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Application of DNA filter hybridization and PCR to distinguish between human and non-human tissues of poor quality

Roman Müllenbach[1], Darius Makuch[2], Hans-Joachim Wagner[2], and Nikolaus Blin[1]

[1]Institut für Humangenetik and [2]Institut für Rechtsmedizin, Universität des Saarlandes, W-6550 Homburg/Saar, Germany

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Summary. The present report describes a novel approach for the identification of human or non-human specimens after long-term storage in a badly preserved state. The application of the PCR-technique (polymerase chain reaction) using human-specific primers as well as Southern blot (filter) hybridization of the sample DNA to a primate-specific DNA probe enabled us to extend the positive identification beyond the limits of conventional methods such as serological or morphological examinations.

Key words: Species identification – Long-term storage – Human or non-human origin – PCR – Southern blot hybridization

Zusammenfassung. Der vorliegende Bericht beschreibt eine neuartige Strategie für die Identifizierung von menschlichen oder nicht-menschlichen Geweben nach Langzeitlagerung in schlecht erhaltenem Zustand. Die Anwendung der PCR-Technik (Polymerase Kettenreaktion) mit Hilfe humanspezifischer Starter sowie mit Hilfe der Southern Blot-Hybridisierung der Proben-DNA mit einer Primaten-spezifischen DNA-Sonde versetzte uns in die Lage, die positive Identifizierung jenseits der Nachweisgrenzen konventioneller Methoden, wie serologische und morphologische Untersuchungen auszudehnen..

Schlüsselwörter: Spezies-Identifizierung – Langzeitlagerung – Menschliche oder nicht-menschliche Herkunft – PCR – Southern-Blot-Hybridisierung

Introduction

Several samples were received by the Institute of forensic medicine for analysis to determine the species origin of the tissues. The material had been stored in an aromatic alcoholic fluid, possibly a perfume-like solution (Eau de Cologne) for at least 6 months in the refrigerator. A glass vessel containing the specimens had been left behind by a person who was known to be violent and who was on the run. Considering this history and the possibility of an act of violence, it became necessary to determine the origin of these specimens to decide further legal steps. The tissues were an eye with some attached pieces of muscles, 4 teeth and one less defined tissue fragment, probably an earlobe. The morphology of the teeth clearly proved their human origin, probably from an adult individual over 35 years of age. Morphological and immunohistological investigations of the soft tissues yielded no clear results due to decomposition caused by the unusual method of conservation. The few remaining characteristics were not species-specific. Further serological examinations [croos immuno-electrophoresis using human specific antisera; subtyping of group specific component (GC) by isoelectric focusing] led to inconclusive results. Therefore, our task was to analyze the species origin of the 2 remaining specimens.

Correspondence to: R. Müllenbach at the present address: Institut für Anthropologie und Humangenetik, Universität Tübingen, Wilhelmstrasse 27, W-7400 Tübingen, Germany

Materials and methods

Blood typing. Serological analysis was performed by (i) crossover immuno-electrophoresis [1] using a dilution series of antisera [anti-human Ig, heavy chain specific, Atlantic Antibodies, Hamburg) and (ii) subtyping of group specific component (GC) by isoelectric focusing [2].

PCR analysis. 100 ng of template DNA and a 2 μM concentration of primer IV (5'GAGAATTCGCGACAGAGCGAGACTCCG-TCTC-3') [3] were used per reaction in standard TaqI polymerase buffer (50 mM KCl, 10 mM Tris HCl, pH 8.3, 1.5 mM $MgCl_2$ and 0.01% gelatin) and 200 μM of dATP, dCTP, dTTP and dGTP. The initial denaturing step (10 min at 94°C) was followed by 36 cycles of annealing for 30 s at 57°C, extension for 2 min at 72°C and denaturing for 45 s at 94°C. The final extension period was lengthend to 10 min. Aliquots from the reaction were analysed on a 2% agarose gel, DNA was stained by ethidium bromide and visualized by UV.

DNA hybridization. DNA was extracted using a non-phenol method developed in our laboratory [4], involving only one transfer to a new tube and thereby minimizing additional shearing as well as loss of limited material. Aliquots of each extraction were analyzed on a 2% agarose minigel and transferred to Genescreen nylon membrane (Dupont NEN) by alkali blotting followed by extensive neutralisation and UV-crosslinking according to standard methods [5] or the manufacturers' instructions. The DNA probe D22Z3 [6, 7] was labeled by nick translation [8] and hybridized to the filter overnight at 65°C in Church buffer (500 m*M* Na_2HPO_4/NaH_2PO_4 buffer pH 7.2, 1 m*M* EDTA, 7% SDS) [9]. The filter was washed in 250 m*M* and 100 m*M* phosphate buffer, pH 7.2 at 65°C for 15 min each. Autoradiography was done overnight at −70°C using amplifying screens and Kodak XAR film.

