

# ReliaPrep™ Miniprep Systems - Nucleic Acid Purification from FFPE Tissues

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## Abstract

Formalin fixation and paraffin embedding (FFPE) is a commonly used method for archival of pathological specimens. The ability to extract high-quality DNA and RNA from these samples provides the potential for correlating disease state and tissue morphology with genotype and gene expression. The ReliaPrep™ FFPE Miniprep Systems from Promega offer rapid purification of high quality genomic DNA, total RNA, and total nucleic acid without the use of organic solvents.

## Background

Purification of high quality nucleic acids from formalin-fixed and paraffin embedded tissues has, historically, been a difficult task. Formalin fixation of tissues can cause nucleic acid damage and introduce modifications that make downstream analysis difficult. These issues, coupled with the preciousness of the samples and the small amount of nucleic acids traditionally purified from FFPE samples have made purification of high-quality nucleic acids in sufficient amounts the limiting factor in analyzing these archival tissue types.

We describe two new ReliaPrep™ FFPE Miniprep Systems for extraction of nucleic acids from FFPE tissues that allow scientists to purify nucleic acids from FFPE samples **without** the use of organic solvents.

- ReliaPrep™ FFPE gDNA Miniprep System
- ReliaPrep™ FFPE Total RNA Miniprep System

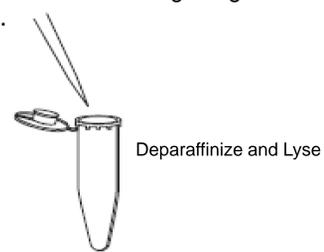
Both kits feature optimized lysis conditions designed to reverse modifications introduced by formalin fixation without the need for overnight digestion. Purified nucleic acids can be used in a number of downstream applications including:

- PCR/qPCR
- Cloning
- Microarray Analysis
- Bisulfite Conversion
- Sequencing

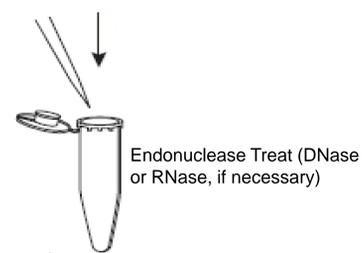
## Protocol

The ReliaPrep™ FFPE Miniprep Systems deliver quality nucleic acids from FFPE tissues with minimal hands-on time, without the need for overnight digests or harsh organic compounds.

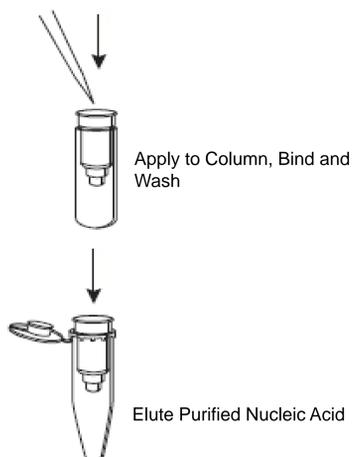
**Deparaffinize without organic compounds**



