MagMAX FFPE DNA/RNA Ultra Kit

Introduction

Rapid and accurate cancer genome analysis is in high demand for research into the detection and therapeutic management of cancer. However, clinical cancer research samples are routinely formalin-fixed, paraffin-embedded (FFPE) for histopathological analysis and tissue archiving. This method preserves the tissue and allows for long-term storage, but the extensive chemical crosslinking of nucleic acids can complicate downstream processing for applications such as next-generation sequencing (NGS).

The Applied Biosystems™ MagMAX™ FFPE DNA/RNA Ultra Kit provides fast, reliable nucleic acid isolation from FFPE samples. DNA and RNA are sequentially extracted from the same sample in a single workflow, enabling versatile sample analysis. In addition, samples can be processed either manually or in an automated system using open robotics to process up to 96 samples simultaneously. The magnetic bead–based protocol and on-bead nuclease treatment help reduce sample loss, making this kit ideal for small or limited tissue specimens. The kit is also compatible with CitriSolv™ clearing agent, a deparaffinization alternative to xylene that is less toxic and can be used without a chemical fume hood.

The MagMAX FFPE DNA/RNA Ultra Kit has been validated for multiple research sample types, including solid tumor biopsies, core needle biopsies, and fine-needle aspirates. Using this kit, nucleic acids can be isolated from a single 5 µm FFPE sample section with yield and quality comparable to other commercially available kits that use magnetic beads or spin columns, but with ~40% less hands-on time than that required when using the leading

supplier's kit. The DNA, RNA, and microRNA isolated with the MagMAX FFPE DNA/RNA Ultra Kit are suitable for downstream molecular applications, including qPCR, analysis by the Agilent Bioanalyzer™ system, and NGS, even when using FFPE tissue over 25 years old.

This application note describes the process of extracting DNA and RNA from 5–40 µm sections of human FFPE tissue using the MagMAX FFPE DNA/RNA Ultra Kit for manual or automated extractions on the Thermo Scientific™ KingFisher™ Duo system or KingFisher™ Flex Magnetic Particle Processor. Multiple FFPE sample types were tested, including solid tumor resections, core needle biopsies, and fine-needle aspirates. Nucleic acid yield, quality, and functionality were tested for downstream applications, including Ion Torrent™ NGS sequencing.

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Methods

Figure 1 shows the steps in the workflow for isolation of nucleic acids from FFPE samples. The FFPE samples were first deparaffinized and then digested with Proteinase K solution. Extractions with the MagMAX FFPE DNA/RNA Ultra Kit were processed either manually in microcentrifuge tubes or automatically with the KingFisher Duo system or KingFisher Flex Magnetic Particle Processor. Nucleic acid yield was quantified using Invitrogen™ Quant-iT™ dsDNA and RNA Assay Kits with a 96-well plate reader.

For qPCR analysis, samples were reverse-transcribed using the Invitrogen™ SuperScript™ VILO™ cDNA Synthesis Kit and followed by the use of Applied Biosystems™ TaqMan® Gene Expression Assays. For miRNA analysis, RNA samples were reverse-transcribed using the Applied Biosystems™ TaqMan® MicroRNA Reverse Transcription Kit and followed with Applied Biosystems™ TaqMan® MicroRNA Assays. Samples were run on an Applied Biosystems™ 7900HT Fast Real-Time PCR System, and C_t values were determined automatically.

To test for NGS compatibility, FFPE tissue samples were extracted using the MagMAX FFPE DNA/RNA Ultra Kit or a commercially available column-based extraction kit. For both kits, DNA and RNA were isolated from consecutive FFPE tissue sections, in duplicate. The samples extracted using the other supplier's kit were processed manually on columns according to the kit's protocol, while the MagMAX FFPE DNA/RNA Ultra Kit samples were processed on a KingFisher Flex processer with a 96 deep-well head.

