Use of PCR for Forensic Analysis of DNA from Formaldehyde fixed and Paraffin embedded Human Tissues

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Some fixation methods damage DNA and avoid subsequent classical fingerprint analysis.
Previous authors (Dubeau, 1986 ; Goelz, 1985) showed that high molecular weight DNA can be yielded from paraffin embedded tissues and analysed by the RFLP technic.
The polymerase chain reaction is a powerfull tool for the analysis of the fixed tissues. DNA in treated tissue can serve as a template for amplification of the HLA DQ alpha region.
The results from our study indicate that PCR results can be achieved from two years old paraffin embedded muscle and heart blocks. This kind of fixation is used in medical studies conducted in remote regions where samples require fixation and storage for extended periods of time before analysis. The storage require fixation methods which preserve the DNA of specimens for future study.

MATERIAL AND METHODS

1) Paraffin blocks

The skeletic muscle and heart paraffin blocks have been prepared for routine histopathologic examination of surgical resection specimen. Their ages varied from 24 to 36 months.

2) Preparation of the samples

For the embedded samples, it was important to cut away as much of the paraffin as possible before weighing and to mince the tissue extremely finely.

The minced pieces were less than 0.5 mm in any dimension and weighed 0.30 gr. They were placed in a 1.5 ml microfuge tube. Deparaffinization of the sections was performed with a twice xylene (1 ml) extraction followed by two ethanol rinses and a centrifugation. Pellets were air dried. The pellets were suspended in 200 µl of digestion buffer (50 mM Tris, 10 mM EDTA, pH = 7.4, 1 % SDS, 10 mM NaCl, 300 µg/ml proteinase K) and incubated during 60 hours at 37°C. Fresh aliquots of proteinase K were added every 16 hours.

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Edited by Ch. Rittner and P. M. Schneider

After this step, the samples underwent under classical phenol-chloroform extraction and a yield gel was performed.

Aliquots of 2 µl of DNA were amplified in duplicate according to the amplitype™ kit method. Cycling parameters were 32 cycles, 94°C for denaturation (1 mn), 60°C for annealing (30 sec) and 72°C for elongation (30 sec) in a DNA thermal cycler (Perkin Elmer Cetus®). 10 ng from K 562 cell line are used as a human DNA control.

RESULTS

From each specimen 15-20 ng DNA/mg tissues were extracted. The variation in yields were partially due to different densities within the tissues and to the quantity of paraffin left which contributed to the mesured weight. Amplification products (1/20e of the reaction) were electrophoresed on a 4 % Nusieve (3:1) gel, stained with ethidium bromide and photographed under UV light. Aliquots of 20 ng were amplified and hybridized onto the nylon strip where the allele specific probes of the HLA DQ alpha region were disposed. As an example, we present the analysis of a two years old breast carcinoma biopsy fixed and paraffin embedded after contestation by the patient of the diagnostic on the base of a possible inversion of samples from different patients by the laboratory. Therefore a control of the origin of the samples was required and refered to our laboratory for DNA identification.

DISCUSSION AND CONCLUSION

In previous studies, several authors (Greer 1991) studied different fixation methods found in clinical settings. Goelz and coworkers (1985) showed that even if DNA is not completely intact, it is double stranded, cleavable with restriction endonucleases and hybridized efficiently with labeled probes.

In our study, the material was unusable for Southern blot analysis but the remaining DNA was a sufficient template for successfull amplification. Therefore, DNA from routinely fixed and embedded tissue was suitable for the analysis of the HLA DQ alpha region by PCR. These results were in agrement with the work of Greer and coll (1991) who succeded to amplify the human B-globin gene.

In our case work the donnor of the biopsy was the right patient. The routine histopathologic fixation and conservation method provided acceptable histology analysis and suitable DNA for PCR analyse. This fixation procedure might be a good method for specimen storage for extended periods of time before analysis.

REFERENCES

Goelz SE, Hamilton SR, Vogelstein B (1985) Purification of DNA from formaldehyde fixed and paraffin embedded human tissue. Biochem Biophysic Res Commun, 130:188-126

Greer CE, Lund JK, Manos MM (1991) PCR amplification from paraffin-embedded tissues : Recommandations on fixatives for long-term storage and Prospective studies. PCR methods and application, 1:46-50

Greer CE, Peterson SL, Kiviat NB, Manos MM (1991) PCR amplification from paraffin-embedded tissues : Effects of fixative and fixation time. Am J Clin Pathol, 95:117-124