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Higher DNA Recovery from Eppendorf twin.tec® PCR plates 96 LoBind During Long-term Storage

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Executive Summary

The storage of DNA samples is widely discussed and many recommendations exist e.g., storage in dried form or in liquid nitrogen at -196 °C [1, 2]. But these techniques need either pre-treatment like rehydration before usage, or are expensive and need lab space for liquid nitrogen tanks. Thus, laboratories tend to store the DNA at -20 °C in standard consumables which however might lead to degradation and binding of DNA molecules to the vessel wall. This causes loss of sample and unsatisfactory results in downstream experiments.

Quantitative real-time PCR (qPCR) experiments of human genomic DNA libraries stored for 6 months at -20 °C in PCR plates showed that the DNA recovery was stable at around 84 - 100 % in Eppendorf twin.tec PCR plates 96 LoBind while in standard plates, the recovery showed much greater variability and eventually decreased to only 16 %.
These results show that the choice of storage vessel has a significant impact on sample quantity. Eppendorf LoBind consumables offer a viable option for laboratories with restricted space and budget on long-term sample storage.

Introduction

Storing DNA samples is essential for almost every laboratory either to verify an experiment during a paper review process or due to revisions in forensics or medical studies. The quality and quantity of the stored DNA samples is critical for further analysis and thus needs to be stable over a long period of time, sometimes even for decades. Many methods for DNA storage exist, of which two methods have the advantage to minimize the molecule movement and thereby prevent chemical and nuclease degradation (1, 2). One option is to store dehydrated DNA at room temperature. But this method includes rehydration of the DNA sample prior to usage. The other option is long-term storage of DNA in solution in liquid nitrogen to fully guarantee DNA quality and quantity (1). But not every laboratory has the resources to adopt either option. However, low-temperature freezer (-20 °C and/or -80 °C) is commonplace in many laboratories.

Storage of precipitated DNA in ethanol at -80 °C has advantages over -20 °C storage, but it also means that the DNA has to be re-isolated from ethanol and transferred to buffers prior to use (1). Therefore it is very common to store DNA samples in Tris/EDTA buffer at -20 °C. Due to multiple freeze-thaw cycles DNA shearing can be an issue with these conditions. Consequently, over time the DNA quantity and quality suffers from degradation and when the sample is actually needed, its recovery can be difficult or even impossible. If DNA recovery is reduced, re-measuring concentration becomes necessary which means additional time and consumption of valuable samples. Maximizing DNA recovery becomes even more important when a low starting amount of the sample is present, such as in FFPE, saliva, or crime scene samples.

One method to prevent loss of quality and quantity is to prepare multiple aliquots of the DNA sample to avoid multiple freeze-thaw cycles. This method is widely adopted but often leads to many tubes lying in the freezer in addition to the ever-increasing space requirement. Changing the vessel type used could be another option to improve long term storage of samples. Many commercially available micro tubes are often found to adsorb DNA molecules which lowers the quantity of the sample. Furthermore, DNA might be degraded by leachables deriving from plastics used, which lowers the quality of the DNA (3). Concluding, the ability to minimize sample storage requirement while protecting DNA from degradation and from loss of quantity is highly desired.

Solutions & Benefits

A solution to store DNA samples over a long time by preserving the quality and quantity of the sample is given by surfaces that show low affinity to DNA to guarantee maximum recovery. This can be achieved by different technologies. One is chemical treatment to create a surface where DNA molecules cannot bind. But these chemicals may leach into samples stored in common solutions and organic solvents. These chemicals can lead to degradation of the DNA or even PCR inhibition when the sample is used for a PCR set up. A more sophisticated option is to create a DNA low-binding surface by changing the molecular structure of the used plastic. This technology offers low-binding characteristics with the same purity as non-treated plastics to provide reliable recovery of DNA. Because of the absences of chemical treatments, DNA samples can be used for any application, even for sensitive ones like qPCR.
To demonstrate the advantages of LoBind plates in long term storage, qPCR experiments with libraries of low starting amounts of human genomic DNA have been performed. This sensitive application was used to compare the quality and quantity of a DNA sample stored in vessels with low-binding DNA surface such as the Eppendorf twin.tec PCR plates 96 LoBind (LoBind plate) and standard surface. Three DNA libraries from human genomic DNA (Promega®, Madison, USA) were created (Table 1) according to the standard "automated" library preparation protocol of GATC Biotech AG (Konstanz, Germany).

Table 1: Sample name and concentration of each human genomic DNA library used for real-time qPCR comparison prior to long-term storage. Each library was stored in both standard and LoBind plates.

Sample name	Concentration [ng/µl]	Fragment length [bp]
Library 1	6.19	473
Library 2	8.25	470
Library 3	4.31	509

The DNA libraries were aliquoted into eight separate standard plates and eight Eppendorf twin.tec PCR plates 96 LoBind. All plates went into storage at -20 °C and were thawed for qPCR analysis at different time points (Table 2).

Table 2: Plate name and respective time point of storage for measurement.

