Rapid, Easy Extraction of DNA and RNA from Archival Samples

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Introduction

Many attempts have been made to simplify and speed up the extraction of nucleic acids from archival samples. Traditional protocols require organic reagents to remove paraffin, SDS for cell lysis, and days of overnight proteinase K digestion followed by further purification.\(^1,^2\) In particular, the analysis of nucleic acids from formalin-fixed, paraffin-embedded (FFPE) tissues is challenging, due to the extensive cross-linking of nucleic acids during the fixation process. Other challenges include chemical modification and degradation of the nucleic acids, and the limited amount of nucleic acid in the samples.

The QuickExtract™ FFPE DNA and RNA Extraction Kits provide the simplest and fastest method to reliably produce PCR-ready DNA and RT-PCR-ready RNA from FFPE tissues. The kits allow nucleic acid amplification from a wide variety of archival tissue samples without chemical solvents, spin columns, or tube transfers that could result in sample loss.

The QuickExtract FFPE DNA and RNA Extraction Kits are optimized to release the maximum amount of PCR-ready nucleic acid. Efficient lysis and extraction conditions allow PCR-ready DNA preparation in about an hour, while RNA extraction takes just over 30 minutes. Extracted DNA can be used in many PCR-based analysis methods including microsatellite detection, SNP detection, tumor heterogeneity studies, copy number detection, and methylation analysis. Extracted RNA can be used in both end-point and real-time RT-PCR.

Methods and Results

**DNA Extraction and PCR Amplification**

Slide-mounted, FFPE-preserved, skeletal muscle tissue slices (US Biomax, Inc.) were wet with 100 µl of QuickExtract FFPE DNA Extraction Solution, scraped off the slide with a sterile blade, and transferred to the bottom of small microcentrifuge tubes. The samples were incubated at 56°C for 1 hour and then at 98°C for 2 minutes (Fig. 1). The 0.9 cm\(^2\) skeletal muscle tissue sample yielded 1.8 µg of extracted DNA as determined by Hoechst dye fluorimetry. The extracted DNA was directly amplified with the FailSafe™ PCR System (EPICENTRE).

Two microliters of extracted DNA was amplified in an optimized FailSafe PCR PreMix with a series of primers that detect regions of three different genes: tumor protein 53 (TP53), dystrophin (DMD), and tumor necrosis factor (TNF). The DNA was denatured at 95°C for 2 minutes, and then PCR amplified for 40 cycles: 92°C for 15 seconds, 55°C for 15 seconds, and 72°C for 20 seconds. The resulting PCR amplicons vary from 162 bp to 802 bp in length, span different regions of the genes, and demonstrate that these gene targets (used for tumor heterogeneity studies, and deletion and insertion mutagenesis studies) can easily be amplified from DNA extracted from FFPE samples (Fig. 2).

**Fig. 1. Procedure for QuickExtract™ DNA or RNA Extraction from slide-mounted FFPE Tissue.**

**Fig. 2. PCR amplification of DNA from a slide-mounted, FFPE-preserved human skeletal muscle tissue section.** Following the QuickExtract™ protocol, 2 µl of undiluted, extracted DNA was amplified with primers for three different loci: TP53, DMD, and TNF. The products were separated on a 3% agarose gel and were visualized with SYBR® Gold. Lane M, 100-bp DNA ladder; lanes 1-3, exons 2, 3, and 11 of TP53; lanes 4-6, exons 6, 50, and 3 of DMD; lane 7, exon 4 of TNF.
of randomly-primed cDNA was amplified with an optimized FailSafe PCR PreMix and 10 pmol of each primer. The cDNA was denatured at 95°C for 2 minutes, and then PCR amplified for 40 cycles: 92°C for 12 seconds, 58°C for 15 seconds, and 72°C for 20 seconds. Eight different messages were easily detected in the extract with amplicons ranging from 116 bp to 308 bp in length (Fig. 4).

RNA is extensively modified and fragmented during tissue preservation procedures and storage. The extent of fragmentation varies depending on the type and length of fixation as well as the tissue type, but has been reported to average 200 bases in length.3 Primers were designed to detect a series of these fragments within one specific message. A 3-µl aliquot of randomly-primed cDNA was amplified with an optimized FailSafe PCR PreMix and a series of primers that detect different regions of the 15.4 kb ryanodine receptor 1 (RYR1) message. The cDNA was denatured at 95°C for 2 minutes, and then PCR amplified for 40 cycles: 92°C for 15 seconds, 55°C-60°C for 15 seconds, and 72°C for 20 seconds. The resulting PCR amplicons spanned the length of the message, indicating that even though only fragments of RNA are recovered during extraction, the entire message is represented in the RNA extract (Fig. 5).

The cDNA produced from DNase-treated, QuickExtract FFPE RNA is also a suitable template for real-time PCR. A 1-µl aliquot of skeletal muscle cDNA was amplified to produce short amplicons from a number of different messages. The products were separated on a 3% agarose gel and were visualized with SYBR® Gold. Lane M, 100-bp DNA ladder; lane 1, a 116-bp region of RYR1; lane 2, a 162-bp region of ACTA; lane 3, a 166-bp region of RSP18; lane 4, a 226-bp region of GAPDH; lane 6, a 232-bp region of OAZ1; lane 7, a 307-bp region of ACTB; lane 8, a 308-bp region of TNF.

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DNA & RNA Purification

70°C for 15 seconds. Both messages amplified reproducibly; data for ACTB are shown in Fig. 6.

Even though only fragments of RNA are recovered during extraction, the entire message is represented in the RNA extract.

Conclusions
The QuickExtract FFPE DNA and RNA Extraction Kits provide a simple, one-tube, rapid DNA or RNA extraction protocol for PCR- or RT-PCR-based analysis methods. Once the preserved tissue sample is placed in the extraction solution, heat treatment is all that is required to free the desired nucleic acid for amplification. No organic extractions, spin columns, or centrifugation steps are required.

References

Ordering Information

QuickExtract™ FFPE RNA Extraction Kit
QFR82805 5 ml (50 rxns)
QFR82050 50 ml (500 rxns)
Includes Extraction Solution and optional DNase Reagents.

QuickExtract™ FFPE DNA Extraction Kit
QEF81805 5 ml (50 rxns)
QEF81050 50 ml (500 rxns)

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