Maximize recovery of quality nucleic acid from formalin-fixed paraffin-embedded tissue samples using a novel, flexible purification technology.

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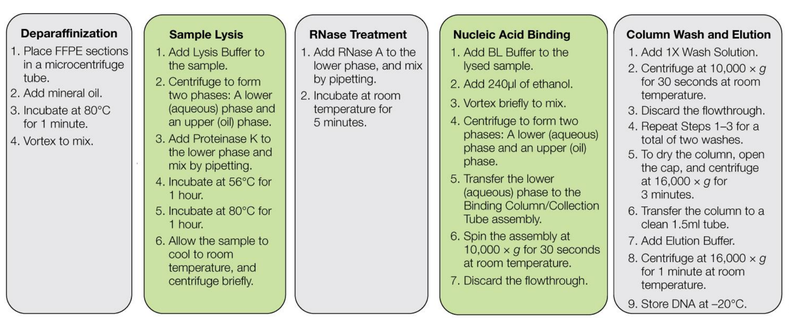
Abstract #4738

1. The ReliaPrep™ FFPE gDNA Miniprep System

Formalin-fixed paraffin-embedded (FFPE) tissue samples are a valuable source of genetic information for gene expression and clinical research. Extraction of nucleic acid (NA) from FFPE tissues is a challenge because the fixation process results in cross-linking between proteins and nucleic acid, as well as between different strands of DNA or RNA molecules. Recent advances in sample preparation enables access to the valuable information contained within these difficult samples.

The ReliaPrep™ FFPE gDNA Miniprep System uses a novel purification technology that does not rely on use of any harsh organic solvents and concentrates the NA for low volume elution on a specially designed spin column, thus maximizing concentration. Using this technology, we examined several aspects of the purification protocol for flexibility and adaptability: effect of decrosslinking time on the quality and length of nucleic acids released from diverse FFPE tissues, effect of overnight storage between lysis and extraction steps, and whether the technology could be adapted to extract both DNA and RNA from the same FFPE tissue sample.

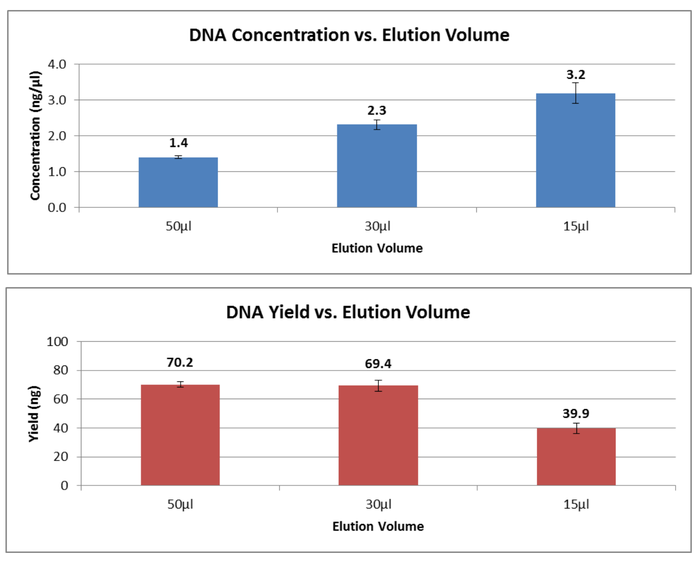
2. Purification without the Use of Organic Solvents



The ReliaPrep™ system includes nontoxic mineral oil to safely and effectively deparaffinize FFPE sections and in a shorter period of time than organics. ReliaPrep™ features optimized lysis conditions designed to reverse the modifications introduced by formalin fixation, without the need for overnight digestion.

