Simultaneous Isolation of RNA and DNA from One FFPE Sample

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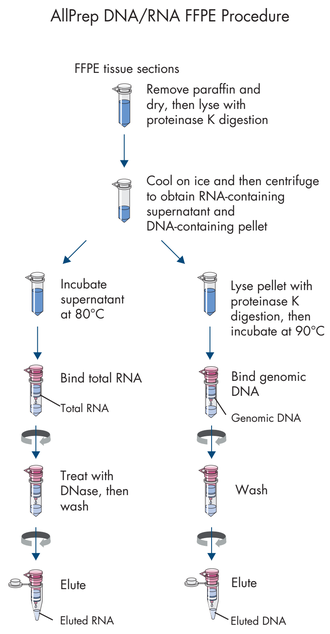
Introduction

Worldwide, there are millions of tissue samples archived in tissue biobanks and biorepositories. These samples are extremely valuable for pharmacological and biomedical research and companion diagnostics, due to the linkage to patient history. The vast majority of archived tissue samples are formalin-fixed and paraffin-embedded (FFPE), since formalin is the standard fixative for tissue samples.

FFPE blocks serve as an excellent source for histomorphology studies, but their use in molecular studies is challenging, due to crosslinking and fragmentation caused by fixation, processing, embedding, and storage conditions.

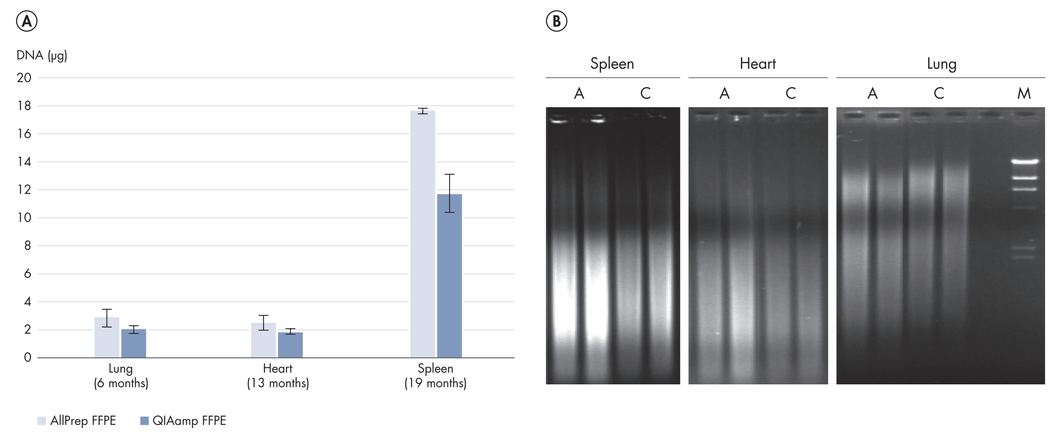
For reliable comparison of genomic and transcriptomic data from heterogeneous samples and to spare sample material, purification of DNA and RNA from the same sample is essential. This is particularly important when working with tumorous tissues, which contain a heterogeneous distribution of healthy and malignant cells.

The AllPrep® DNA/RNA FFPE Kit is designed to simultaneously purifiy genomic DNA and total RNA from FFPE tissue sections. FFPE samples are incubated in an optimized lysis buffer, resulting in the release of RNA and precipitation of DNA. After centrifugation, the RNA-containing supernatant and DNA-containing pellet are processed separately to purify RNA and DNA.



Genomic DNA: Yield and Fragment Length

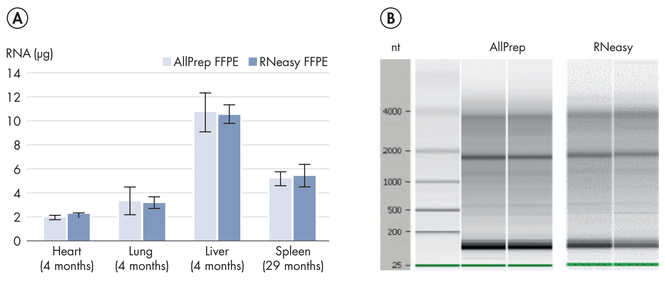
Since FFPE samples contain DNA molecules that are crosslinked to each other, as well as to RNA and protein molecules, breakage of these crosslinks is necessary in order to release DNA for subsequent purification. After differential solubilization, RNA is removed with the supernatant and DNA remains in an insoluble pellet, which is then further lysed. Chemical modifications due to crosslinking are reversed by subsequent incubation, as chemically modified DNA is less efficiently recovered during the purification procedure and represents a poor substrate for PCR and other enzymatic assays.



