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A rapid RT–PCR method for detection of intact RNA in formalin-fixed paraffin-embedded tissues

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The molecular analysis of formalin-fixed and paraffin-embedded tissues has application in both investigative studies and clinical practice (1). It is now possible to analyze gene expression in formalin-fixed and paraffin-embedded tissues by *in situ* reverse transcriptase-polymerase chain reaction (*in situ* RT–PCR) (2). Degradation of RNA, however, can occur at various stages of tissue preparation, fixation and paraffin-embedding. To date, a rapid technique designed to determine RNA quality in formalin-fixed and paraffin-embedded tissues is lacking. The most common method for monitoring RNA involves tissue extraction using guanidinium thiocyanate or acid guanidinium thiocyanate–phenol–chloroform (3). Unfortunately, for many applications, this approach is time-consuming and, more importantly, results in a significant loss of material during RNA extraction. In contrast, the use of diethyl pyrocarbonate (DEPC) water has been described as a means for rapidly determining gene expression in fresh cells, thereby eliminating an RNA extraction step (4). However, residual DEPC is undesirable because of its tendency to inhibit reverse transcription. We have therefore developed a rapid and sensitive method which can determine RNA quality in formalin-fixed, paraffin-embedded tissues. Using this procedure, tissue scraped from slides was used as a direct substrate for RT–PCR in order to amplify K-*ras* and protein kinase Cζ (PKC-ζ). Using this straightforward method, RNA template can be obtained in 1.5 h, making it feasible to reverse transcribe and amplify cDNA in a single day, a time frame appropriate for both the diagnostic and research laboratories.

Experiments described here were performed on formalin-fixed and paraffin-embedded rat colon tissue sections, using specific primers for K-*ras* and PKC ζ genes (Table 1). Tissue sections on slides were deparaffinized in xylene and rehydrated by washing in serial dilutions of ethanol. Slides were stored at –80°C with desiccant for up to one year. After rehydration, 20 µl of solution A [1.25× PCR buffer (200 mM Tris–HCl, 500 mM KCl), 6.25 mM $MgCl_2$, 5 U RNasin (Promega, Madison, WI), 2 mM DTT, 1 U RQ 1 RNase-free DNase (Promega)] was directly applied to a single 5 µm thick section. The section was completely scraped off the slide using a pipette tip and transferred to a 500 µl microcentrifuge tube. In order to compare alternative methods for cell lysing, selected samples were treated with proteinase K (Ambion, Austin, TX) at a final concentration of 0.1 mg/ml, or probe sonicated 3 times, for a duration of 3 s each using an output control of 50% (Vibra Cell VC50 T, Danbury, CT) prior to incubation at 37°C for 1 h to allow for DNA digestion. Cell lysates were then heated to 95°C for 15 min in order to inactivate DNase and proteinase K. Following centrifugation at 13 600 *g* for 5 min, 16 µl of supernatant was transferred to a separate tube and 4 µl of RT mixture [5 mM dNTPs, 2.5 µM random hexamer, 5 U RNasin, 100 U SuperScript II reverse transcriptase (Promega)] were added. The RT reaction was performed at 45°C for 45 min, followed by heating to 99°C for 5 min. Finally, the PCR reaction was initiated by adding 30 µl of the PCR mixture containing 1× PCR buffer, 50 pmol of each of the appropriate 5′ and 3′ primers for K-*ras* or PKC-ζ and 2.5 U *Taq* DNA polymerase (Gibco-BRL, Gaithersburg, MD) to each tube. After denaturation at 94°C for 5 min, 50 cycles of 94°C for 30 s, 59°C for 45 s and 73°C for 45 s were performed using a GeneAmp PCR System 2400 (Perkin Elmer, Foster City, CA). Final extension was for 7 min at 73°C. Products were electrophoresed on precast 4% NuSieve agarose gels (FMC Bioproducts, Rockland, ME), stained with ethidium bromide and visualized with UV illumination.