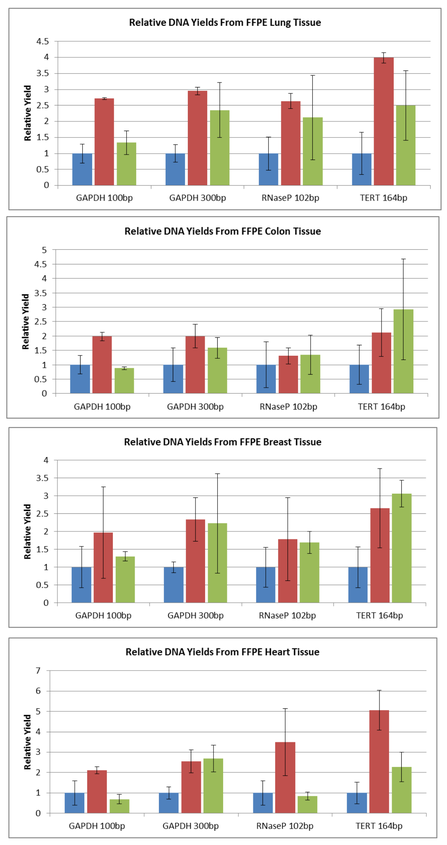
3. Low Elution Volume to Maximize Concentration

As most FFPE samples are unique and often have limited amounts of tissue, it may be important to maximize DNA concentration without sacrificing DNA yield. We examined the effects of varying the elution volume on DNA concentration and yield. DNA was purified from lung FFPE tissue (N=3) and quantitated using the QuantiFluor® ONE dsDNA System on the Quantus™ fluorometer.



DNA purified with the ReliaPrep™ system can be eluted in 30µl – 50ul without a loss in overall yield. The design of the column allows for elution in as little as 15µl, providing a concentrated DNA sample for use in downstream applications where concentration may be critical to success.

4. Longer Decrosslinking Times Maximize Amplifiable Yields

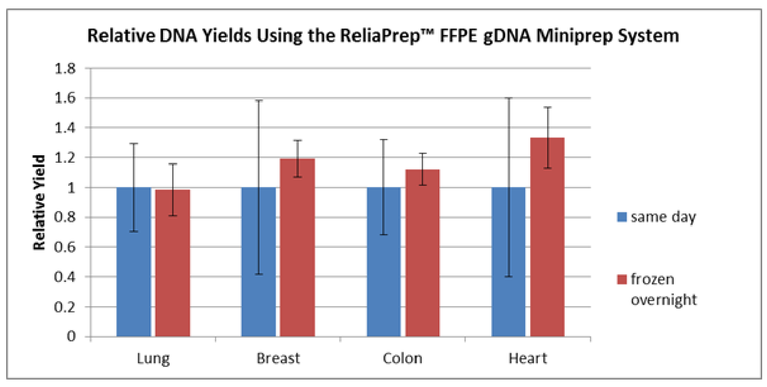


Relative DNA recoveries from FFPE lung, breast, colon, and heart tissues using three purification methods as determined by qPCR with each of four primer sets.

Increasing the decrosslinking time for the ReliaPrep™ FFPE gDNA Miniprep System from 1 hour to 4 hours resulted in increased DNA recovery based on the amount of amplifiable DNA.

5. Flexible Protocol for Convenient Sample Processing

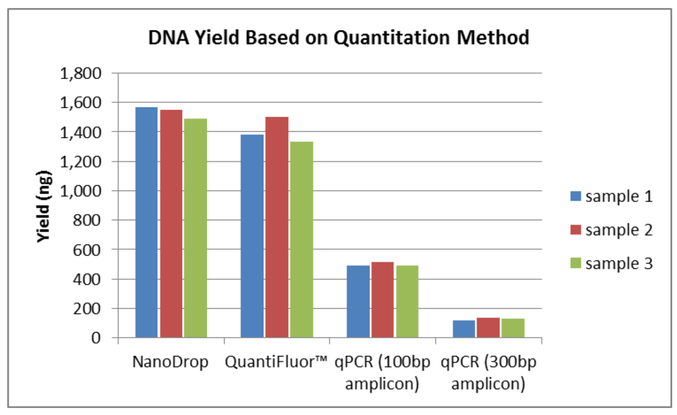
Following pre-processing (deparaffinization, tissue lysis, and RNase treatment), BL buffer and ethanol were added to the samples and stored overnight at -20°C. The following day, the remaining purification steps (nucleic acid binding, column wash, and elution) were completed.



No significant difference in DNA recoveries was observed between same day processing and next day processing following overnight storage of samples at -20°C. The ability to pause between sample pre-processing and DNA purification allows for the flexibility to perform the protocol at times that are most convenient for the researcher.

