

Short report

Direct multiplex amplification of DNA from a formalin fixed, paraffin wax embedded tissue section

L Cawkwell, P Quirke

Abstract

The extraction of DNA from formalin fixed, paraffin wax embedded tissue can be problematical, with long protocols producing low yields. This report describes a very simple and useful method for amplifying DNA from formalin fixed, paraffin wax embedded tissue without the need for prior DNA extraction. This method allows direct polymerase chain reaction (PCR) based molecular analysis of fixed tissue. It is an invaluable method if clinical biopsy specimens are to be investigated, because extraction of uncontaminated DNA from such small samples can be very difficult or even impossible. It will also facilitate the study of intratumour heterogeneity, with the analysis of multiple small areas from within a single tumour section. In addition, this method can be used for other samples where only a few tests are to be carried out and a stock of DNA is not required, thus shortening the analysis time.

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Keywords: archival tissue; multiplex amplification; DNA extraction

Most clinical tissue samples are routinely fixed in formalin and embedded in paraffin wax. This process is essential for archiving purposes and to maintain excellent cell morphology. Thus, formalin fixed, paraffin wax embedded clinical samples are widely used for molecular genetic research. Molecular analyses of human tissue often entails the use of the polymerase chain reaction (PCR) with previously extracted DNA as the template. The extraction of DNA requires time and sufficient material. There are several methods available for the extraction of DNA from formalin fixed, paraffin wax embedded samples, some of which take over a day to complete.^{1–3} However, a stock of DNA is not always necessary if only a limited number of analyses are to be carried out, making the time and expense of the extraction procedure wasteful in such situations. Results can also be generated more rapidly if time is not required for the extraction of DNA. In addition, most extraction protocols rely on relatively large

amounts of starting material. Molecular analyses using small amounts of clinical tissue, especially biopsy material, can be problematical because the extraction of DNA from limited quantities of tissue can be unreliable, produce poor yields, and be prone to excessive contamination with external DNA. Therefore, it is not always possible to produce a pure DNA stock from such clinical samples and molecular analyses become impossible.

Methods for direct PCR from blood and fresh solid tissue have been reported.⁴ We now describe a simple method that allows the amplification of DNA directly from small fixed tissue samples without the need for prior DNA extraction and purification. This procedure will save time and reagents if a DNA stock is not required and also allows the contamination free analysis of small amounts of tissue. It has been developed using colorectal and gastric tissue in the form of small biopsies or microdissected areas from larger sections. Conventional PCR and multiplex fluorescence PCR have been used. Each amplification is a once only procedure, but multiplex fluorescence PCR allows the co-amplification of more than one target.

Materials and methods

A single formalin fixed, paraffin wax embedded section (4 µm thick) was floated on to a glass microscope slide and attached by incubation for 30 minutes on a hot plate set at 65°C. After dewaxing in xylene, rehydrating using a graded alcohol series, and rinsing in sterile distilled water, the tissue was air dried. A total of 20 µl of sterile distilled water was pipetted on to the whole biopsy or the chosen area (identified using a consecutive haematoxylin and eosin stained section) of a larger tissue section. The tissue to be used as a template was then dislodged from the slide and into the water, using a sterile pipette tip. The water containing the tissue was transferred carefully to a 0.2 ml PCR tube using a pipette tip that had been cut to provide a wider tip diameter, thus facilitating the uptake of the fragmented tissue. To ensure that the maximum amount of dislodged material was recovered, the water was agitated with the tip to aid suspension of the tissue. Alternatively, 10 µl of water could be used in the first

