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Long-term storage of nucleic acids

Permanent storage below the temperature of recrystallization

White Paper

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Content:

1. Importance of the long-term storage of nucleic acids

Due to the development of different genetic methods for clarifying biological and medical problems it is becoming more and more important, to store nucleic acids in a long-term stable manner. If it is clear from the outset, that the designed biobank should clarify genetic issues exclusively, it makes sense for the sake of storage volume efficiency to extract the nucleic acids from the tissue or cells. The importance of the nucleic acid analyzes for both the biological and medical research increased recently due to new methods, such as Next Generation Sequencing or continuously improved quantitative RT-PCR[1]. Fixed or inappropriate cryopreserved material is only accessible to a very limited number of genetic methods. For this reason DNA banks are created worldwide to preserve nature historically valuable material or nucleic acids stemming from tumors to maintain these for later analysis or for future genetic approaches as effectively as possible. Especially for cancer patients an optimal storage of genetic material from tumor tissue for further prospective analysis can be advantageous and positively influence their course of therapy. Also in the field of forensics a high quality long-term storage of nucleic acids is of utmost importance.

2. Methods and Problems in long-term storage of nucleic acids

The currently most widely used method for the storage of nucleic acids is the storage of the material in a trehalose or β-mercaptoethanol stabilized solution at +4 to -80°C. Whereas the extent of degradation is significantly lower at lower temperatures as shown in Figure 1[2]. The figure also shows the storage of nucleic acids in the lyophilized state. This method is inappropriate for a long-term storage as well. There are data showing that even under these conditions a degradation of nucleic acids by depurination, hydrolysis and oxidation occurs despite of the very low water content[3]. As the used tubes and vials for storage are not completely sealed, humidity and oxygen penetrates and causes the degradation[4]. Furthermore the harmful activities of nucleases remain in these storage conditions[5]. The best way for storing nucleic acids is storage in the gas phase of liquid nitrogen. Due to a maximally

[1] Zetzsche, Dröge, und Gemeinholzer, „Die Etablierung eines DNA-Bank-Netzwerkes in Deutschland“; Mardis, „The impact of next-generation sequencing technology on genetics“.

[2] Smith und Morin, „Optimal storage conditions for highly dilute DNA samples“.

[3] Hofreiter u. a., „Ancient DNA“; Lindahl, „Instability and decay of the primary structure of DNA“.

[4] Bonnet u. a., „Chain and conformation stability of solid-state DNA“.

[5] Lee, Crouse, und Kline, „Optimizing storage and handling of DNA extracts“.

inhibited thermal motion of all the molecules in the solution, the shear forces acting on the relatively large nucleic acids are the lowest[6]. All storage methods in the temperature range from -20°C to -80°C cannot reach the long-term storage quality in the gas phase of liquid nitrogen[7]. There are no more recrystallization processes, which would cause migratory growth of large ice crystals[8]. In a permanent storage of the aqueous samples below the transition temperature from the crystalline to the vitreous phase (<-135°C), they are protected from any harmful influences[9]. The majority of DNA banks and institutes worldwide still use ultra-cold electric freezers, which offer in addition to the high temperatures significantly less protection for the valuable material during a power cut[10]. To ensure a constant cooling of the samples in case of failure, which often occurs in the mechanical elements of compressors, additional freezers are necessary as a backup system. A freezer for maintaining temperatures of around -80°C will heat up by around 20°C in 2 hours if it is not functioning properly. Furthermore the degradation of nucleic acids is accelerated due to frequent changes in temperature, occurring by sample manipulation in conventional systems[11]. A frequent access to the samples leads to icing of the storage system which progressively complicates the sample handling and necessitates a regular defrosting.

[6] Smith und Morin, „Optimal storage conditions for highly dilute DNA samples“; Crowe u. a., „Stabilization of Dry Mammalian Cells“; Sheldon, „Molecular collections for basic research“; Prendini, Hanner, und DeSalle, „Obtaining, storing and archiving specimens and tissue samples for use in molecular studies“.

[7] Baust, „Strategies for the Storage of DNA“.

