A80 Comparison DNA Preservation Buffers for Low Quantity DNA 4°C Storage

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After attending this presentation, attendees will have a better understanding of the DNA preservation capability of several commonly used buffers for DNA storage at 4°C.

This presentation will impact the forensic science community by assisting other laboratories in selecting the optimal DNA preservation buffer for short term storage at 4°C.

The amount of DNA recovered from some forensic evidence samples such as those from handled items is usually very low. Following extraction, DNA samples are typically stored at 4°C for a few days and sometimes a few weeks until completion of the testing process, and then transferred to a -80°C freezer for long term storage. Protecting DNA from potential loss or degradation during this period is critically important in order to obtain as much information as possible for genetic profiling. For this reason, it is necessary to evaluate the DNA preservation performance of buffers commonly used for 4°C wet storage.

Historically, forensic DNA has been suspended or eluted in water, 0.1xTE or 1xTE with or without additional reagents. For this study, two reagents, trehalose and fish sperm DNA, were considered. Trehalose is considered a very effective DNA protection agent in the dry condition. Fish sperm DNA is sometimes used as carrier DNA to enhance DNA recovery from a Microcon™ purification column. The following ten different DNA buffers were evaluated: water, 0.1xTE, 1xTE; 15ng/µl fish sperm DNA in water, 0.1xTE or 1xTE; 10% trehalose in water, 0.1xTE, or 1xTE, and 15ng/µl fish sperm DNA in 10% trehalose with 0.1xTE. Human blood was extracted with the Qiagen MagAttract kit and diluted with each of the ten different buffers to a final concentration of 10pg/µl or less. Six replicates for each condition for six different time points were stored for up to 15 weeks at 4°C. Six replicates of DNA stored in 0.1xTE buffer alone were also stored at -80°C for each of six time points as a control. For each time point, DNA was measured with an in-house Alu based qPCR system.

Results from this study show a significant difference in the amount of DNA recovered from the various buffers used. Un-buffered DNA in water degraded quickly; however, the addition of fish sperm DNA increased the yield. Fish sperm also enhanced the performance of 0.1X TE buffer alone. Similarly, 10% trehalose was not effective when dissolved in water, but DNA was preserved better when trehalose was dissolved in 0.1xTE and 1xTE. With both fish sperm DNA and trehalose, 0.1xTE outperformed 1xTE. In order to investigate the difference between 0.1xTE and 1xTE, the affect of extra EDTA on qPCR was explored. Although qPCR was slightly inhibited with 1xTE, this effect cannot account for the dramatic difference between the two stored buffers.

Interestingly, there was no significant difference among the top performing buffers, 0.1xTE with 10% trehalose and/or 15ng/µl fish sperm DNA. More DNA was recovered from all of these buffers than from the controls stored in 0.1xTE alone at -80°C. This study proved that DNA preservation buffers have significant impact on the DNA recovery of low amounts of DNA stored at 4°C for even only a few weeks. Since it is critical to select optimal DNA preservation buffers to ensure the robust downstream genotyping, additional studies are in progress to examine the effect of these buffers on long term storage in both the wet and
dry conditions.

DNA Preservation, Trehalose, TE

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