difference in DNA adsorption for different polypropylene grades and/or different surface treatments.

For long-term and low-temperature storage of low amounts of DNA, in for example Bio-banks it is important to identify a polymer surface with low adsorption of DNA.

We have analysed several polypropylene resins/modifications and identified a polypropylene based approach that offers low DNA adsorption. The performance of the identified polypropylene candidate has been compared to a number of different commercially available DNA containers manufactured from polypropylene.

Method

To avoid differences in surface/volume conditions when comparing containers from different manufactures, test samples (d=4 mm) were die cut from the containers. 50 µl DNA solution (4,5 ng non-labelled Lambda DNA and 0,5 ng ^{32}P-labelled Lambda DNA) was dispensed into NUNC Polypropylene Modules (Cat. No. 232034) and the test samples were placed on top. Polypropylene has a density of approximately 0.9, and will therefore float on the liquid and only one side of the test sample will be exposed to the DNA solution. The modules were sealed and incubated over night. After incubation, the test sample was removed, washed in TE-buffer, radioactivity remaining on the test sample recorded by Packard Cyclone Phosphor Imager. In order to calculate the amount of DNA adsorbed to the test sample, a DNA standard curve was made based on 2-fold dilutions of the DNA solution. 8 µl was applied to reference test samples and after evaporation of the liquid, they were incubated and exposed together with test samples.

DNA adsorption were tested under different conditions such as high and low ionic strength buffers, high and low DNA concentration and storage at –20°C for 1 and 7 days, respectively.

Preparing of test material

Lambda DNA was digested with Hind III and end-labelled with α^{32}P-dGTP using Nick Translation Kit (Amersham). Labelled fragments were purified by ProbeQuant G-50 column (Amersham) and purified DNA quantitated by measuring adsorption at 260 nm (Envision Instrument 2100). Standard procedures were used.

High ionic strength buffer: 2.5 M NaCl in TE-buffer

Low ionic strength buffer (TE-buffer): 10 mM Tris-HCl, 1 mM EDTA

Conclusion

For storage of low amounts of DNA it is important to identify a polymer surface with low adsorption of DNA. Test samples have been die cut from commercial available containers commonly used for DNA storage as well as from containers produced *in-house* from different polypropylene grades and/or different surface treatments. We have identified a polypropylene modification (**container 2 in the figures**) that has a remarkably low adsorption of DNA. Containers produced with this particular polypropylene were tested against several commercial available containers used for DNA storage, some of which are specific sold as being low-DNA binding. The polypropylene resin identified by NUNC was throughout all experiments among the best performing polypropylene. Under common storage conditions (low ionic strength buffer) these container posses a DNA adsorption corresponding to **less than 5%** of the container with the highest DNA adsorption (Figure 2).

It was also investigated if low DNA adsorption was related to the amount of DNA incubated with the sample. However, Figure 3 shows that even at high DNA concentration the same tendency for DNA adsorption between the various PP containers is still observed. Long-term storage of DNA (7 days, -20°C, Figure 4) does not change the picture. Container 2 is still among the best performers.

It is important to inform that the polypropylene based approach identified here improves DNA recovery without the use of any additive or coatings that can interfere with the biological material.

Results

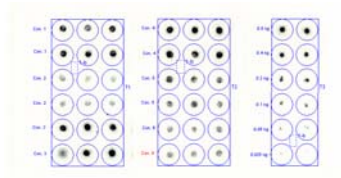

Figure 1: Autoradiogram of DNA adsorption to various polypropylene containers (Con.). The intensity and size of the spot is proportionally to the amount of adsorbed DNA. DNA was incubated in high ionic strength buffer at RT for 20 hours. DNA dilution (T3) was included with all exposures. For data analysis a grid specifying a definite area around each test samples was applied and total DLU (Digital Lights Unit) counted.

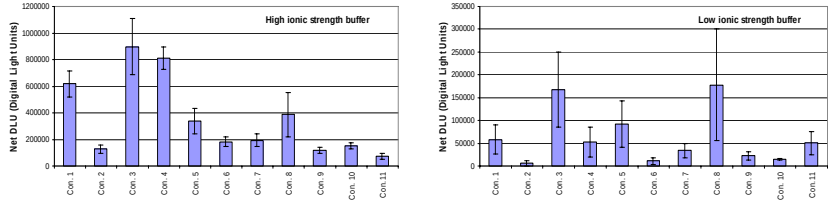

Figure 2: Adsorption of DNA to test samples at high and low ionic strength buffer, respectively. Low values indicates low DNA adsorption. High ionic strength promote binding to polypropylene, however adsorption does also take place at low ionic strength buffer. Test samples were die cut from various Polypropylene containers and incubated in a solution of ^{32}P-labelled DNA (0,1 ng/µl). Test samples incubated over night at 20°C, and radioactivity adsorbed to the test samples was measured. DLU = Digital light units. Con. = Container. Container 2 is die cut from cryotubes produced by NUNC with a polypropylene based approach. DNA adsorption in low ionic strength buffer to container 2 corresponds to 5% of the container with highest DNA adsorption.

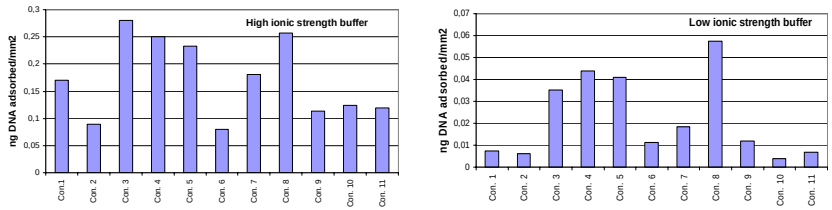

Figure 3: Incubation of test samples in high concentration DNA solution. Test samples were incubated in a solution of ^{32}P-labelled DNA (0,4 ng/µl) dissolved in high and low ionic strength buffer, respectively. Samples were incubated over-night at 20°C, and radioactivity adsorbed to the test samples was measured. A DNA dilution was included in the experiments and was used to calculate the amount of DNA adsorbed to the test samples. Container 2 is die cut from cryotubes produced by NUNC with a polypropylene based approach.

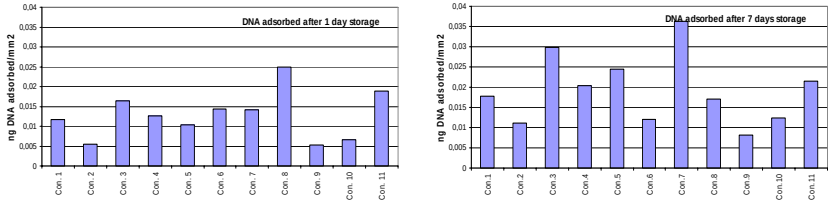

Figure 4: DNA adsorption after 1 and 7 days of storage at –20°C. Test samples were incubated in a DNA solution (0,1 ng/µl) in low ionic strength buffer for 1 and 7 days, respectively. A DNA dilution was included in the experiment and incubated under the same conditions as test samples. The DNA dilution was used to calculate the amount of DNA adsorbed to the test samples. Container 2 is die cut from cryotubes produced by NUNC with a polypropylene based approach. There is still a pronounce difference in DNA adsorption between the various containers after 7 days of storage, however Container 2 is still among the containers with lowest DNA adsorption.