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AB0 blood group genotyping of ancient DNA by PCR-RFLP

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Abstract The paper presents an PCR-RFLP-based method to determine AB0 blood groups at the genotype level. In order to ensure the applicability of the method to severely degraded DNA, new sets of primers were designed that amplify 103/104 bp and 64 bp sequences on exon 6 and exon 7 of chromosome 9, respectively. The amplification of the two PCR products and the subsequent RFLP analysis with four endonucleases was revealed to be an effective and reliable way to determine AB0 bloodgroups at the genotype level, distinguishing the alleles A, B, 0_1, 0_{1v}, and 0_2. PCR analysis of severely degraded sample material may possibly require higher cycle numbers. Therefore, the experiments presented here including those on positive control samples, were carried out employing 45 amplification cycles in order to ensure the validity of the amplification and RFLP analysis. As positive controls, small amounts of modern intact DNA extracted from saliva samples of 12 individuals with known AB0 phenotypes were used. The protocols for the AB0 typing were then applied to ancient degraded DNA extracted from 15 archaeological bones and teeth about 250 and 3,000 years old, respectively. The results presented for the archaeological sample material are based on repeated analysis derived from two independently processed DNA extracts of each sample. Moreover, the authentification process for the results derived from the archaeological samples included repeated multiplex STR genotyping of the extracts, showing the genetic uniqueness of the extracts which is the strongest possible indicator for the authenticity of an unknown DNA sample. Additionally, it was possible to compare the STR typing results to those from previous studies using the same material. Both the AB0 typing and the STR typing revealed fully reproducible results in all cases.

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Introduction

The scientific interest in human identification and palaeoserology initiated the very first attempts to analyse forensic evidence and archaeological materials on a molecular level. The analysed sample materials were mummified soft tissues, keratinous tissues, body fluids and bone (e.g. Boyd and Boyd 1933; Kirst et al. 1971; Borgonini Tarli 1980). In particular, the analyses focused on the determination of AB0 bloodgroups. Besides the aspects of human identification from forensic evidence material, paleopathology was strongly interested in AB0 traits since they are thought to be closely related to an individuals suceptibility to infectious diseases. In general, it is bloodgroup 0 which seems to increase an individuals risk to severely suffer from an infectious agent as is demonstrated by the empirical findings on morbidity and mortality rates of many epidemiological studies of modern populations (e.g. van Loon et al. 1991; Faruque et al. 1994; Swerdlow et al. 1994). However, the serological methodology that was used was finally shown to reveal unreliable results when applied to decayed biological material (e.g. Berg et al. 1983). In contrast, PCR-based methods of determining genetic markers are known to reveal valid results even from degraded biological sample materials.

AB0 blood group typing of forensic evidence material by means of PCR was made possible by the description of the molecular genetics of the AB0 blood group system even down to the subgroup level (Yamamoto et al. 1990; Lee and Chang 1992; Grunnet et al. 1994; Fukumori et al. 1995; Nishimukai et al. 1996; Ringel et al. 2000; Tsai et al. 2000; Yip 2000). AB0 genotyping may reveal invaluable data in certain identification cases although genetic typing by short tandem repeats (STRs) generally provides a superior discrimination power. This was shown through the analysis of the remains of victims of a plane crash where whole families died (Hsu et al. 1999). The identifi-

Table 1 Sequence polymorphisms, PCR and RFLP product lengths for the different AB0 alleles

[a]Nomenclature of the allelic variants of blood group 0 follows Nishimukai et al. 1996.
del deletion.
Bold faced letters represent the genotypes that are RFLP digested
bp basepairs, + restriction site, *np* nucleotide position

Chromosome 9	Exon 6		Exon 7	
PCR product	103/104 bp		64 bp	
Restriction endonuclease	Rsa I	HpyCH4IV	Nla III	Mnl I
RFLP products	66+37 bp	73/72+31 bp	37+27 bp	42/41+23/22 bp
Allele[a]	np 261	np 297	np 796	np 802
A	G	A	C	G
B	G	**G**	**A**	G
0_1	**del**	A	C	G
0_{1v}	**del**	**G**	C	G
0_2	G	**G**	C	**A**

cation was hindered by the lack of living relatives and of ante-mortem samples. Instead, the AB0 genotyping results of the victims were compared to their AB0 records.

With this paper we present an improved PCR-based method to determine AB0 bloodgroups from degraded DNA at the genotype level. By involving only two amplification products of 103/104 basepairs and 64 basepairs in length and 4 RFLP analyses, the amount of sample material that is necessary is reduced considerably. Additionally, due to the short length of the amplification products the analyses were also successful in those samples where, for example a full STR typing failed. The newly designed primers and the RFLP analysis were initially tested on modern intact DNA extracted from saliva samples of individuals with known AB0 phenotype. The analysis was then successfully transferred to severely degraded archaeological skeletal material.