**Minutes of hands-on time**

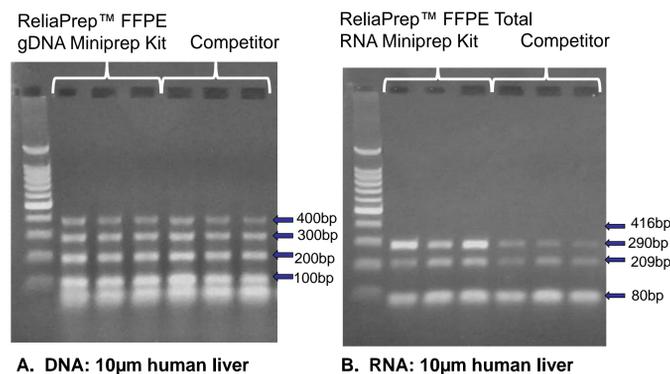


**Total protocol time of less than 2 ½ hours**



## Assessing Nucleic Acid Fragmentation by Multiplex PCR

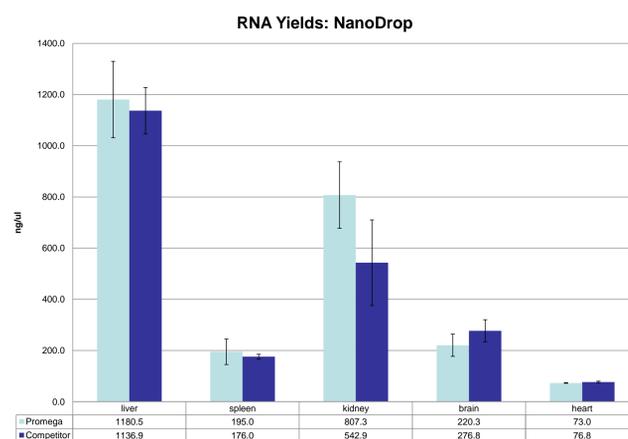
The degree of nucleic acid fragmentation following purification can have profound effects on downstream analyses. The degree of nucleic acid fragmentation from FFPE tissue was assessed using multiplex PCR, comparing the ReliaPrep™ FFPE Miniprep Systems and a leading competitor. The ReliaPrep™ FFPE Miniprep Systems showed equivalent gDNA integrity and superior RNA when compared to the competitor.



- A. Comparison of purified DNA integrity from ReliaPrep™ FFPE gDNA Miniprep System and an industry competitor by multiplex PCR of variable length fragments of GAPDH gene from 10µm slices of FFPE human liver. 10µl of purified DNA was used as template for the PCR (GoTaq® Hot Start Polymerase, Cat.# M5001).
- B. Comparison of purified RNA integrity from ReliaPrep™ FFPE Total RNA Miniprep System and an industry competitor by multiplex end-point PCR of variable length fragments of GAPDH gene from 10µm slices of FFPE human liver. Purified RNA (5µl) was used as template for reverse transcription (GoScript™ Reverse Transcription System, Cat.# A5001) for a total reaction volume of 20µl. cDNA (5µl) was used as template for the Multiplex PCR for a total reaction volume of 50µl (GoTaq® Hot Start Polymerase, Cat.# M5001).

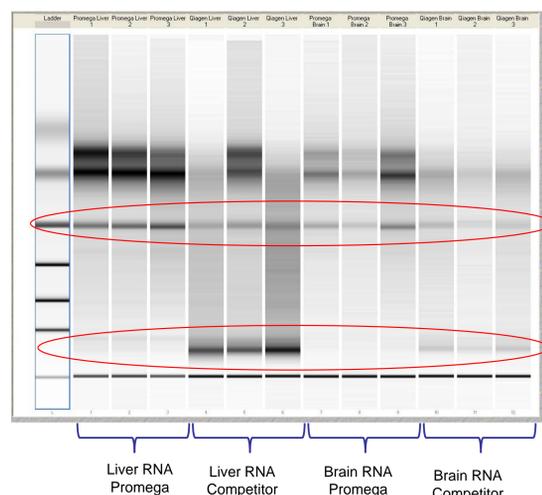
For each experiment, 10µl of the PCR product was analyzed by electrophoresis on a 2.0% agarose gel.

## Purify RNA From a Variety of Tissues



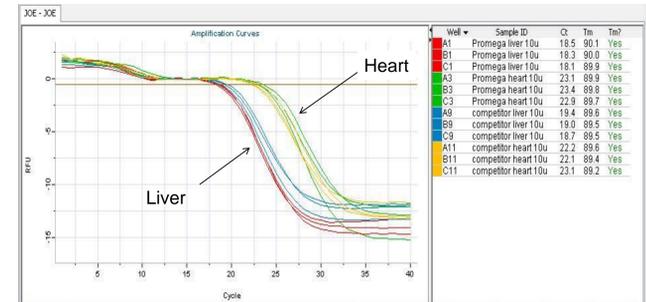
Total RNA was purified from 50µm mouse tissue sections using either the ReliaPrep™ FFPE Total RNA Miniprep kit or a competitor. The ReliaPrep™ System purified amounts of RNA comparable to the competitor across all tissue types.