Library preparation and sequencing

NGS libraries were constructed from 10 ng of input DNA or RNA using the Ion AmpliSeq™ Library Kit 2.0 with either the Oncomine™ Focus Assay panel or the Ion AmpliSeq™ Cancer Hotspot Panel v2 and RNA Cancer Panel. Samples were templated on the Ion Chef™ System and subsequently sequenced with the Ion PGM™ System. The coverage analysis plug-in, Torrent Variant Caller on Torrent Suite™ Software or Ion Reporter™ Software, was used to analyze the data and detect variants across the genes assayed in these panels.

Results and discussion

Recovery and reproducibility

To examine the reproducibility of recovery of nucleic acids, archived FFPE tissues were processed using the MagMAX FFPE DNA/RNA Ultra Kit and a commercially available column-based kit (Kit Q). All samples were eluted in equal volumes (50 µL) and quantified using the Quant-iT dsDNA and RNA Assay Kits. The MagMAX FFPE DNA/RNA Ultra Kit consistently produced DNA and RNA yields that were equivalent to or higher than those obtained using the other supplier's kit (Figure 2). In addition, the sample processing with the MagMAX FFPE DNA/RNA Ultra Kit was automated on a KingFisher Flex system, resulting in approximately 40% less hands-on time compared to manual isolation by Kit Q.

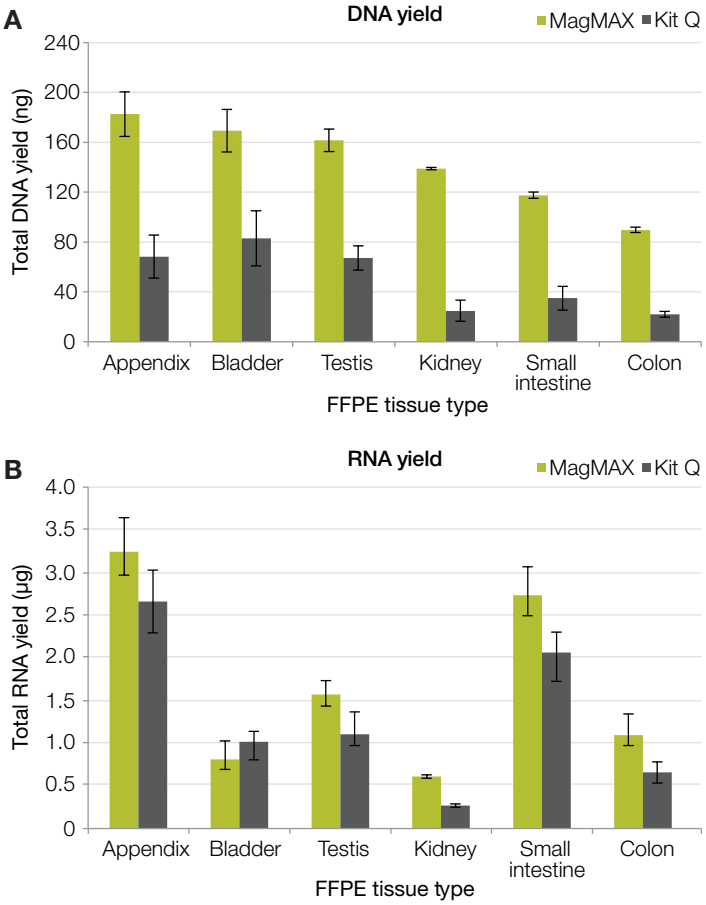

Figure 2. Reproducibility of nucleic acid recovery. Yields of **(A)** DNA and **(B)** RNA extracted from FFPE tissues (year of embedment 1981–2004) using either the MagMAX FFPE DNA/RNA Ultra Kit or a commercially available column-based kit (Kit Q).

Figure 1. MagMAX FFPE DNA/RNA Ultra sample extraction and sequencing workflow.

To determine the suitability of the extracted nucleic acids for downstream molecular applications, real-time PCR analysis was performed. RNA samples were reverse-transcribed using the SuperScript VILO cDNA Synthesis Kit, and qPCR analysis was performed using a TaqMan Gene Expression Assay for the *GAPDH* gene (amplicon length 93 bp) on a 7900HT Fast Real-Time PCR System, using equal input volumes for all samples. The RNA samples extracted using the MagMAX FFPE DNA/RNA Ultra Kit had consistently lower C_t values compared to those obtained by extraction using Kit Q (Figure 3).