AB0 bloodgroups at the DNA level

The molecular genetics behind human AB0 bloodgroups are defined by a number of sequence polymorphisms located on exon 6 and exon 7 of chromosome 9 (Nishimukai et al. 1996; Ringel et al. 2000; Grunnet et al. 1994; Tsai et al. 2000; Yip 2000). At least 13 sequence polymorphisms that code for the different amino acids determining the AB0 serological traits have been well investigated. Based on the findings of these studies that investigated large numbers of individuals from different human populations, we chose four sequence polymorphisms for the analysis from degraded material, that fulfil the following criteria:

- Discrimination between the alleles coding for the genotypes of bloodgroups A, B, and 0
- Location on the exon sequences as near as possible in order to allow short amplification products
- Allows RFLP analyses of the PCR amplification products, i.e. restriction endonucleases for the particular sequence must be commercially available.

The sequence polymorphisms at the nucleotide positions listed in Table 1 fulfil these criteria and enable a discrimination between alleles coding for A, B, 0_1, 0_{1v} and 0_2. For amplification, two PCR reactions are performed with product lengths of 103/104 bp and 64 bp, respectively. The amplification products are analysed by two separate RFLP digestions (Table 2).

Table 2 Restriction patterns of AB0 genotypes

Phenotype	Genotype	Rsa I	HpyCH4IV	Nla III	Mnl I
A	AA	–/–	–/–	o	o
A	$A0_1$	+/–	–/–	o	o
0	0_10_1	+/+	–/–	o	o
0	0_10_{1v}	+/+	+/–	o	o
0	$0_{1v}0_{1v}$	+/+	+/+	o	o
A	$A0_2$	–/–	+/–	–/–	+/–
AB	AB	–/–	+/–	+/–	–/–
0	0_20_2	–/–	+/+	–/–	+/+
B	$B0_2$	–/–	+/+	+/–	+/–
B	BB	–/–	+/+	+/+	–/–
A	$A0_{1v}$	+/–	+/–	–/–	–/–
0	0_10_2	+/–	+/–	–/–	+/–
B	$B0_1$	+/–	+/–	+/–	–/–
0	$0_{1v}0_2$	+/–	+/+	–/–	+/–
B	$B0_{1v}$	+/–	+/+	+/–	–/–

–/– PCR product remains undigested.
+/– one allele of the PCR product is digested, the other remains undigested.
+/+ PCR product is completely digested.
o not necessary for analysis, since the Rsa I / HpyCH4IV patterns are already unique. If carried out the pattern for both of the enzymes is –/–.

Actually, the most common genotypes (AA, $A0_1$, 0_10_1, 0_10_{1v} and 0_{1v} 0_{1v}) can already be identified by two RFLP analyses of the first PCR amplification product. Only if genotype $A0_{1v}$ or any genotype involving a B or an 0_2 allele are present, is it necessary to perform the second PCR and RFLP. That is due to the fact that allele 0_2 could be misinterpreted as B if it occurs in the respective allelic combinations.

Materials and methods

Materials

The AB0 bloodgroup analysis was carried out on archaeological skeletal material from an early modern cemetery (Bramanti et al. 2000a, 2000b) and a Bronze age cave (Hummel and Schultes 2000; Schultes 2000; Schultes et al. 2000). The DNA extracts were prepared from compact bone samples derived from femora and from tooth roots. The extracts were STR genotyped with the AmpF*l* STR Profiler Plus of Applied Biosystems (Weiterstadt) in order to demonstrate the authenticity of the extracted ancient DNA (aDNA).

From the early modern site with burials of about 250 years old, we randomly chose 8 of the 30 individuals which had already

revealed reproducible autosomal STR fingerprints earlier (cf. Bramanti et al. 2000a). From the Bronze age site, which is about 3,000 years old, we took 7 individuals that had been reproducibly typed by autosomal and mitochondrial haplotypes, and in case of male individuals by Y-chromosomal STRs earlier (cf. Schultes 2000; Schultes et al. 1999).

The preliminary tests for adapting and optimising all amplification and restriction enzyme analysis parameters with the newly designed primers were performed on DNA extracts prepared from saliva samples of 12 individuals with known AB0 phenotypes (A, B, 0, and AB). Within those samples we found all types of alleles that were planned to be discriminated although not all possible genotypes (allele combinations) were represented.