Genomic DNA purified from various FFPE rat tissues stored at room temperature for the times indicated. Purification was performed using either the AllPrep DNA/RNA FFPE Kit or, as a control, the QIAamp® FFPE Tissue Kit including RNAse digestion during sample preparation. Ⓐ DNA yields from 20 µm sections were determined by OD measurement. Ⓑ Agarose gel analysis of the same volume of eluates. **A**: AllPrep DNA/RNA FFPE Kit; **C**: Control; QIAamp FFPE Tissue Kit. **M**: Lambda *Hin*d III marker.

RNA: Yield and Integrity

During differential solubilization, RNA is released and subjected to reverse crosslinking using optimized conditions that avoid additional fragmentation. This yields high-quality RNA, whilst maintaining RNA integrity for demanding downstream applications.

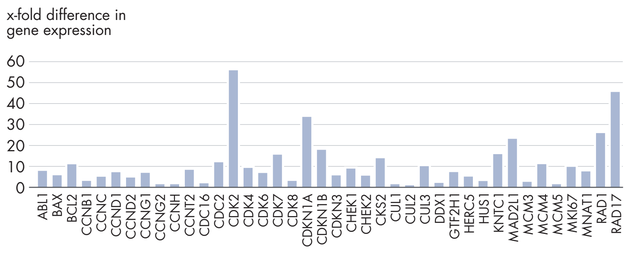


Total RNA was purified from various rat FFPE tissues, stored at room temperature for the durations shown, using either the AllPrep DNA/RNA FFPE Kit or the miRNeasy FFPE Kit. nt: Nucleotide.

Ⓐ RNA yield from one (spleen, liver) or two 10 µm sections (heart, lung) per sample determined by OD measurement.

Ⓑ The same volume of RNA purified from one 10 µm section of rat kidney was analyzed on an Agilent® 2100 Bioanalyzer.

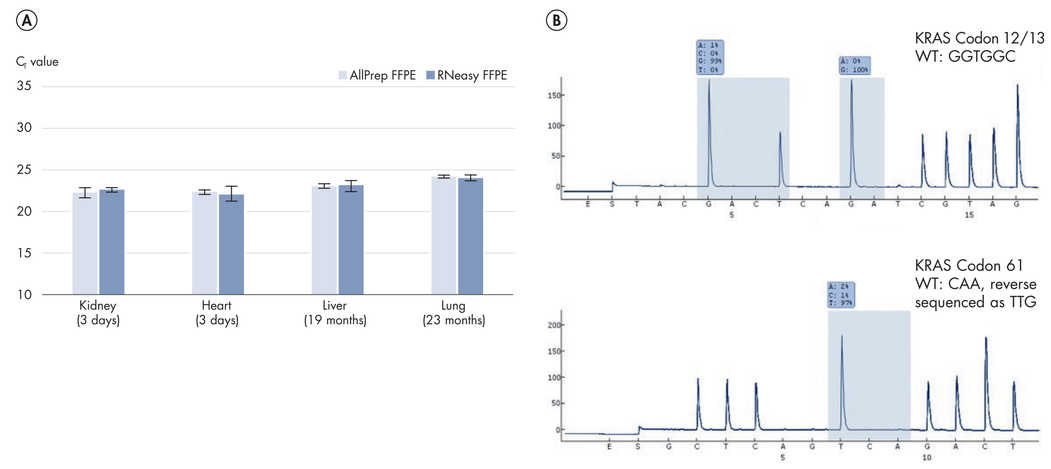
RNA: RT²™ FFPE PreAmp technology



Total RNA was purified from human breast FFPE tissue using the AllPrep DNA/RNA FFPE Kit. RNA was reverse-transcribed using RT² FFPE PreAMP technology. Gene expression analysis by real-time PCR was performed using the Human Cell Cycle RT² Profiler™ PCR Array, comparing a tumor and non-tumor sample. $\Delta\Delta C_T$ analysis shows the x-fold difference in gene expression of the tumor sample compared with the non-tumor sample.