Controls. As a positive control for all techniques performed, a human earlobe was kept in an aliquot of the storage solution for 4 months prior to DNA extraction. An additional sample of human muscle was used for all serological examinations. For DNA studies, DNA freshly extracted from human placenta and canine and porcine tissues was included.

Results

DNA extraction resulted in randomly degraded fragments showing a smear in gel electrophoresis. In the critical samples the size distribution varied from 2 kb (lane 2) and 10 kb (lane 3) down to less than 100 bp in all tissues (Fig. 1a).

Due to degradation and very limited yields of DNA a direct Southern blot of the control agarose gel was performed. Hybridization with the probe D22Z3 resulted in positive signals in DNA lanes from the human control earlobe (Fig. 1b, lane 1), the presumed ear fragment of unknown origin (lane 2) but not the eye muscle (lane 3).

PCR with Alu-primer IV yielded visible products from human placenta DNA, even when it was sheared by sonication to fragments of 2–0.2 kb and thus adjusted to the fragment length of the DNA to be probed (Fig. 2, lane 7). From the investigated tissues, only the human control sample (Fig. 2, lane 4) and the presumed ear sample of unknown origin (lane 5) showed PCR products. The eye muscle (lane 6) and the porcine and canine control samples were negative (lanes 2 and 3).

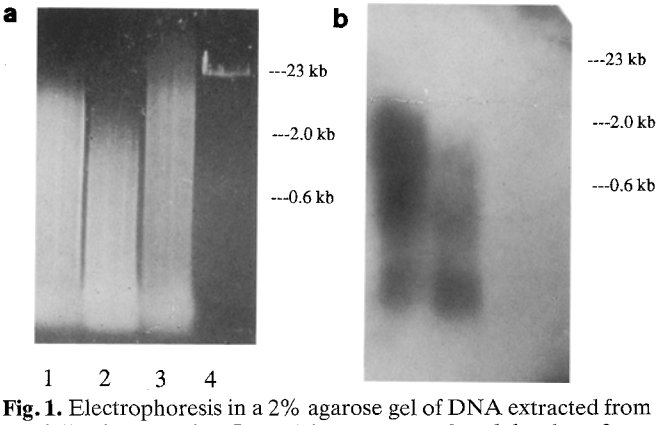

Fig. 1. Electrophoresis in a 2% agarose gel of DNA extracted from the following samples: Lane 1 human control earlobe; lane 2 unknown tissue sample; lane 3 eye muscle; lane 4 undigested lambda-DNA (500 ng). **b** Southern blot of the gel in Fig. 1a hybridized to probe D22Z3 showing positive signals (visible as a smear) in lanes 1 and 2 but no hybridization reaction and signal in lane 3

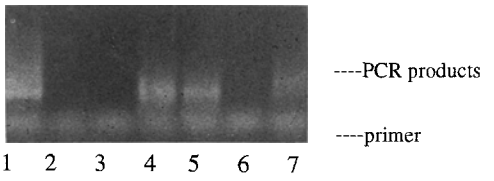

Fig. 2. Electrophoresis in a 2% agarose gel of PCR products from the following samples: Lane 1 human placenta DNA as control; lane 2 porcine DNA; lane 3 canine DNA; lane 4 DNA from human control earlobe; lane 5 unknown tissue sample; lane 6 eye muscle; lane 7 sonicated human control DNA

Thus we had confirmed the human origin of the presumed ear fragment and the non-human origin of the eye by 2 different techniques, confirming that the PCR products were not falsified by contamination or wrong experimental conditions.

Discussion

PCR using Alu-primers has proved to be a valuable tool in the generation of region-specific DNA libraries [3]. We took advantage of a special property of primer IV to specifically amplify human sequences from a rodent background in somatic cell hybrids. This specificity is based on the observation that although most parts of the ubiquitous Alu-sequences are homologous in many mammalian species, the 3′-region displays interspecies variation. Therefore, a human-specific oligonucleotide from this region was synthesized as a priming molecule. Control experiments proved that bovine and canine DNA were not amplified.

Our second approach, which was added to avoid false conclusions caused by possible PCR artifacts, was based on the exclusive presence of a centromeric repetitive DNA sequence in primate genomes. For many years, chromosome-specific repetitive DNA probes have been used for the localization of translocated parts of chromosomes, additional chromosome analysis, clinical diagnosis and recently, even in studies of interphase nuclei [10]. In our case, a repetitive probe was the only appropriate tool as it resulted in distinguishable positive and negative reactions in spite of the limited amount and highly degraded state of the probed samples.

Here, we describe a novel application of both techniques for very different means. The determination of human or non-human origin of DNA in a badly conserved state might be of general interest in forensic analysis. In this case immunological as well as morphological examinations did not aid in species identification. However, molecular biology allowed a new approach.

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