DNA extraction and DNA yields

The treatment of the ancient samples followed an established laboratory routine. For sample preparation, the outer surfaces of the bone samples were removed. After grinding the samples to a fine powder, aliquots of about 0.3 g of bone powder were suspended in 0.5 M EDTA for 24 h. The supernatants were transferred to an automated nucleic acid extractor (Gene Pure Typ 341A, Applied Biosystems) which performs a proteinase K digestion followed by phenol and chloroform extraction. The DNA is concentrated by binding to silica powder (Glassmilk, Dianova) in the presence of isopropanol and sodium acetate. The Glassmilk-DNA was collected on filtration membranes, washed with ethanol and then eluted into sterile water (Ampuwa, Fresenius; for details of sample treatment and DNA extraction see e.g. Lassen et al. 1994; Baron et al. 1996; Schultes et al. 1999). The yields of the ancient DNA (aDNA) extracts were determined by UV spectrophotometry in order to prevent amplification failures due to DNA overloads which may occur independently of the taxonomic origin of the DNA. Additionally, the extracts were separated by electrophoresis in order to check for possibly remaining humic acids that would inhibit the amplification process.

The DNA extraction from the saliva samples was done by the Chelex 100 method basically following the protocol of Walsh et al. (1991).

Authenticity of ancient DNA

Precautions commonly taken for aDNA work (i.e. the use of protective clothing and the processing of various types of control samples such as mock extractions and no-template samples) were followed during all steps of sample preparation, DNA extraction and amplification in order to minimise the risk of false positive results due to modern contamination (Schmidt et al. 1995). All archaeological sample material was extracted twice in independent processes. Additionally, the extracts were STR typed through multiplex PCRs employing the AmpF*l*STR Profiler Plus amplification kit (Applied Biosystems). Due to the individual-specific nature of STR-based genetic fingerprints, any possible contamination that might still have entered the analysis process at any stage could have been identified positively through the STR typing (Hummel et al. 2000) that was performed on all extracts used for AB0 typing. Additionally, the STR genotypes were compared to those that had been processed from the same samples during earlier studies (e.g. Bramanti et al. 2000a; Schultes et al. 2000). All AB0 and STR typing results were based on at least two amplification results from the independently prepared aDNA extracts.

By carrying out the authentification directly on the extract and by individualising the data in a way that enables identification and direct assignment, this authentification strategy is superior to other commonly practiced strategies (Hummel in press) which are based on proxy-data (e.g. amino acid racemization, morphological features) and which, e.g. by involving a second laboratory, are designed to serve the needs of mitochondrial DNA analysis in case studies, i.e. non-individual specific marker analysis of a single sample (e.g. Hofreiter et al. 2001).

Primers

The sequences of the ABO bloodgroup alleles were downloaded from GenBank (http://www.ncbi.nlm.nih.gov/Genbank/index.html). EDIT SEQ and MEG ALIGN of the software package DNA STAR were used for sequence alignments and the primer design was performed with PRIMER SELECT. The primer sequences including nps 261 and 297 are:

- AB0-exon 6-upper: 5' – ctctctccatgtgcagtaggaagg
- AB0-exon 6-lower: 5' – gaactgctcgttgaggatgtcg

The amplification product including nps 796 and 802 of exon 7 is partially identical with that of Nishimukai et al. (1996). The primer sequences for exon 7 including np 796 and np 802 are:

- AB0-exon 7-upper: 5' – aaggacgagggcgatttcta
- AB0-exon 7-lower: 5' – gctgcaactcttgcaccgacc

Amplification

The amplifications for the AB0 determinations from exon 6 and exon 7 sequences were carried out as singleplex reactions in a 50 µl reaction mixture. Both amplification mixes contained the same concentrations of the following components: 50 mM KCl, 10 mM Tris-HCl (Buffer II, Perkin Elmer Cetus), 1.5 mM $MgCl_2$ (Perkin Elmer Cetus), 200 mM dNTPs (Boehringer, Mannheim), 0.12 µM of each primer of the respective pair (AB0-exon 6-upper/lower or AB0-exon 7-upper/lower) and 2.5 U AmpliTaqGold. The amount of DNA was variable: for the ancient samples it varied between 2–10 µl DNA extract depending on the total DNA yield (i.e. indigenous DNA of the individual and possible DNA derived from microorganisms) of the samples (30–250 ng/µl); for the saliva samples 1 µl (90–140 ng/µl) was used. The reaction volumes were made up to 50 µl with DNA-free water (Ampuwa).