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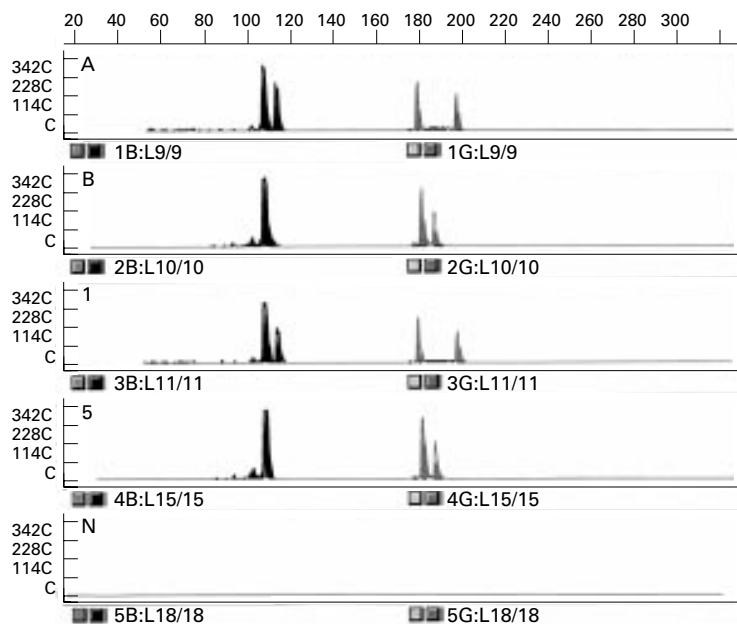


Figure 1 Direct multiplex amplification of formalin fixed, paraffin wax embedded human gastric biopsies analysed using a model 377 automated DNA sequencer. The x axis indicates size in base pairs and the y axis indicates peak height. The multiplex consists of the microsatellite MycL1 (grey peaks) and a sex determination assay based on the amelogenin gene (black peaks). It is apparent that sample A (originating from a 2×2 mm biopsy) and sample 1 (1×2.5 mm) share the same genotype for these markers. Sample B (0.5×1 mm) and sample 5 (0.5×1 mm) share a second genotype. Panel N represents the negative control (no template).

instance to remove some of the tissue fragments, followed by a further $10 \mu\text{l}$ of water to wash the area and collect the remnants. Tissue fragments in water could be stored at 4°C for at least 48 hours. A standard PCR reagent cocktail, made up in accordance with the manufacturer's instructions for Amplitaq Gold™ DNA polymerase (Perkin Elmer, Foster City, California, USA), was added to the tube containing the tissue in $20 \mu\text{l}$ of sterile distilled water (making a total reaction volume of $50 \mu\text{l}$). Reactions were performed in a model 9600 thermal cycler (Perkin Elmer). The Amplitaq Gold enzyme requires activation by heating at 95°C for 15 minutes and thus provides a "hot start" to the reaction. After this preincubation step a two step amplification strategy was used (95°C for 30 seconds and then one minute at the appropriate annealing temperature). If the product was to be visualised using agarose gel electrophoresis with ethidium bromide staining, a total of 45 cycles was performed and 50% of the reaction mix was evaluated. For multiplex fluorescence PCR, two fluorescently labelled microsatellites were co-amplified from each sample, and a total of 35–40 cycles was performed. Evalua-

tion of fluorescent products was performed using a model 377 automated DNA sequencer with Genescan software (PE Applied Biosystems, Foster City, California, USA).

Results and discussion

The direct tissue PCR protocol described here provided a robust amplification for the tissue types tested (colon and gastric tissues) and has now been used successfully for over 100 samples (fig 1). Successful, contamination free amplification has been obtained when using very small biopsies (1 mm^2), as well as tissue taken from selected small areas from within a large section. The method can be used with single small biopsy sections or large sections if a chosen small area ($1\text{--}2 \text{ mm}^2$) is to be microdissected. However, the amount of tissue to be used in each PCR should not exceed approximately 2 mm^2 or inhibition of the reaction may occur. The amplification of large segments from DNA that has been extracted from formalin fixed, paraffin wax embedded tissue is not reliable and, therefore, only PCR assays that yield products of less than 250 bp should be used.

Our method has been used for the amplification of DNA for fluorescence assays such as direct sequencing, genetic fingerprinting, and the detection of allelic imbalance and microsatellite instability. PCR products up to 250 bp have been obtained. The method requires one biopsy section or piece of tissue for each reaction, but multiplex fluorescence PCR can be used. This direct PCR protocol will facilitate the molecular analysis of very small amounts of tissue and will be ideally suited to the analysis of intratumour heterogeneity. With modification, the method could be used in conjunction with a finer microdissection procedure.^{5–7}

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