In 30 Minutes Obtain PCR-Ready DNA from FFPE Tissue with QuickExtract™ DNA Extraction Solution

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Introduction

Conventional histopathology techniques include the microscopic observation of thin sections of preserved tissues that are stained to reveal anatomical structures. A commonly used method for tissue preservation has been formalin-fixation followed by paraffin-embedding (FFPE). In recent years the desire for molecular information from these preserved, archival tissues has emerged. One of the most notable successful examples of nucleic acid extraction from such an archived FFPE tissue was the retrieval of influenza viral RNA sequence data from the FFPE lung tissue of soldiers who died in 1918.[1]

QuickExtract™ DNA Extraction Solution enables the rapid extraction of PCR-ready DNA from formalin-fixed, paraffin-embedded tissues. The DNA can be used in end-point or real-time PCR (qPCR).

Methods

FFPE tissue from a slide (2 months old) of thin sections (0.5 micron) of three mouse embryos (at day 12 of gestation; courtesy of Dr. Igor Prudovsky, Maine Medical Center Research Institute) was scraped with a flamed scalpel blade into 100 µl of QuickExtract Solution and heated at 65°C. At 0.5, 1, and 3 hour time intervals, 30 µl aliquots were withdrawn from the solution—the amount of DNA extracted was quantified by fluorescence with Hoechst dye 33258. The DNA was also amplified by end-point PCR with β-actin primers, and by qPCR with SYBR® Green I dye using beta-2-microglobulin primers.

Results

Table 1 shows the data for the amounts of DNA detected at each time point. As one might expect, the average amount of DNA extracted increased with the length of time of incubation in QuickExtract DNA Extraction Solution.

Incubation Time in QuickExtract™ DNA Extraction Solution (hours)	0.5	1.0	3.0
Total Yield of DNA (ng)	ND*	30	90
*None Detected, i.e. < 1 ng			

Table 1. Time Course of DNA Extracted from FFPE Tissue.

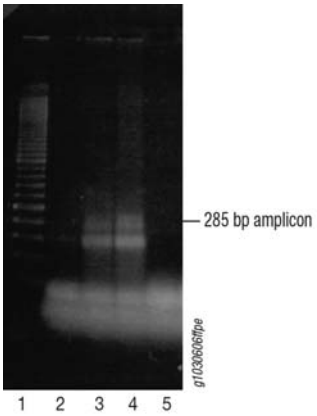

FIG 1. End-point PCR amplification of DNA extracted from FFPE mouse embryos. Lane 1, 100 bp ladder; **Lane 2**, 0.5 hr extraction; **Lane 3**, 1 hr; **Lane 4**, 3 hr; and **Lane 5**, no template control PCR. The reaction used EPICENTRE Biotechnologies FailSafe™† PCR System with PreMix D, and β-actin primers. The primer sequences were, 5′ primer: 5′-TCA TGA AGT GTG ACG TTG ACA TCC GT and 3′ primer: 5′-CTT AGA AGC ATT TGC GGT GCA CGA TG. Cycling conditions were: 95°C (2 min) and 35 cycles of 95°C (30 sec), 55°C (30 sec), 74°C (30 sec). The amplicon was 285 bp in length.

In FIG 1 it is also possible to spot the trend of increasing amounts of amplicon produced by end-point PCR with increasing length of incubation in QuickExtract Solution.

FIG 2 displays the results from qPCR analysis of cyclophilin A (peptidylprolyl isomerase A) from DNA extracted at varying times from a mixture of FFPE human tissues (at least 3.5 years old; Sigma H-2286) scraped from a slide as described above. Cyclophilin A was readily amplified from all samples by qPCR with SYBR Green I dye. As predicted, the C_T (threshold cycle) obtained from the three hour sample was significantly lower (indicating greater abundance) than the C_T of the 0.5 hour extracted DNA sample. However, there was clearly DNA present after the 0.5 hour digestion.

Conclusion

QuickExtract provides a rapid, simple method for obtaining PCR-ready DNA from archived FFPE tissues in 30 minutes.

References

1. Reid, A.H. *et al.*, (2000) *Proc. Natl. Acad. Sci.* **97**(12), 6785.

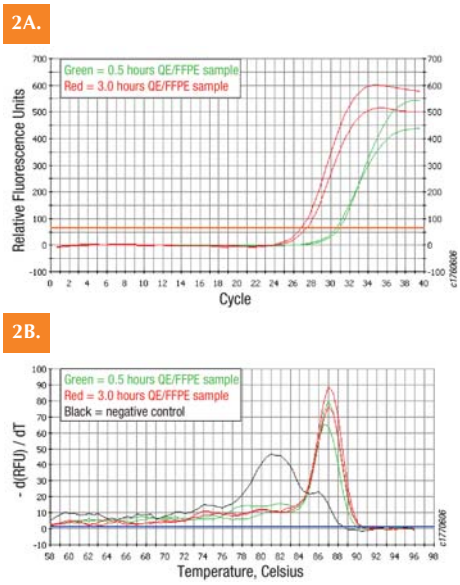

FIG 2. qPCR of DNA extracted from FFPE tissues using QuickExtract™ DNA Extraction Solution. Duplicate 25 µl qPCR reactions were set up containing: 12.5 µl TAQurate™ GREEN Real-Time PCR MasterMix, 10 pmoles of forward and reverse primers for cyclophilin A, 1 U TAQurate™ Real-Time PCR Enzyme Blend, and 1 µl of appropriate DNA. Cycling conditions were: 95°C (2 min), and 40 cycles of 95°C (20 sec), 57°C (20 sec), and 72°C (30 sec). **A. Amplification plot.** The DNA template for PCR was extracted from FFPE tissue 0.5 hours (green traces), or 3 hours (red traces) before PCR amplification in duplicate. No DNA template control, and positive controls performed as expected, data not shown. **B. Melt curve analysis of the amplicons of panel A.** No DNA template negative control shown as black trace.

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QuickExtract™ DNA Extraction Solution 1.0

QE09050 50 ml

Bulk solution, sufficient to perform 100 extractions.

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