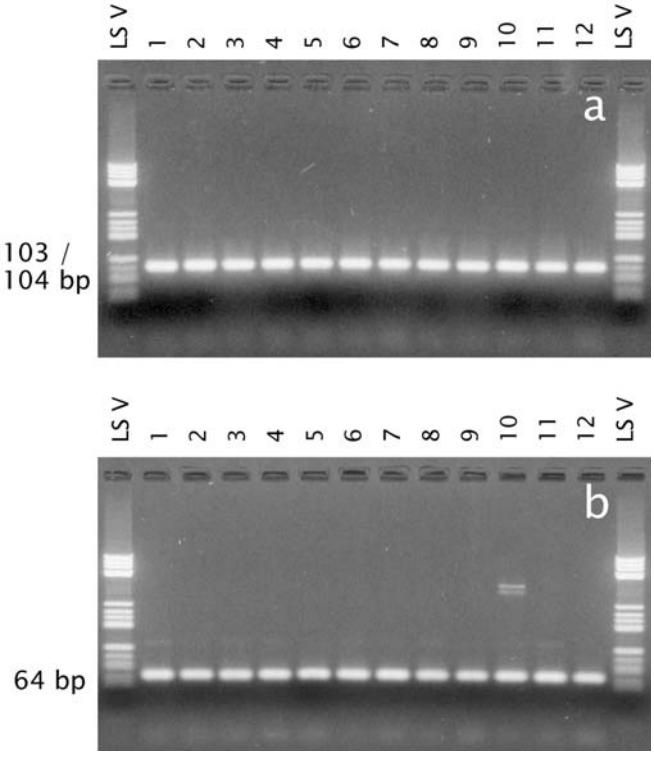

Fig. 1a, b Amplification products (8 µl) of the DNA extracted from saliva samples. **a** The 103/104 bp products are amplified from the exon 6 sequence, **b** the 64 bp products from the exon 7 sequence

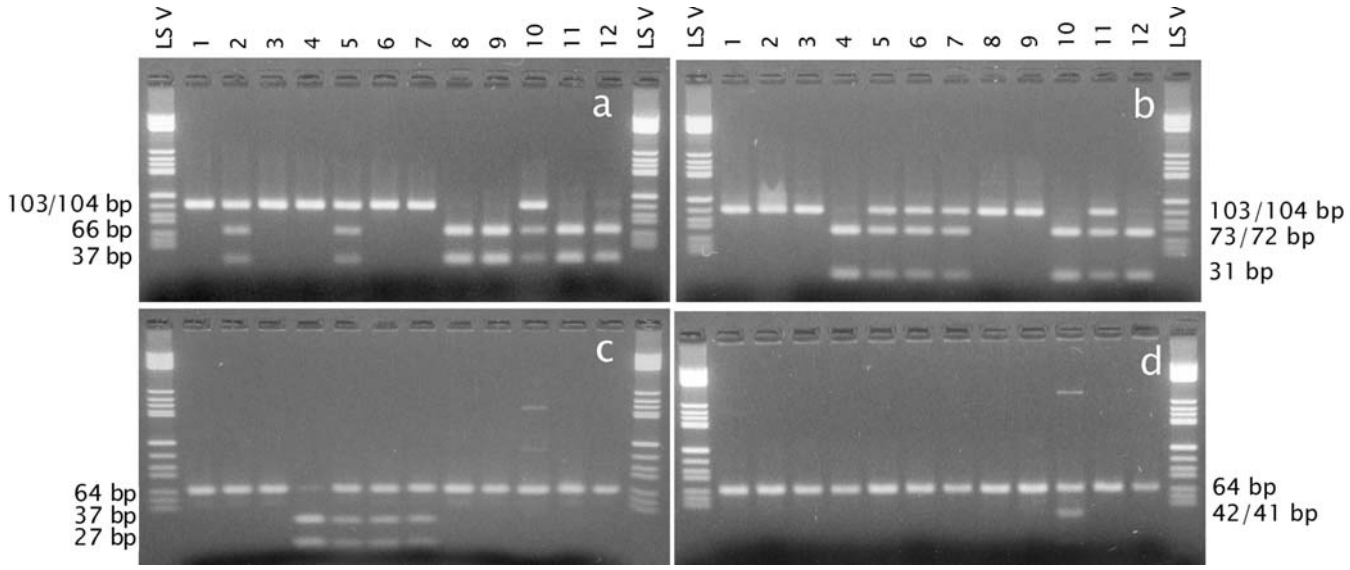

Fig. 2 RFLP analysis of the saliva sample amplification products by the endonucleases **a** Rsa I, **b** HpyCH4IV, **c** Nla III, **d** Mnl I

The control amplifications employing the AmpF*l*STR Profiler Plus and the respective analyses (e.g. electrophoresis conditions and allele determinations) were performed as described by Bramanti et al. (2000a) or Schultes et al. (2000).

