

# A comparison of methods for retrieval of DNA and RNA from FFPE tissues

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## Summary

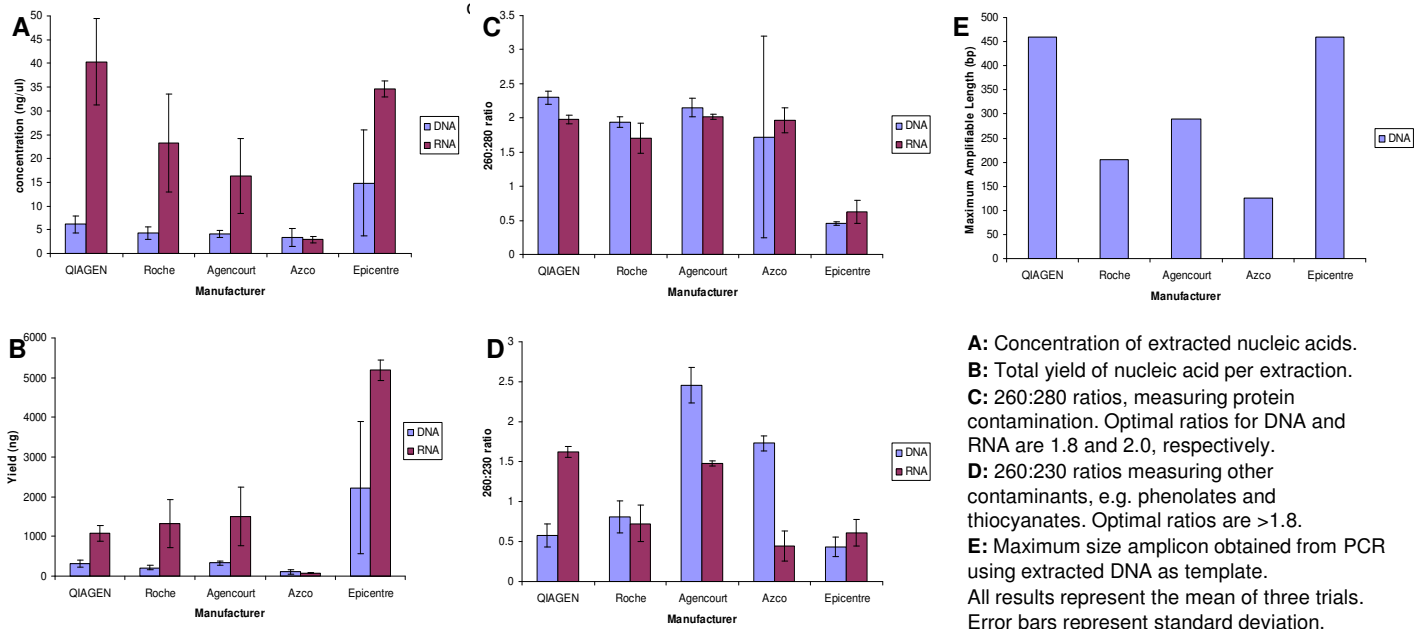
A variety of commercially available kits designed for the purpose of extracting DNA/RNA from formalin-fixed, paraffin-embedded (FFPE) samples were tested under controlled conditions. The kits were selected based on the method of extraction: Column-based, lysis only, solid-phase reverse binding, and magnetic based extraction. Kits were compared on the basis of resulting nucleic acid concentration, yield, protein and chemical contamination, and maximum amplifiable fragment length. The results of this study indicate that each type of kit has distinct advantages and a careful consideration should be given to ultimate experimental requirements prior to purchasing the kit.

## Introduction

Formalin-fixation followed by paraffin-embedding has been the paradigm for preserving tissue samples for histological analysis for over a century. More recently, strategies have been developed to extract the nucleic acids contained within FFPE samples, and the large archives of these samples provides an extremely valuable resource for molecular biological research. However, RNA and DNA from these tissues is of much lower quality than that obtained from fresh frozen samples<sup>1</sup>. Factors having a profound effect on the quality of the nucleic acid preparation include fixation time and conditions, and age of the FFPE sample. Although there is little that can be done to compensate for the inevitable reduced quality of aged FFPE samples, methods have been devised to improve the quality of the final nucleic acid prep<sup>2</sup>. Usually, archived tissue is first deparaffinised by use of xylene or other solvent. Next, incubation with proteinase K at elevated temperature reduces and reverses protein:DNA and DNA:DNA cross-links and digests the tissue. Extraction of nucleic acid can then generally proceed as with standard non-FFPE samples. A number of commercial kits are now available for the extraction of DNA/RNA from FFPE tissues, utilising varying technologies. Our aim was to identify the advantages and short-comings of some of these technologies using a standardised tissue sample model, created by fixation and embedding of cultured cells.