## Purify More Intact RNA



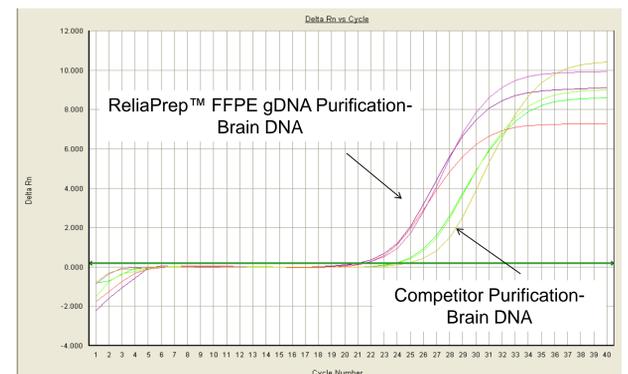
Total RNA purified from 10µm mouse liver and brain FFPE sections was analyzed on an Agilent Bioanalyzer. More small-fragment nucleic acid was purified with the competitor kit, indicating increased degradation in the sample. Higher molecular weight product was purified with the ReliaPrep™ Total RNA FFPE kit.

## Detect RNA From Various Tissues using RT-qPCR



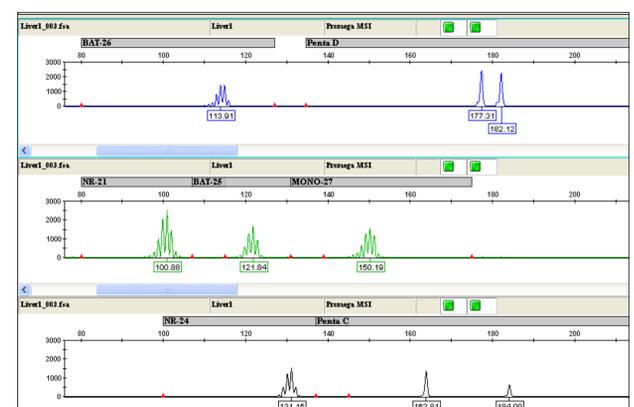
Total RNA was purified from single 10µm mouse liver and heart sections using the ReliaPrep™ FFPE Total RNA Miniprep System and a competitor kit. cDNA was generated using the GoScript™ Reverse Transcription System (Cat.# A5001) and mouse beta-Actin gene product was detected using Plexor® System (Cat.# A4021). RNA was detected in both liver and heart tissues.

## Detect gDNA Sooner



Genomic DNA was purified from a single 10µm mouse brain FFPE sections using either the ReliaPrep™ FFPE gDNA Miniprep System or a competitor kit and analyzed using GoTaq® qPCR System (Cat.# A6001). Earlier Ct values were observed with Promega's ReliaPrep™ FFPE gDNA Miniprep System than with the competitor product. Comparable results were achieved with Plexor®, TaqMan®, SYBR chemistries (data not shown).

## Detect Microsatellite Instability



Genomic DNA was purified from 10µm human liver FFPE sections using the ReliaPrep™ FFPE gDNA Miniprep System. STR analysis was performed using Promega's Microsatellite Instability (MSI) Analysis System v. 1.2 (Cat.# MD1641). This system is compatible with STR amplification and MSI detection.

## Summary

- The ReliaPrep™ FFPE Systems effectively purify amplifiable nucleic acid from FFPE tissues.
- The ReliaPrep™ FFPE Systems feature optimized lysis conditions designed to reverse modifications introduced by formalin fixation without the need for overnight digestion; shortening protocol time and resulting in higher quality nucleic acids.
- The ReliaPrep™ FFPE Systems utilize a deparaffinization method that does not use xylene or other organic solvents.
- The ReliaPrep™ FFPE Systems allow scientists to quickly and easily purify nucleic acids that can be used in a many different workflows and downstream applications.