[8] Schmitz, Der Experimentator; Zhmakin, Fundamentals of Cryobiology.

[9] Baust, „Strategies for the Storage of DNA“.

[10] Hanner, Corthals, und Dessauer, „Salvage of genetically valuable tissues following a freezer failure“.

[11] Zetzsche, Dröge, und Gemeinholzer, „Die Etablierung eines DNA-Bank-Netzwerkes in Deutschland“.

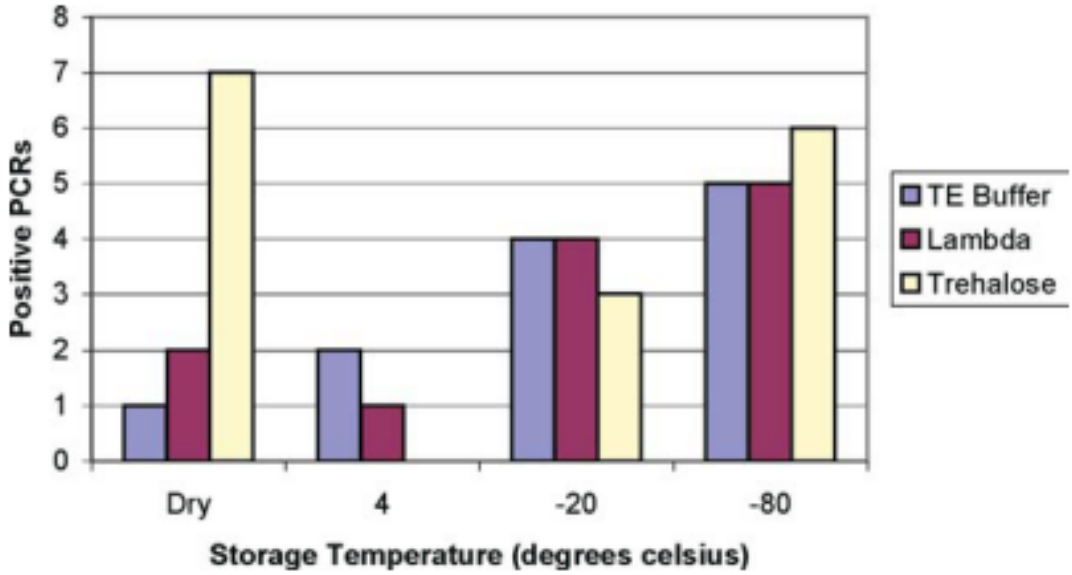

Figure 1: PCR success for a 747 bp fragment of the 18s ribosomal gene amplified from gorilla fecal DNA stored for 12 months. Data represent number of successful PCRs from all 4 individual extracts in duplicate for each treatment.[12]

3. The advantages of using the Askion C-line® system for the storage of nucleic acids

Storage of nucleic acids in the gas phase of liquid nitrogen is the accepted method, which ensures the integrity of the material and thus its methodical use most suitably. The temperature in the storage container is safely below -150°C and thus below the transition temperature of aqueous solutions from the vitreous to the crystalline phase. In addition, there are all the benefits of storage in the storage tank of the HS 200 as minimizing of icing, temperature stability during sample handling and the long interim period in the case of a power cut of at least 4 days. In addition, the C-line® system provides the option of sample tracking and recording of all temperatures to which the sample was exposed throughout their storage period. Due to the optional automation everyday procedures can be simplified

[12] Smith und Morin, „Optimal storage conditions for highly dilute DNA samples“.

even in case of high sample throughput. Furthermore, prior to the examination of the aliquots it is possible to identify inappropriate aliquots with the recorded temperature data and thus their cost and time-consuming analysis can be saved. The running costs (electric energy and liquid nitrogen) for this system are in a comparable range such as the energy costs for an electrically powered freezer for maintaining temperatures of -80°C. It is not yet included that the waste heat of electrical freezers must be compensated by an air conditioning system, which causes additional costs. Additionally the noise pollution of the C-line® system is substantially less than that produced by the compressors of electrical freezers. The C-line® system meets all stated needs of long-term stable storage in the most comprehensive manner basing on the current knowledge.

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