Table 1. Nucleotide sequence of K-*ras* and PKC-ζ primers

Gene (rat)	Fragment size (bp)	Primer sequence
K-*ras* 5′	238	5′-GCCTGCTGAAAATGACTGAG-3′
K-*ras* 3′		5′-TCCCCAGTTCTCATGTACTG-3′
PKC-ζ 5′	681	5′-CGATGGGGTGGATGGGATCAAAA-3′
PKC-ζ 3′		5′-GTATTCATGTCAGGGTTGTCTG-3′

The specificity of the RT–PCR reaction was confirmed by size determination on agarose gels in comparison to a positive control, rat colonic mucosal RNA which had been extracted using standard techniques (5). For K-*ras*, only one band of ~240 bp (Fig. 1) was observed. The predicted size of the K-*ras* product is 238 bp. This size product can only be derived from K-*ras* RNA since the primers were designed to span an intron between exon 1 and exon 2. For PKC-ζ, similar results were obtained. Only a single sized product of 680 bp (Fig. 2) was detected. The predicted size of the PKC-ζ product is 681 bp. This product is exclusively derived from PKC-ζ RNA since the primers were designed to span an intron. Examination of the data indicates that the sonication step plus DNase digestion is critical in order to generate clear product bands (Figs 1 and 2, lanes 3 and 4), whereas proteinase K treatment, to release RNA trapped by

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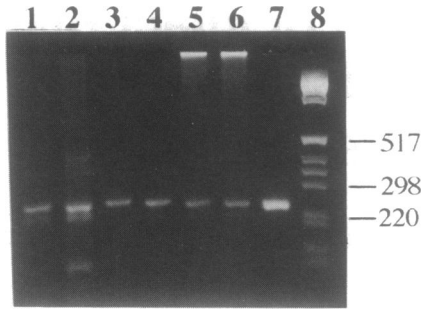

Figure 1. RT–PCR analysis of K-*ras*. Samples were electrophoresed in a 4% NuSieve Agarose (FMC). Lanes 1 and 2, samples treated with proteinase K; lanes 3 and 4, samples treated with DNase and sonicated; lanes 5 and 6, samples treated with DNase; lane 7, rat colonic mucosal RNA. M, 1 kb marker (Gibco). DNA was visualized by ethidium bromide staining.

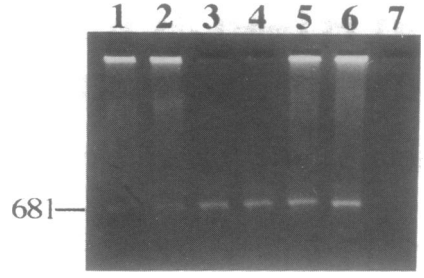

Figure 2. RT–PCR analysis of PKC-ζ. Samples were electrophoresed in a 4% NuSieve Agarose (FMC). Lanes 1 and 2, samples treated with proteinase K; lanes 3 and 4, samples treated with DNase and sonicated; lanes 5 and 6, samples treated with DNase; lane 7, negative control, sample treated with DNase and sonicated without RT. DNA was visualized by ethidium bromide staining.

formalin cross-linking, is not necessary. In addition, the sonication step prior to DNase treatment is essential to allow complete destruction of DNA (Figs 1 and 2, lanes 3 and 4 compared with lanes 5 and 6).

In conclusion, this simple and rapid method is very useful for determining the integrity of RNA in archived formalin-fixed, paraffin-embedded tissues since PCR products will not be generated from degraded RNA. In addition, this technique can be utilized for gene expression studies on archived tissues, as it circumvents the time-consuming RNA isolation procedure using guanidium thiocyanate.

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REFERENCES

1 Mies, C. (1992) *Diagn. Mol. Pathol.* **1**, 206–211.
2 Nuovo, G. (1994) *Am. J. Pathol.* **144**, 659–666.
3 Chomczynski, P. *et al.* (1987) *Anal. Biochem.* **162**, 156–159.
4 Ferre, F. *et al.* (1989) *Nucleic Acid Res.* **17**, 2141.
5 Davidson, L. *et al.* (1994) *Arch. Biochem. Biophys.* **312**, 547–553.