Deparaffinizing agents

Traditionally, xylene has been used to deparaffinize FFPE samples prior to extraction of the nucleic acid. Xylene effectively removes paraffin wax from tissue sections, but a chemical fume hood is required for safe handling of this solvent. CitriSolv clearing agent, a less toxic deparaffinization alternative, was tested to determine compatibility with the MagMAX FFPE DNA/RNA Ultra Kit. Consecutive 10 µm sections from three different FFPE samples were deparaffinized with either xylene or CitriSolv solution. Following deparaffinization, samples were digested and extractions were automated on a KingFisher Flex Magnetic Particle Processor with a 96 deep-well head. DNA and RNA yields were quantified using the Quant-iT dsDNA and RNA Assay Kits (Figure 4).

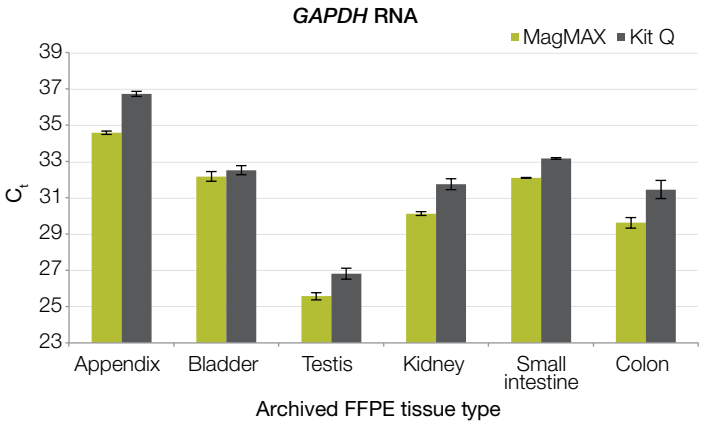

Figure 3. Real-time PCR analysis of RNA samples. From FFPE tissue sections, C_t values from a *GAPDH* assay are consistently lower for RNA isolated with the MagMAX FFPE DNA/RNA Ultra Kit than for RNA obtained using a commercially available column-based kit (Kit Q).

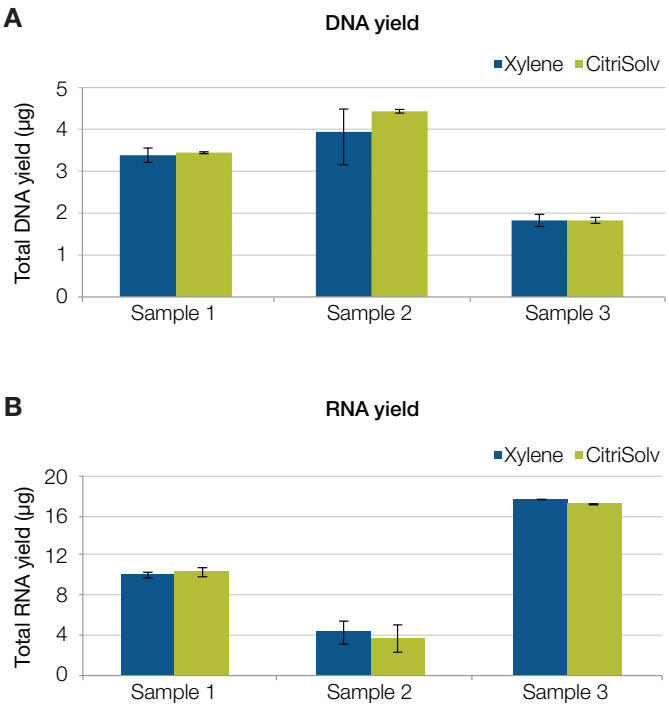

Figure 4. Compatibility of CitriSolv deparaffinizing agent with the MagMAX FFPE DNA/RNA Ultra Kit. Yields of **(A)** DNA and **(B)** RNA from 10 µm FFPE tissue sections are equivalent when deparaffinized with either xylene or CitriSolv solution.