## Materials and methods

**Generation of FFPE blocks:** FFPE material was created by embedding cultured MDA-MB-231 cells in agarose plugs, followed by 16 hr fixation in 10% neutral buffered formalin prior to processing. **DNA/RNA extraction:** Sequential 5 µm sections were cut using a clean microtome blade and 2 sections were used per extraction. Commercial kits used for extraction of DNA were QIAamp (QIAGEN), QuickExtract DNA Extraction Solution (Epicentre Biotechnologies) and Just-a-Plate DNA (Azco). Kits for RNA extraction were RNeasy FFPE (QIAGEN), QuickExtract RNA Extraction Solution (Epicentre Biotechnologies) and Just-a-Plate RNA (Azco). Kits used for simultaneous extraction of DNA and RNA were High Pure (Roche) and Formapure (Agencourt). The QIAGEN and Roche kits are all column based. The Epicentre kits are lysis only, no purification. The Azco kits are 96-well plate based and utilise solid-phase reversible binding technology. The Agencourt kit utilises magnetic bead-based DNA reversible binding technology. All extractions were carried out according to manufacturer's instructions in triplicate. Results below represent the average of these repeats for each kit. **Nucleic acid quantification:** All DNA and RNA samples were quantified fluorometrically using the Quant-iT™ assay on a Qubit™ Quantitation Platform (Invitrogen). **Contamination detection:** Protein and chemical contamination was determined by obtaining the 260:280 and 260:230 ratios for each DNA and RNA sample (NanoDrop - Thermo Scientific). **Maximum Amplifiable Fragment Length:** Primer pairs were designed complementary to the human GAPDH housekeeping gene. Primer pairs were designed specifically to amplify fragments of increasing length: 125, 204 and 460bp. PCRs were performed on DNA samples (50 ng) using HotStarTaq DNA polymerase (QIAGEN). Products were



## Conclusions

- The two column-based technologies (QIAGEN and Roche) provided good concentrations and yields of nucleic acid with low protein contamination. However, as indicated by the sub-optimal 260:230 ratios, the samples were contaminated with other chemicals, presumably from carry-over during wash steps.
- The results of the magnetic bead based kit (Agencourt) are comparable to those of the column-based kits, but with improved contamination removal and reduced maximum amplifiable fragment length. Whether purity or fragment length is most important, would help determine which kit type is preferable.
- The plate-based solid-phase reversible binding kit (Azco) offered the lowest yield and concentration of nucleic acids, shortest fragments and variable levels of protein and chemical contamination. One advantage offered by this kit is that many samples can be processed simultaneously, offering a good solution in situations where high-throughput is important, but yield and quality less so.
- The kit utilising lysis but no nucleic acid purification (Epicentre) offered large quantities of amplifiable but contaminated nucleic acids. This kit is highly suitable for very rapid and cheap extractions, where downstream applications do not require pure nucleic acids.
- These results demonstrate that that no single kit outperforms all other kits; the choice of kit should be heavily based on the ultimate application.

## References

- Scicchitano MS, Dalmas DA, Bertiaux MA, Anderson SM, Turner LR, Thomas RA, Mirable R, Boyce RW., Preliminary comparison of quantity, quality, and microarray performance of RNA extracted from formalin-fixed, paraffin-embedded, and unfixed frozen tissue samples, *J Histochem Cytochem.* 2006 Nov;54(11):1229-37.
- Mark Abramowitz, Maja Ordanic-Kodani, Yuefang Wang, Zhenhong Li, Charles Catzavelos, Mark Bouzyk, George W. Sledge Jr., Carlos S. Moreno, and Brian Leyland-Jones. Optimization of RNA extraction from FFPE tissues for expression profiling in the DASL assay, *BioTechniques* 44:417-423 (March 2008)