The PCR amplifications were carried out in a DNA Thermal Cycler 480 (PE Applied Biosystems), starting with an initial heating to 94°C for 11 min followed by 45 amplification cycles. The large amount of PCR product that was generated from ancient and modern samples by this high cycle number enabled vizualisation of even the lowest amounts of restriction products after the RFLP analysis although even fewer cycles (35) showed amplification products in most of the samples. The annealing temperatures for the primer pairs are different: the cycling parameters for AB0 exon 6-upper/lower consists of 94°C for 1 min, 56°C for 1 min and 72°C for 1 min, while for AB0 exon 7-upper/lower it consists of 94°C for 1 min, 53°C for 1 min and 72°C for 1 min.

The electrophoresis of the PCR products was carried out in 2.5% agarose gels (Agarose Ultra-Qualität, Roth).

RFLP analysis

All restriction enzymes with the respective buffer solutions were purchased at New England Biolabs (Frankfurt a.M.). Deviating from the manufacturers instructions, the restriction analyses were carried out in a diluted reaction medium by using water (Ampuwa) and elongating the incubation time. These measurements proved to enhance the specificity of the restriction results. The digestions were performed as follows:

- Rsa I: 2 µl enzyme, 2 µl NEB1 buffer, 3 µl Ampuwa, 5–7 µl amplification product
- HpyCH4IV: 2 µl enzyme, 2 µl NEB1 buffer, 3 µl Ampuwa, 5–7 µl amplification product
- Nla III: 2 µl enzyme, 2 µl NEB4 buffer, 3 µl Ampuwa, 1.5 µl BSA (10 mg/ml), 5–7 µl amplification product
- Mnl I: 2 µl enzyme, 2 µl NEB2 buffer, 3 µl Ampuwa, 1.5 µl BSA (10 mg/ml), 5–7 µl amplification product.

All incubations were carried out for 3 h at 37°C. For Mnl I this step was followed by 1 h at 65°C.

The electrophoresis of the total amounts of the Rsa I and HpyCH4IV restriction products was carried out on 3.8% agarose gels, the analysis of the Nla III and Mnl I restriction products on 4.4% agarose gels (Agarose Ultra-Qualität, Roth).

Results and discussion

The preliminary tests for optimising and validating the high cycle number amplifications and restriction enzyme

Table 3 AB0 genotype determination for the positive control samples

Saliva sample nr.	Phenotype (previously known)	Restriction analysis results				Genotype (analysed)
		Rsa I	HpyCH4IV	Nla III	Mnl I	
1	A	+/–	–/–	–/– (o)	–/– (o)	$A0_1$
2	A	+/–	–/–	–/– (o)	–/– (o)	$A0_1$
3	A	–/–	–/–	–/– (o)	–/– (o)	AA
4	B	–/–	+/+	+/+	–/–	BB
5	B	+/–	+/–	+/–	–/–	$B0_1$
6	AB	–/–	+/–	+/–	–/–	AB
7	AB	–/–	+/–	+/–	–/–	AB
8	0	+/+	–/–	–/– (o)	–/– (o)	0_10_1
9	0	+/+	–/–	–/– (o)	–/– (o)	0_10_1
10	0	+/–	+/+	–/–	+/–	$0_{1v}0_2$
11	0	+/+	+/–	–/– (o)	–/– (o)	0_10_{1v}
12	0	+/+	+/+	–/– (o)	–/– (o)	$0_{1v}0_{1v}$

–/– PCR product remains undigested.
+/– one allele of the PCR product is digested, the other remains undigested.
+/+ PCR product is completely digested.
–/– (*o*) carried out although not necessary for analysis, since the Rsa I/HpyCH4IV patterns are already unique (cf. Table 2).