Automation and scalability

The MagMAX FFPE DNA/RNA Ultra Kit has built-in flexibility and scalability, allowing for simultaneous processing of up to 12 samples with the KingFisher Duo system, or up to 96 samples with the KingFisher Flex Magnetic Particle Processor. To determine whether automated and manual extractions produced equivalent nucleic acid yields and quality, consecutive FFPE samples were processed in tubes using the MagMAX FFPE DNA/RNA Ultra Kit (manual extraction), and on the KingFisher Flex and KingFisher Duo magnetic particle processors. Total nucleic acid yields were comparable between all three methods, as was the nucleic acid quality for downstream applications such as RNA analysis using the Agilent Bioanalyzer system (Figures 5 and 6).

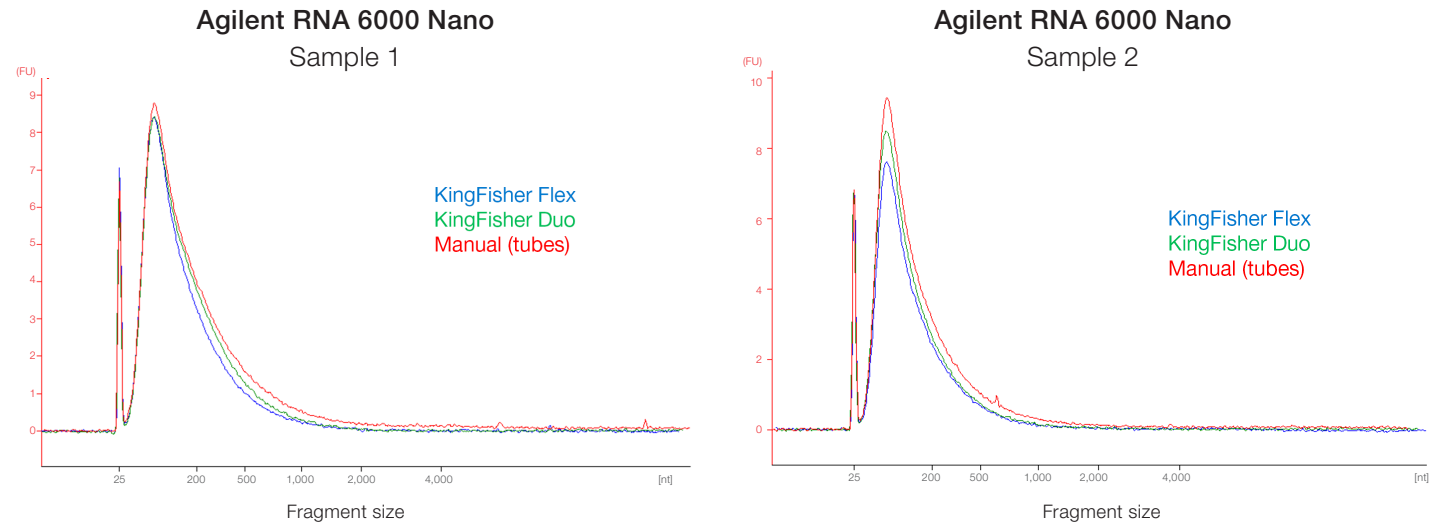

Figure 5. RNA analysis of FFPE samples with the Agilent RNA 6000 Nano kit. Samples extracted manually, or using automated protocols on the KingFisher Flex Magnetic Particle Processor or KingFisher Duo system, had comparable RNA yields and fragment sizes.

