Tech Note No. 50

Low DNA adsorption to Thermo Scientific Nunc Bank-It vials

Introduction

Labware made of polypropylene is often used to store DNA samples for prolonged periods of time, which may total many years. Adsorption of DNA to the containing vessel decreases the sample concentration.

Polypropylene is a hydrophobic polymer and will therefore inhibit adsorption of the very hydrophilic DNA molecule. However, it has been observed ^{1,2} 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 the Thermo Scientific Nunc Bank-It vials for storage of DNA at ambient temperatures, we have identified a polymer surface with very low adsorption of DNA.

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-labeled Lambda DNA and 0.5 ng ³²P-labeled Lambda DNA) was dispensed into polypropylene modules, and the test samples were placed on top. Polypropylene has a density of approximately 0.9 g/cm³ 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, and the radioactivity remaining on the test sample was measured.

Preparing of test material

Lambda DNA was digested with Hind III and end-labeled with α ³²P-dGTP using Nick Translation Kit. Labeled fragments were purified by ProbeQuant G-50 column and purified DNA quantitated by measuring adsorption at 260 nm. Standard procedures were used.

High ionic strength buffer: 2.5 M NaCl in TE-buffer. Low ionic strength TE-buffer: 10 mm Tris-HCl, 1 mm EDTA.

Results

Test samples have been die cut from commercially available containers commonly used for DNA storage as well as from containers produced in-house from different polypropylene grades and/ or different surface treatments. DNA adsorption to test samples was recorded by Packard Cyclone Phosphor Imager (Fig. 1). The intensity and size of the spot is proportional to the amount of adsorbed DNA. In total 11 different containers were analyzed, however only results from 6 containers are shown. Fig. 1 clearly shows that the amount of DNA adsorption varies significantly from one polypropylene to another, and that the polypropylene used to

produce Nunc™ Bank-It™ vials has a very low DNA adsorption.

It is known that high ionic strength buffer (2.5 M NaCl in TE-buffer) can promote binding of DNA to plastic. As depicted in Fig. 2 the amount of DNA adsorbed varies quite a lot, depending on the polypropylene resin/modification. There are two polypropylene containers, which throughout all the experiments have a substantial higher DNA adsorption (container 3 and 4 in Fig. 2), meanwhile there is a group (Bank-It, container 9, 10, and 11) having a low DNA adsorption, corresponding to 10-14% of the container with the highest DNA adsorption.

Long-term storage of DNA usually takes place at low ionic strength such as TE-buffer. Under these conditions, DNA also adsorbs to the polymer surface, though not to the same extent as for high ionic strength conditions. The adsorption of DNA under these conditions can be highly variable, the difference between manufacturers seems to be quite pronounced. Fig. 3 shows DNA adsorption at low ionic strength. Under these conditions Bank-It vials have the lowest DNA adsorption, corresponding to approximately 3.5% of the candidate with highest DNA adsorption (container 8 in Fig. 3).

Conclusion

For storage of small amounts of DNA, it is important to identify a polymer surface with low adsorption of DNA. We have identified a polypropylene modification that has proven



to have a remarkably low DNA adsorption. Our Bank-It vials are produced using this polypropylene and have been tested against several commercially available containers used for DNA storage, some of which are specifically sold as being low DNA binding. Under common storage conditions (TEbuffer) Bank It vials adsorp 95% less DNA than the highest binding containers.

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

Additional results can be found on the poster "A modification to polypropylene to reduce adsorption of DNA when used as a storage medium for prolonged periods of time" posted on www.thermoscientific.com.

References

1. Gaillard D and Strauss F. Eliminating DNA loss and denaturation during storage in plastic microtubes. IBL. Aug. 2000, p. 6.

2. Belotserkovskii BP and Johnston BH. Denaturation and association of DNA sequences by certain polypropylene surfaces.

Anal Biochem. 1997, 251:251-62.

Fig. 3. Adsorption of DNA to polypropylene test samples at low ionic strength.

Test samples were die cut from various polypropylene containers and incubated in a solution of 32P-labeled DNA (0,1 ng/µL) dissolved in 10 mm Tris-HCI, 1 mm EDTA. Test samples incubated over-night at 20°C, and radioactivity adsorbed to the test samples was measured. DLU = Digital Light Units.

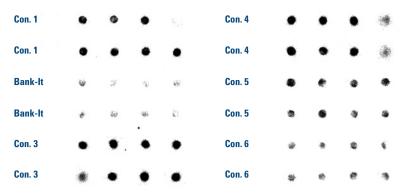


Fig. 1. Autoradiogram of DNA adsorption to various polypropylene surfaces. The intensity and size of the spot is proportional to the amount of adsorbed DNA. For each container (Con.), eight test samples were assayed. DNA was incubated in high ionic strength buffer at RT for 20 hours. For data analysis a grid specifying a definite area

around each test samples was applied and total DLU (Digital Light Units) counted.

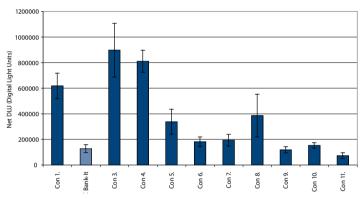
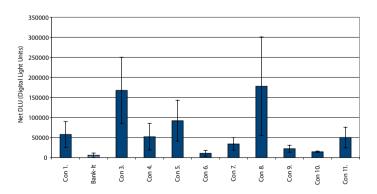


Fig. 2. Adsorption of DNA to polypropylene test samples in high ionic strength buffer. Test samples were die cut from various polypropylene containers and incubated in a solution of 32P-labeled DNA dissolved in 2.5 mm NaCl. Test samples incubated overnight at 20°C, and radioactivity adsorbed to the test samples was measured. DLU = Digital Light Units. Con. = Container.



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