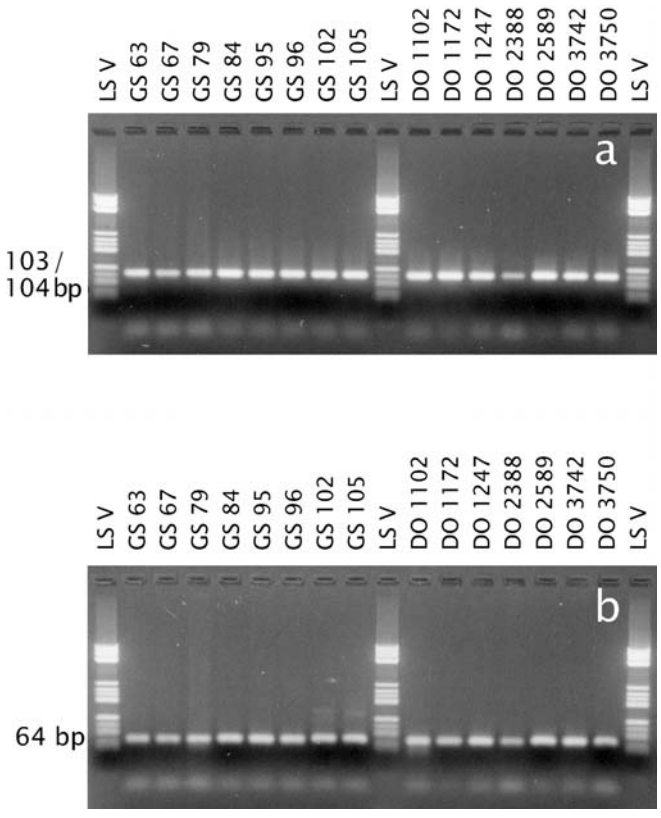

Fig. 3a, b Amplification products (9 µl) of the aDNA extracted from ancient skeletal samples. **a** The 103/104 bp products are amplified from the exon 6 sequence, **b** the 64 bp products from the exon 7 sequence. As for the STR typing the AB0 allele amplifications resulted in amplification products of slightly varying intensity reflecting the different amounts of intact target sequences

analysis parameters that were carried out on DNA extracts prepared from saliva samples with known AB0 phenotypes, revealed fully consistent and reproducible results. In all cases the amplification products were of the expected length (Fig. 1) and the restriction products that enabled the allelic genotypes to be determined (Fig. 2) corresponded to the serological phenotypes (Table 3). Since these findings on the positive controls were fully consistent not only with the serological genotypes but also with validated applications of PCR-RFLP strategies to determine AB0 blood groups from the respective point mutations on exon 6 and exon 7 (e.g. Grunnet et al. 1994; Nishimukai et al. 1996; Ringel et al. 2000; Tsai et al. 2000), it was not necessary to perform a direct sequencing analysis.

The amplification results of the degraded DNA samples are shown in Fig. 3. It can be seen that there are no unspecific amplification products and no extensive primer-dimer formation, which is a prerequisite for a reliable RFLP analysis.

The results of the restriction analyses of the aDNA amplification products are shown in Fig. 4. The analysis revealed fully reproducible restriction results for all individuals. The restriction analysis results and allele determinations are given in Table 4. The interpretation of the RFLP fragments derived from the exon 6 amplification product (103/104 bp) did not cause any difficulties. In principle, the same held true for the restriction analysis of the short exon 7 product (64 bp). This might be different if the amplification products would reveal extensive primer-dimer formation since this could be mistaken for the short length restriction products of Nla III and Mnl I. Therefore, the amplification reaction parameters must be kept to high

Fig. 4 RFLP analysis of the aDNA amplification products by the endonucleases **a** Rsa I, **b** HpyCH4IV, **c** Nla III, **d** Mnl I. The Rsa I and HpyCH4IV restriction analysis were carried out on 3.8% agarose gels. Difficulties in the interpretation of the restriction results may occur in cases of primer-dimer formation (~50 bp) as has happened in samples GS 79 and DO 1102 (also cf. Fig. 2), since they could be misinterpreted as a restriction product. However, if the electrophoretic separation is carried out on high percentage agarose gels (here 4.4%) the restriction products (GS 84 and GS 105) are distinguished unambigously