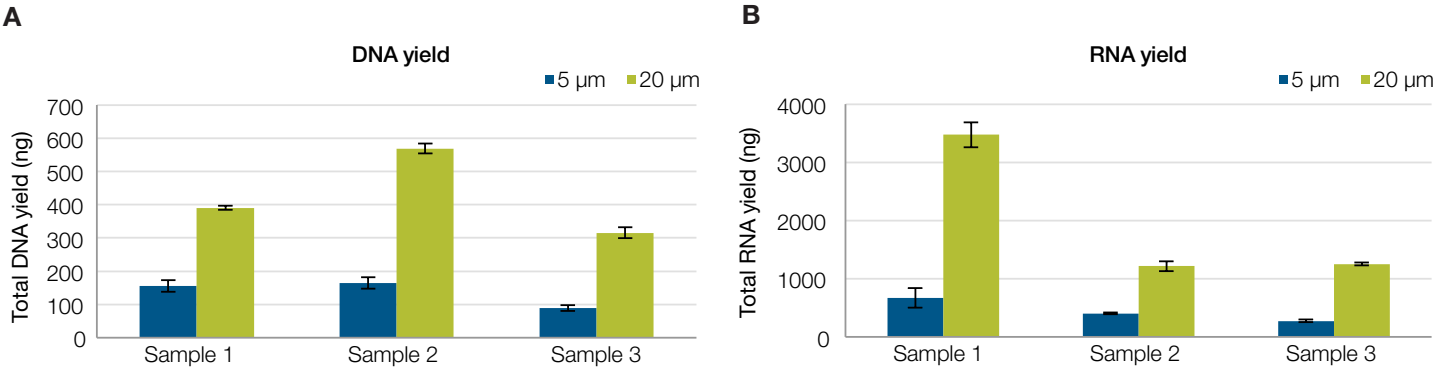

Figure 6. Sample input scalability. Matched 5 µm and 20 µm FFPE sections were processed on the KingFisher Flex system. The total yields of **(A)** DNA and **(B)** RNA increased accordingly for all samples, indicating scalable functionality for samples across a range of FFPE section thicknesses.

Core needle biopsy and fine-needle aspirate samples

To determine the versatility of the MagMAX FFPE DNA/RNA Ultra Kit for common samples used in cancer research, extractions were performed on multiple core needle biopsies and fine-needle aspirate samples. Nucleic acids were extracted from matched samples using the automated Kingfisher Duo protocol. Libraries were prepared with the Oncomine Focus Assay panel using 10 ng of input sample. Samples were templated on the Ion Chef System and sequenced on the Ion PGM System.

Consistent sequencing metrics were obtained across all donors and tissue types. On average, the DNA mean read length was ≥109 bp with 97% uniformity and no strand bias. For RNA, mean read length was ≥87 bp with all 5 endogenous control genes detected (Figure 7).

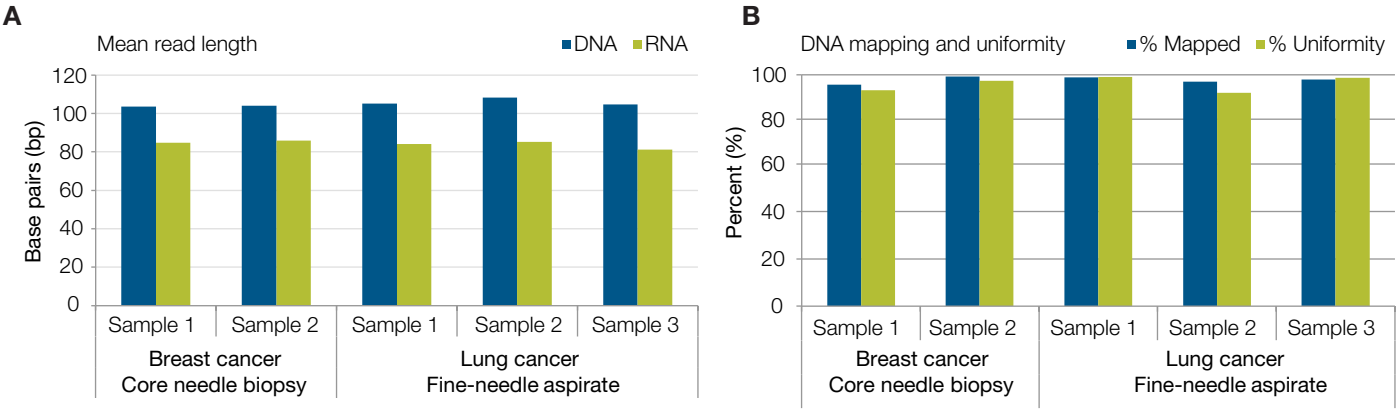

Figure 7. Sequencing of cancer research samples. Sequencing metrics such as **(A)** mean read length and **(B)** DNA mapping and uniformity, obtained from core needle biopsy and fine-needle aspirate samples, are consistent, demonstrating functional recovery of nucleic acids from different specimen types. A 60 pM library was prepared using the Oncomine Focus Assay panel on a 10 ng sample input. Ion Chef and Ion PGM Systems were used to template and sequence the samples, respectively.