Plate name = time point (in weeks)	Plate no.
0	1
2	2
4	3
8	4
12	5
16	6
20	7
24	8

An initial qPCR performance test showed that a 1:100,000 dilution of the libraries was ideal for all following quantification. This sample was used as "start" sample and the DNA concentration measured was set to 100 % (Figure 1). All following samples were compared to the "start" sample. After initial measurements after 2 and 4 weeks, additional measurements were performed at 4 week intervals until a total of 24 weeks (5.5 months). At each time point a standard and LoBind plate were thawed and five replicates from each library were measured via qPCR. The mean of each plate was calculated to analyze the DNA recovery.
Already after two weeks of storage, differences in DNA recovery became evident. The percentage of DNA recovered from a standard plate drops down enormous already after 2 weeks of storage (Figure 1). After 16 weeks all three libraries in standard plates showed large variations leading to an error bar with 44.7 % deviation (Figure 1). In total the lowest recovery of the DNA dropped down to 16.3 %. In comparison, samples stored in LoBind plates show a minimum DNA recovery of 84.0 %.

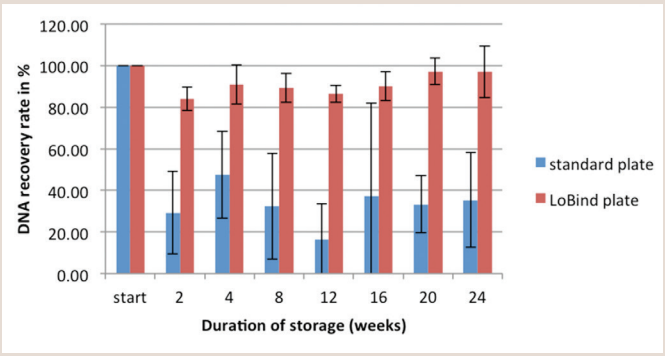

Figure 1: Percentage of DNA recovered in standard and LoBind plates after different time intervals of storage at -20 °C. Five replicates of each library were analyzed. The diagram shows the average DNA recovery in % of all three libraries.

Not only the high DNA recovery, but also the consistency was better in LoBind plates as the DNA recovery varied only between 84.0 – 97.2 % (Figure 1). Compared to that in standard plates the actual DNA recovery varies between 16.3 to 47.4 % indicating that the DNA recovery from standard plates is not reproducible. These huge variations indicated by the error bars can originate either from partial degradation over time or uncontrolled binding of DNA molecules to the surface.

The results of these experiments clearly show that using Eppendorf twin.tec PCR plates 96 LoBind for long term storage of DNA samples has advantages compared to standard plates. As one of the most sensitive tools, qPCR showed that the overall DNA recovery from the Eppendorf LoBind plates is higher and more consistent than DNA recovery from standard plates. This would give the peace of mind to store DNA samples for a longer period without suffering loss of quantity. Furthermore, routine procedure may be simplified because repeated quantification of the DNA is not necessary when LoBind consumables are used for storage. Precious and expensive samples can be reliably stored and maintain their quality for further experiments without the concern of degradation or adsorption. Using LoBind plates for the storage of your DNA samples facilitates your every day work. There is no need to buy liquid nitrogen tanks, a new freezer or perform additional working steps. What is easier than just using a different plate to be sure of keeping your sample stable and reliable?

In general, when using standard laboratory consumables for the storage of your DNA samples at -20 °C, you risk an insecure and irreproducible recovery of your sample, never knowing how much yield is still in the tube when the sample is needed. Furthermore, degradation issues may affect your DNA sample over long term storage leading to unusable DNA samples. In standard plates, the risk of complete sample loss is given. These extensive aftermaths can be avoided by simply using the right consumable, such as Eppendorf twin.tec PCR plates 96 LoBind for the storage of your DNA samples. You will receive a higher DNA recovery that is stable over at least 6 months storage at -20 °C. High-quality DNA offers you the choice to do your experiments whenever needed. Eppendorf twin.tec PCR plates 96 LoBind are the optimal choice for DNA storage and give you the peace of mind to work with stable, reliable samples.

Promotion

Your next step into reliable, stable and high DNA recoveries after long term storage at -20 °C is to try our Eppendorf twin.tec PCR plates 96 LoBind and see the benefits for yourself. Contact your local sales rep for more opportunities in DNA and Protein saving options.

> Or directly order your free sample at www.eppendorf.com/sample-order

References

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About Eppendorf

Eppendorf is a leading life science company that develops and sells instruments, consumables, and services for liquid-, sample-, and cell handling in laboratories worldwide. Its product range includes pipettes and automated pipetting systems, dispensers, centrifuges, mixers, spectrometers, and DNA amplification equipment as well as ultra-low temperature freezers, fermentors, bioreactors, CO_2 incubators, shakers, and cell manipulation systems. Associated consumables like pipette tips, test tubes, microtiter plates, and disposable bioreactors complement the instruments for highest quality workflow solutions. Eppendorf was founded in Hamburg, Germany in 1945 and has more than 3,000 employees worldwide. The company has subsidiaries in 25 countries and is represented in all other markets by distributors.

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