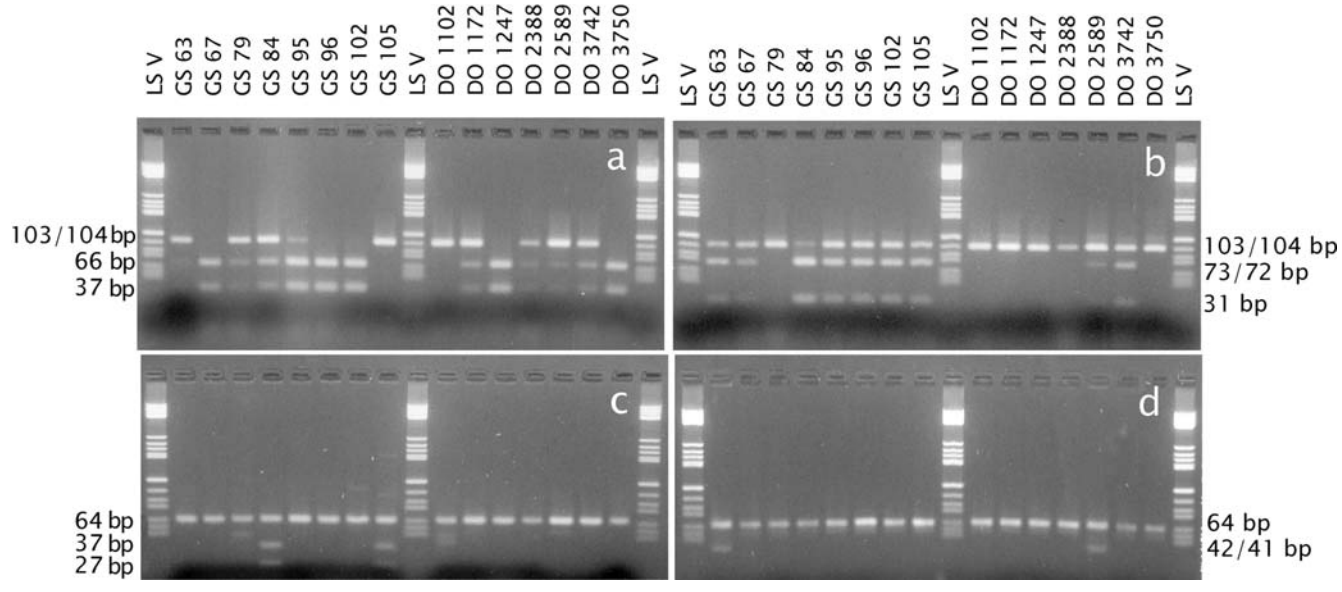

Table 4 AB0 genotype determination from ancient bone and teeth samples

Sample no.	Restriction analysis results				Genotype
	Rsa I	HpyCH4IV	Nla III	Mnl I	
GS 63	+/–	+/–	–/–	+/–	$0_1 0_2$
GS 67	+/+	+/–	–/– (o)	–/– (o)	$0_1 0_{1v}$
GS 79	+/–	–/–	–/– (o)	–/– (o)	$A0_1$
GS 84	+/–	+/–	+/–	–/–	$B0_1$
GS 95	+/–	+/–	–/–	–/–	$A0_{1v}$
GS 96	+/+	+/–	–/– (o)	–/– (o)	$0_1 0_{1v}$
GS 102	+/+	+/–	–/– (o)	–/– (o)	$0_1 0_{1v}$
GS 105	–/–	+/–	+/–	–/–	AB
DO 1102	–/–	–/–	–/– (o)	–/– (o)	AA
DO 1172	+/–	–/–	–/– (o)	–/– (o)	$A0_1$
DO 1247	+/+	–/–	–/– (o)	–/– (o)	$0_1 0_1$
DO 2388	+/–	–/–	–/– (o)	–/– (o)	$A0_1$
DO 2589	+/–	+/–	–/–	+/–	$0_1 0_2$
DO 3742	+/–	+/–	–/–	–/–	$A0_{1v}$
DO 3750	+/+	–/–	–/– (o)	–/– (o)	$0_1 0_1$

–/– PCR product remains undigested.
+/– one allele of the PCR product is digested, the other remains undigested.
+/+ PCR product is completely digested.
–/– (o) carried out, although not necessary for analysis, since the Rsa I/HpyCH4IV patterns are already unique (cf. Table 2).

stringency. Also, in the case of weak amplification products that are to be expected if fewer amplification cycles are carried out with extracts consisting of highly degraded DNA, it may be necessary to carry out the electrophoretic separation of the restriction products on PAA or Metaphor agarose gels instead, in order to ensure the clear visualisation of the restriction products.

The authenticity of the results was ensured by the control amplifications. All negative control samples included in STR and AB0 typing revealed neither products of the expected length nor unspecific products. Additionally, the STR genotyping revealed individual and reproducible allele determination results from the two independent extracts and amplifications each (Table 5). By the individuality of the STR typing results any systematic contamination through laboratory handling or carry-over can be excluded. Therefore, the extracts used for the AB0 typing were considered to consist of authentic ancient DNA. Furthermore, all STR typing results matched those of earlier studies. The possibility of non-systematic contamination in the individual PCR reactions can be considered to be highly unlikely due to the full reproducibility of the AB0 genotyping results.