Ion Torrent sequencing accuracy and sensitivity

Many FFPE samples are in limited supply, demanding isolation methods that not only are reproducible but also can produce accurate results from minimal sample input. To test assay sensitivity, FFPE samples were digested and subsequently diluted to as low as 10% of the sample input for the remainder of the isolation protocol. Nucleic acid yield was highly linear across the full range of sample input titration, indicating high DNA and RNA recovery rates for the assay (Figure 8).

To determine whether the samples were of high functional quality, NGS libraries were prepared with the Ion AmpliSeq Cancer Hotspot Panel v2. All libraries were prepared with 10 ng input and sequenced on the Ion PGM System. DNA variants were analyzed using the Torrent Variant Caller plug-in. Titration of the extracted input samples had no effect on mean read length or uniformity. In addition, the same single nucleotide polymorphism (SNP) variants were detected across DNA samples at all input percentages (Table 1).

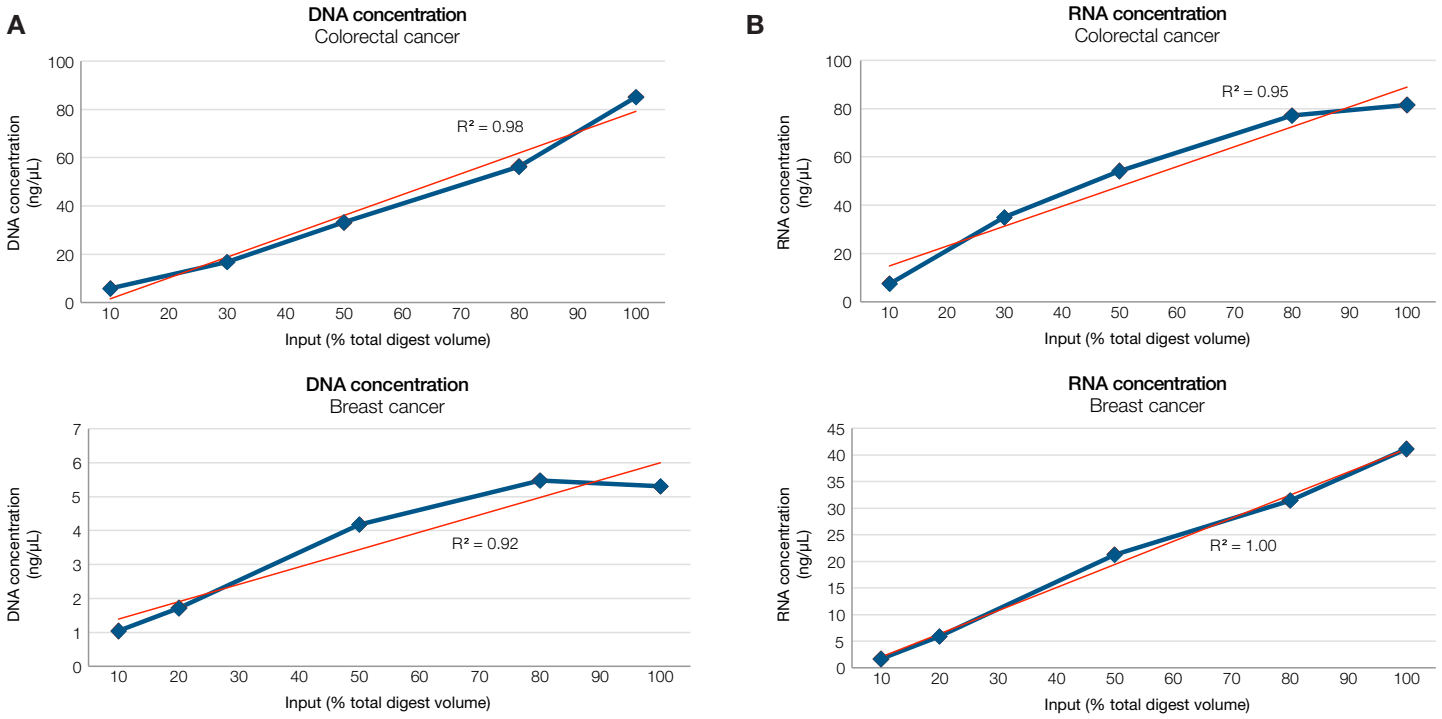

Figure 8. Recovery of DNA and RNA from FFPE cancer samples. Yields of **(A)** DNA and **(B)** RNA from 10 µm FFPE sections of colorectal cancer and breast cancer samples. Following digestion with Proteinase K solution, samples were diluted to as little as 10% sample input for the remainder of the isolation protocol. The linear correlation between yield and percent sample input indicate high nucleic acid recovery rates for both DNA and RNA.

Table 1. Detection of variants in cancer samples. Following Proteinase K digestion, FFPE samples were diluted in fresh digestion buffer to various input percentages. Extractions were carried out according to the protocol, and libraries were prepared with the Ion AmpliSeq Cancer Hotspot Panel v2 for Ion PGM sequencing. For both colorectal and breast cancer tumor types, the exact same cancer hotspot variants were detected, verifying the sensitivity and functionality of the assay across the input titration.

