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Identification of animal skin of historical parchments by polymerase chain reaction (PCR)-based methods

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ABSTRACT

This study deals with establishing of a PCR-based strategy with the aim to recognize the animal origin of different historical parchments. This is one of relatively rare studies on the analysis of ancient DNA from parchments. Robustness of the PCR technology is demonstrated by successful identification of the animal species using only a small amount of DNA isolated from 12 parchment samples. Ten PCR-based assays specific for the detection of different animal species (*Bos taurus*, *Ovis aries*, *Capra hircus*, *Sus scrofa*, *Oryctolagus domestica*, *Cervus elaphus*, *Capreolus capreolus*, *Dama dama*) and two PCR assays utilizing universal primers were evaluated and optimized with the aim to find a rapid parchment identification method, which would be more reliable than the classical microscopic examination. The optimized PCR methods produced satisfactory results. Out of 12 investigated parchments, 9 items were unambiguously identified, DNA from 2 samples could not be amplified with any of the species-specific PCR assays, and only one parchment produced controversial results. The species-specific PCR results were confirmed by direct sequencing and PCR cloning with consequent sequencing. Our approach, including isolation of parchment DNA by chaotropic solid-phase extraction, optimization of the PCR programs and high-stringency annealing temperatures, demonstrated to be effective, easy and reliable for the analysis of historical parchment DNA. We consider this PCR-based strategy potentially useful also for investigation of other types of animal items conserved in museums, galleries or libraries.

1. Introduction

Parchment was for many centuries the most common writing material. It was the vehicle of culture and information in all European countries at least until the end of the Middle Age, when it was gradually substituted by paper. Parchment is usually made from calfskin, sheepskin and goatskin, but other animal skins were used for this purpose as well (Oltrogge and Fuchs, 1989; Moog, 1991; Burger, 2002; Kennedy and Wess, 2003).

The animal species identification of parchments is interesting for several practical restoration and conservation reasons. For example, to see whether it is better to use a specific kind of parchment to restore the historical ones or whether, for quality reproduction, the same, identical parchment as used originally, should be applied in every case. Anyway, it is very important to know what the palaeographic characteristics of the parchment under study is, including the exact identification of animal that was used for its manufacturing.

The traditional optical microscopic analysis, based on examination of hair follicle pattern, veining, natural scars and bruises and, in certain skins, fat deposits, is a common technique of recognition and identification of the animal used for the production of parchment (Reed, 1972; Fuchs et al., 2001). However, this technique has some limitations: for example in case when only very small parchment samples are available for analysis, or when the parchment surface conditions do not permit reliable microscopic analysis for reason of quality degradation such as extensive scrapping of the grain layer or its surface being covered by colours and decorations. In these cases, it is convenient to perform a suitable DNA analysis, and polymerase chain reaction (PCR) method seems to be a promising tool for this purpose.

Until now, only a few scientific publications dealt with this topic, while all of them warned about the correct manipulation of ancient DNA (aDNA) in order to avoid external contamination and the need to prepare it well for subsequent PCR analysis (Burger, 2002; Poulakakis et al., 2007).

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The aDNA on parchment could be degraded for various causes, such as i) chemical processes during parchment preparation (lime-water bath for example), ii) the age of parchment, iii) and many alterations that parchment could have suffered during the centuries as a result of human manipulation or inadequate conservation. It is therefore useful to extract the aDNA the way that can guarantee satisfactory DNA purification as a precondition for reliable PCR analysis (Kemp et al., 2006).

Considering the possible aDNA degradation of parchment, it is convenient to amplify a small fragment of DNA (from 100 to 200 bp). Many researches on aDNA demonstrated that it is useful to orient the PCR detection to mitochondrial DNA (mtDNA), because each cell contains multiple copies of it, and mtDNA seems more resistant and therefore less degraded than the chromosomal one (Robin and Wong, 1988; Bonnichsen et al., 2001; Vuissoz et al., 2007).

The aim of this work was to establish a simple, rapid and reliable PCR-based strategy in order to obtain effective recognition tool capable to complement and surpass the classical microscopic examination for parchment animal species identification. The specificity of 10 different PCR assays, based on species-specific and universal primers, was evaluated by the investigation of 12 historical parchments.

2. Materials and methods

2.1. Parchments, meat samples, and contamination precautions

The parchment samples were provided by the Slovak National Library (Table 1). The animal meat (cow, goat, sheep, pig, rabbit, red deer, roe deer, fallow deer) used for optimization of different PCR assays, were obtained in various butchers' shops.

Standard precautions were taken to minimize the risk of contaminating ancient material with modern DNA. The DNA manipulation, the preparation of PCR master mixes (in laminar flow cabinets) and the consequent PCR products electrophoresis have taken place in two laboratories (one for the aDNA analysis and the other for the modern DNA treatment) in two separate buildings. The aDNA and meat DNA were extracted using independent DNA extraction kits. Deionised water completely free of DNase and RNase (MP biomedicals, Solon, OH, USA) was used in all experiments. For each parchment DNA extracted, separate extraction blank was incorporated. All tubes, including the PCR tubes, were washed with Diethyl Pyrocarbonate (MP biomedicals) and then autoclaved and UV-irradiated. Work surfaces were frequently cleaned with bleach and UV-irradiated before and after each experiment. Filter tips were used for all pipetting activities. Reagents were stored in small aliquots, used once and discarded. Three no-template PCR reactions, with water instead of template DNA, were included in every PCR setup.