In total, the amplification of two PCR products and the subsequent RFLP analysis with four endonucleases re-

Table 5 Autosomal STR profiles of the skeletal samples

Sample	Amel	D3S1358	D8S1179	D5S818	vWA	D21S11	D13S317	FGA	D18S51	D7S820
GS 63 ps	XY	14/15	12/13	9/11	16/17	30/30.2	12/14	20/25	12/–	10/–
GS 63 es	XY	14/15	12/13	9/11	(16)/(17)	(30.2)/–	12/(14)	20/(25)	(12)/–	10/10
GS 67 ps	XY	15/–	10/13	13/–	15/17	29/33.2	12/–	–/–	–/–	–/–
GS 67 es	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–	–/–
GS 79 ps	XX	16/–	12/15	10/–	15/17	29/31	–/–	19/21	–/–	–/–
GS 79 es	XX	16/16	12/15	10/10	(15)/17	29/31	–/–	(19)/(21)	–/–	–/–
GS 84 ps	XY	15/–	13/–	12/–	15/18	29/30.2	11/12	18/20	16/22	12/–
GS 84 es	XY	15/15	13/13	12/12	15/18	29/30.2	12/–	(18)/20	(22)/–	9/12
GS 95 ps	XY	17/–	13/15	11/–	16/17	29/32.2	10/13	22/24	14/–	11/12
GS 95 es	XY	17/17	13/15	11/11	16/17	29/32.2	(10)/13	(22)/24	14/14	11/(12)
GS 96 ps	XY	15/17	13/14	10/12	14/19	30/31.2	13/–	22/24	14/15	9/12
GS 96 es	XY	15/17	13/14	10/12	14/19	30/31.2	13/–	22/(24)	(14)/(15)	(9)/–
GS 102 ps	XY	14/15	11/14	9/12	16/17	29/30	–/–	20/21	15/–	12/–
GS 102 es	XY	14/15	11/14	9/12	17/–	29/30	–/–	20/20	–/–	12/12
GS 105 ps	XY	17/–	11/14	11/–	17/–	30/–	10/–	23.2/25	12/17	8/13
GS 105 es	X(Y)	17/–	11/14	11/11	16/17	28/30	10/–	23.2/25	12/(17)	(8)/–
DO 1102 ps	XY	15/16	12/15	9/10	16/17	29/33.2	11/–	20/21	14/18	7/–
DO 1102 es	XY	15/16	12/15	9/10	16/16	29/33.2	11/11	20/21	14/18	7/9
DO 1172 ps	XY	15/17	12/13/15	10/12	15/16	33.2/–	11/–	21/22/25	–/–	–/–
DO 1172 es	XY	15/17	12/13	10/12	15/16	30.2/33.2	11/–	21/25	–/–	8/–
DO 1247 ps	XX	15/16	11/13	12/–	17/18	29/32.2	8/–	22/25	–/–	10/–
DO 1247 es	XX	15/16	11/13	12/12	17/–	29/–	8/–	22/–	–/–	10/–
DO 2388 ps	XX	15/16	13/15	9/11	17/–	30/33.2	–/–	–/–	–/–	–/–
DO 2388 es	XX	15/16	13/–	11/–	16/17	30/33.2	11/11	20/21	15/–	10/–
DO 2589 ps	XX	15/–	15/–	12/–	–/–	–/–	11/–	22/–	–/–	–/–
DO 2589 es	XX	15/16	13/13	11/12	14/15	28/–	11/–	22/24	–/–	–/–
DO 3742 ps	XX	14/16	13/–	10/13	13/14	29/–	–/–	24/–	12/–	–/–
DO 3742 es	XX	14/16	13/–	10/13	13/14	29/32.2	8/10	24/–	12/15	9/10
DO 3750 ps	XX	16/18	12/13	11/12	17/–	29/32.2	12/–	21/23	17/–	8/10
DO 3750 es	XX	16/18	12/13	11/12	17/–	29/32.2	9/12	21/23	16/17	8/10

alleles in brackets were already determined but were not published in the study of Bramanti et al. (2000b) since they were reproduced only once at that time. (*ps* present study, *es* earlier studies, Bramanti et al. 2000a and Schultes 2000)

vealed this to be an effective and reliable way to determine AB0 bloodgroups at the genotype level from highly degraded sample material. This was proven by testing the method on positive control samples which consisted of DNA extracted from saliva samples of individuals with known AB0 phenotype. The applicability of the presented method to degraded DNA was tested by AB0 typing of archaeological skeletal material which revealed fully reproducible results. Due to the short amplification product lengths of the newly designed primers, the AB0 typing was successful for all of the tested skeletal material. The authenticity of the results was firstly ensured through processing and analysing independent extracts from each individual. Secondly, each extract underwent STR-based fingerprinting which provides indications of authenticity on the highest possible level through the unique nature of the data set.

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