

Protocol Development for Yielding High Quality DNA and RNA from Archived Formalin-Fixed Paraffin Embedded Tissues

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Introduction: While genetic analysis of archived formalin-fixed paraffin embedded (FFPE) tissue specimens would be a significant research resource, extraction of high-quality DNA and RNA from these specimens is a significant challenge. Storage duration, tissue handling and tissue preservation processes as well as their interactions with the nucleic acids could influence the quality and integrity of the DNA or RNA required for genetic analysis.

Objectives: The goal of this study was to establish a protocol to extract high quality DNA and RNA from the FFPE tissues for further use in quantitative PCR analysis.

Methods: For the purposes of this study, human FFPE biopsy tissues from lung, liver, colon and kidney were obtained from a single center biorepository. GeneJET Genomic DNA Purification Kit (Catalog # K0722) obtained from Thermo Fisher Scientific and RecoverAll Total Nucleic Acid Isolation Kit obtained from Life Technologies were modified and used for extraction of DNA and RNA, respectively. Briefly, modifications involved extending reagent incubation times, increasing sample volumes and wash steps, and increased final nucleic acids recovery and concentration steps. Eight sections around 8-10 μm thick were microtomed for each tissue sample and used for extraction. The purity of the nucleic acids obtained was verified using Nanodrop Spectrophotometer.

Results: The average DNA yield from eight sections for each of the tissues was 270 ± 184 ng/ μl and for RNA was 296 ± 188 ng/ μl . Nucleic acid quality was assessed by measuring the 260nm/280nm absorbance ratio for protein contamination as well as the 260nm/230nm absorbance ratio for salt contamination. Both were found to be within acceptable ranges. RNA was reverse transcribed to cDNA and qPCR was successfully performed on both DNA and cDNA samples.

Conclusion: These results indicate that protocols using the silica-based membrane technology can yield high quality DNA and RNA that can be successfully used for downstream genetic analysis.