6. Amplification-Based Quantitation Is the Best Indicator of Nucleic Acid Quality

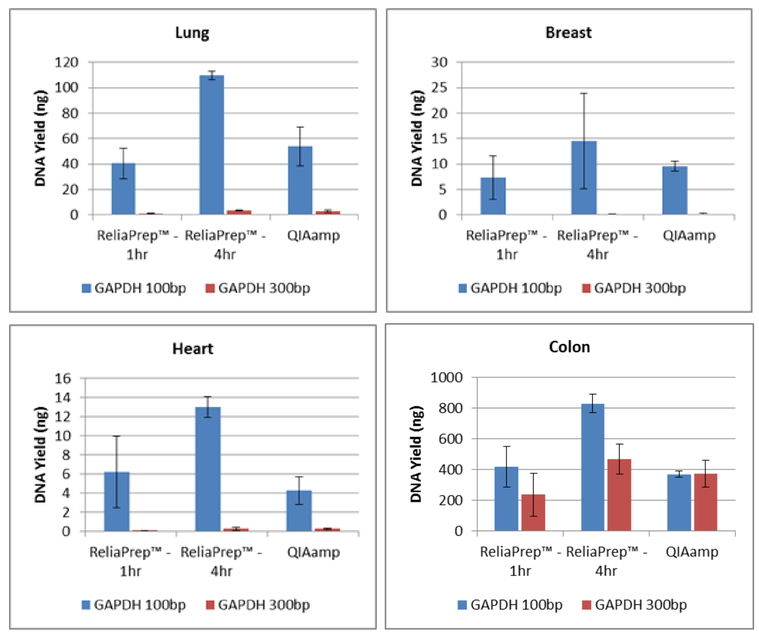
DNA was isolated from human colon FFPE tissues using the ReliaPrep™ FFPE gDNA Miniprep System. DNA yields were determined using a NanoDrop-1000 spectrometer, the QuantiFluor® dsDNA dye on the Quantus™ fluorometer, and by qPCR using GAPDH primer sets for 100bp and 300bp amplicons.



Since DNA from FFPE samples are generally highly degraded, qPCR provides a more accurate method of quantitating amplifiable or functional DNA.

7. Primer Design for qPCR Crucial for Quantitation

We examined the effects of amplicon size for the determination of DNA yields by qPCR from the various FFPE tissues using the GAPDH primer sets for 100bp and 300bp amplicons.



A significant decrease in DNA yield as determined by qPCR is observed when amplifying the 300bp GAPDH target when compared to the 100bp GAPDH target, demonstrating the effect of primer design specifically for FFPE tissue DNA samples. A 15 to 100-fold difference in amplifiable DNA is observed with lung, heart, and breast samples.

8. DNA and RNA from the Same FFPE Tissue Sample

In order to extract both RNA and DNA from a single FFPE tissue section, 200µl of lysis buffer and 20µl of Proteinase K were used to create the lysates. Lysates were split into ~100µl each, and 120µl of lysis buffer was added to adjust the volume for the subsequent RNase or DNase steps.

FFPE Tissue Sample	DNA (ng) 100bp	DNA (ng) 300bp	RNA* (ng)
Colon 1	489.5	119.0	9.5
Colon 2	514.2	135.8	9.2
Colon 3	493.2	129.5	10.5
Average	**499.0**	**128.1**	**9.7**
SD	13.3	8.5	0.7

The GoTaq® qPCR Master Mix (A6001) with DNA-specific GAPDH primers and the GoTaq® Probe 1-Step RT-qPCR System (A6120) with RNA-specific B2M primers were used for quantitation. *RNA was purified using the ReliaPrep™ FFPE Total RNA Miniprep System.

9. Summary

- The ReliaPrep™ FFPE gDNA Miniprep System has many unique features that can overcome the challenges of DNA purification from FFPE sample in a safe, quick and highly effective manner.
- Increasing decrosslinking time resulted in increased DNA recovery.
- No difference in DNA recoveries was observed between same-day processing and next-day processing following overnight storage of preprocessed samples, highlighting the flexibility of the streamlined protocol.
- The importance of quantitation by amplification as well as primer design was demonstrated, as differences in yield were observed with different quantitation methods and when amplicons of different sizes were amplified.
- DNA and RNA was able to be purified from a single FFPE tissue sample.