Genomic DNA: PCR Analysis and Pyrosequencing®

The AllPrep DNA/RNA FFPE Kit yields DNA suitable as template for downstream applications, such as real-time PCR or Pyrosequencing. Pyrosequencing is a technology that allows reliable sequencing of nucleotide sequences, plus sensitive, accurate quantification of genetic variations within the sequences of interest, such as methylation at CpG sites or mutations.

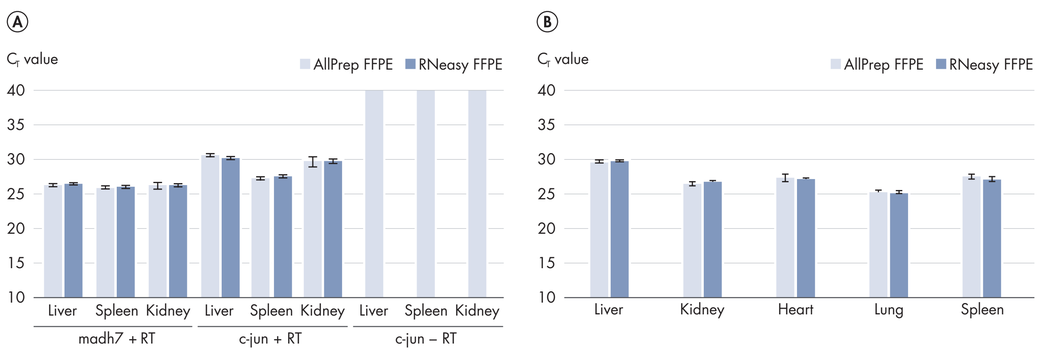


Ⓐ DNA was purified from various FFPE rat tissues stored at room temperature, as indicated, using either the AllPrep DNA/RNA FFPE Kit or, as a control, the QIAamp FFPE Tissue Kit. Real-time PCR was carried out using the QuantiTect® SYBR® Green PCR Kit to analyze a 78 bp amplicon of the Prnp gene.

Ⓑ DNA was purified from breast cancer FFPE tissue using the AllPrep DNA/RNA FFPE Tissue Kit. Pyrosequencing to identify mutation of the KRAS gene was performed using the *therascreen*® KRAS Pyro Kit.

RNA: Real-Time RT-PCR

Genomic DNA contamination in an RNA sample affects the accuracy of gene expression analysis by real-time RT-PCR if the primers used amplify both cDNA and gDNA sequences. Therefore, elimination of gDNA contamination is essential for accurate results. This can be achieved by purifying RNA using the AllPrep DNA/RNA FFPE Kit. Depending on the RNA binding conditions, small RNAs (such as microRNA) are either present or absent in the purified RNA.



Ⓐ Total RNA was purified from various rat FFPE tissue samples using the AllPrep DNA/RNA FFPE Kit or RNeasy® FFPE Kit. Real-time RT-PCR assays for madh7 and cjun each from 30 ng RNA were performed with (+RT) or without (–RT) reverse transcriptase. The –RT samples show that RNA purified using the AllPrep DNA/RNA FFPE Kit is virtually free of gDNA.

Ⓑ Total RNA (including miRNA) was purified from one (liver, kidney, spleen) or two 10 µm sections (heart, lung) of various rat FFPE tissue samples using the AllPrep DNA/RNA FFPE Kit or miRNeasy FFPE Kit. The same amount of purified RNA from each sample was used as template in quantitative, real-time RT-PCR assay for miRNA miR-16.

Conclusions

The AllPrep DNA/RNA FFPE Kit provides:

- Simultaneous purification of RNA and genomic DNA from one FFPE tissue sample
- Separate eluates for DNA and RNA
- High yields of DNA and RNA from every sample without dividing the sample or lysates
- Efficient removal of crosslinks for each nucleic acid
- Purification of RNA including miRNA
- RNA eluates virtually free of genomic DNA due to efficient gDNA removal

For up-to-date licensing information and product-specific disclaimers, see the respective QIAGEN kit handbook or user manual. QIAGEN kit handbooks and user manuals are available at **www.qiagen.com** or can be requested from QIAGEN Technical Services or your local distributor.

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