Table 1
Parchment samples.

Samples	Characteristics	DNA concentration pg/μl
L2-A	Unknown date; historical parchment bookbinding.	133
L2-B	Unknown date; historical parchment bookbinding.	211
C 402	XIII – XIV century; Glagolitic script.	153
J 555/1	XII century; fragment from antiphonary; coloured decorations.	178
J 1159	XV century; fragment from parchment book with rich coloured decorations.	77
J 1741	1709; parchment letter with wax seal.	47
J 1745	1659; parchment letter.	106
J 1748	Unknown date; parchment letter.	72
J 1749/1	Unknown date; parchment letter with wax seal.	307
J 2043/2	XIV century; parchment fragment from bible.	53
J 2733	1909; Doctor of Medicine printed diploma.	84
J 3547/1	Unknown date; fragment from Talmud.	88

2.2. DNA extraction

The DNA from parchment and meat samples was extracted by chaotropic solid-phase extraction (SPE) using Qiagen DNeasy Blood and Tissue kit (Qiagen, Hilden, Germany), which employs proprietary silica columns. The protocol for animal DNA extraction was used.

Small parchment pieces (from 3.5 to 5 mg) were mixed with 180 μl of lysis buffer ATL and 20 μl proteinase K, vortexed, and incubated at 56 °C until the tissue was completely lysed (3 h proved sufficient). After the incubation, 4 μl of RNase A (100 mg/ml) were added to the mixture, mixed by vortexing, and incubated for 2 min at room temperature. Consequently, 200 μl of buffer AL and 200 μl of ethanol (96–100%) were added to the sample and mixed thoroughly by vortexing. The new mixture was pipetted into the DNeasy Mini Spin Column and the DNA washing procedure recommended by the manufacturer, was performed. The only modification regarded the elution of DNA – two consequent elution steps with 30 μl of AE buffer were used. The DNA concentration was determined by using Quant-iT PicoGreen Assay (Invitrogen, Gaithersburg, MD, USA) and fluorescence measurement at 492 nm/520 nm in a Saphire2 microplate reader (Tecan, Grödig bei Salzburg, Austria).

2.3. PCR

The PCR assays were optimized using the DNA from meat samples; the same PCR protocol was also utilized for parchment samples.

The PCR mixture contained 25 pmol of each primer (Table 2), 200 μmol/l of each dNTP, 1.5 U *Taq* DNA polymerase (HotStarTaq plus, Qiagen), 1× PCR buffer, and 3 μl of the template DNA solution

Table 2
PCR primers used.

Species; gene target; reference	Primer sequence
Bos taurus; mtDNA; Shapiro et al., 2004	178F – GCCCCATGCATATAAGCAAG 309R – GCCTAGCGGGTTGCTGGTTTCACGC
Sus scrofa; mtDNA; Lahiff et al., 2001	Pi-fw – GCCTAAATCTCCCCTCAATGGTA Pi-rv – ATGAAAGAGGCAAATAGATTTTCG
Oryctolagus domestica; *cytochrome B*; Yang et al., 2005	F38 – TTGTTAACCACTCCCTAATTGACCT R233 – AGTCAGCCGTAGTTTACRTCTCG
Ovis aries; *cytochrome B*; Newman et al., 2002	Sh-Fw – TATACCCCTCCTCCATACATCA Sh-Rv – GTAGGGGTGTTCAACTGGCTGG
Cervus elaphus, 12S rRNA; Fajardo et al., 2007	12SCE-FW – CAAAAATATATAACGAAAGTAACTTTACAACC 12SCERV-REV – AAAGCACCGCCAAGTCCTT
Capreolus capreolus, 12S rRNA; Fajardo et al., 2007	12SCC-FW – TGAAAATAGATAACGAAAGTAACTTTAAAATA 12SCERV-REV – AAAGCACCGCCAAGTCCTT
Dama dama, 12S rRNA; Fajardo et al., 2007	12SDD-FW – TAAACAACGAAGGTAACCTTATAG 12SCERV-REV – AAAGCACCGCCAAGTCCTT
Capra hircus; *cytochrome B*; Newman et al., 2002	Go-Fw – TCAATCCTAATCTTAGTACTTG Go-Rv – GAGTGTTAATAGATCTGCTACC
Universal primers; *cytochrome B*; Newman et al., 2002	Uni-Fw – TCCCCAACAAACTAGGAGG Uni-Rv – ACTGGTTGTCCTCCAATTCA
Universal primers; *cytochrome B*; Irwin et al., 1991	L15684 – CTCCACACATCCAAACAACG H15760 – TGTTCGACTGGTTGTCCTCC