FFPE tissue type	Sample input (% total digest)	Mean read length (bp)	No. of hotspot variants detected	Hotspot gene ID
Colorectal cancer	100%	119	9	*PDGFRA*, *APC*, *PTEN*, *HRAS*, *KRAS*, *TP53*, *SMARCB1*
	80%	111	9	*PDGFRA*, *APC*, *PTEN*, *HRAS*, *KRAS*, *TP53*, *SMARCB1*
	50%	113	9	*PDGFRA*, *APC*, *PTEN*, *HRAS*, *KRAS*, *TP53*, *SMARCB1*
	30%	112	9	*PDGFRA*, *APC*, *PTEN*, *HRAS*, *KRAS*, *TP53*, *SMARCB1*
	10%	113	9	*PDGFRA*, *APC*, *PTEN*, *HRAS*, *KRAS*, *TP53*, *SMARCB1*
Breast cancer	100%	109	3	*KIT*, *MET*, *HRAS*
	80%	108	3	*KIT*, *MET*, *HRAS*
	50%	109	3	*KIT*, *MET*, *HRAS*
	20%	109	3	*KIT*, *MET*, *HRAS*
	10%	108	3	*KIT*, *MET*, *HRAS*

Conclusions

The MagMAX FFPE DNA/RNA Ultra Kit provides a highly reliable, versatile, and scalable nucleic acid isolation method for a wide range of molecular assays and applications. The kit is compatible with a large variety of FFPE tissue types, even from archived tissue blocks over 25 years old. In addition, nucleic acids are readily recovered from multiple biopsy types, including solid tumor sections, fine-needle aspirates, and core needle biopsies.

The assay parameters are flexible and allow several processing options. For example, samples can be extracted from slides or curls of varied thicknesses with comparable nucleic acid yield and quality. The assay can be conducted manually, or automated to process up to 96 samples simultaneously. In addition, the kit is compatible with alternative deparaffinizing agents such as CitriSolv solution, providing a less toxic procedure that does not require the use of a chemical fume hood.

DNA and RNA isolated with the MagMAX FFPE Ultra kit are suitable for downstream applications including qPCR, analysis using the Agilent Bioanalyzer system, and Ion Torrent NGS. High-quality NGS libraries can also be prepared using Ion AmpliSeq technology with just 10 ng of sample input, maximizing data collection from precious and irreplaceable FFPE samples.

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