Forensic DNA profiling of human bone material by direct PCR

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Introduction

Novel commercial kits based on recombinant DNA polymerases and improved buffer chemistry have recently been introduced for the analysis of samples such as blood, tissue (animal, plant), bird feathers and formalin-fixed paraffin-embedded tissues by direct PCR **[1]**. We tested several kits and/or enzymes from different suppliers (only data for some are presented here) to evaluate the feasibility of a direct PCR approach for forensic investigations of human skeletal remains.

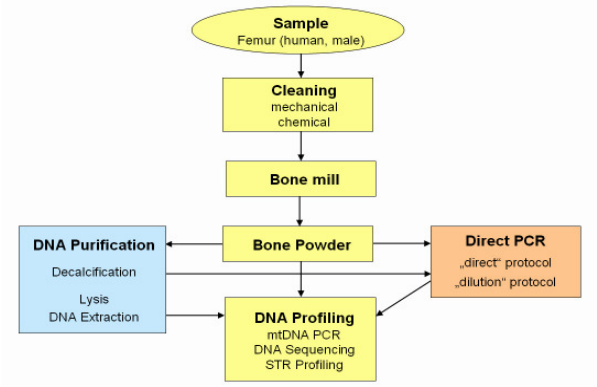

Fig. 1. Sample processing and DNA profiling. A part of a femural shaft was treated mechanically (dremel tool) and chemically (0.5% sodium hypochloride, water and ethanol) to clean the surface and to remove contaminating foreign DNA **[2]**. Dry cleaned bone was crushed and grinded to fine powder with TissueLyser II (Qiagen). Bone powder was utilized to purify DNA in a laborious, time-consuming procedure which included steps of decalcification (EDTA 0.5 M pH 8.0), lysis (EDTA 0.5 M pH 8.0, 0.5% sarcosyl, proteinase K; at 56°C) and DNA extraction (QIAamp DNA Maxi kit, Qiagen) or analysed by direct PCR according to the "direct" or the "dilution" protocol (see below). Aliquots from post-decalcification material were also subjected to direct PCR. Profiling of mitochondrial DNA (mtDNA) and nuclear DNA was carried out as described below.

Component	Phusion, Phire*** (Finnzymes)	AmpliTaq Gold*** (Applied Biosystems)
H_2O	add to 20 µl	add to 25 µl
PCR Buffer	1 X HF Buffer	1 X GeneAmp Buffer
dNTPs (Fermentas)	200 µM	250 µM
BSA (Fermentas)	-	1 µg/µl
Primers**	0.5 µM	0.25 µM
DNA Polymerase***	0.4 U/reaction	2.5 U/reaction
Sample****	see text	see text

	Phusion, Phire (Finnzymes)	Phusion Human Sample (Beta) (Finnzymes)	AmpliTaq Gold (Applied Biosystems)
hot start	98°C, 5 min	98°C, 5 min	95°C, 5 min
amplification	98°C, 5 sec 60°C, 5 sec 72°C, 20 sec	98°C, 1 sec 60°C, 5 sec 72°C, 15 sec	95°C, 30 sec 56°C, 30 sec 72°C, 1 min
no. of cycles	40	40	35
final extension	72°C, 1 min	72°C, 1 min	72°C, 5 min

Tables. PCR conditions. Components for the respective reaction mixes (upper table) and the cycling conditions (lower table) for PCR are shown in the tables:

) Primers have been described previously **[3] and were used to amplify a 443 bp portion of human mtDNA which contains the entire hypervariable region 1 (HV-1).

***) Enzymes used as indicated in the table.

****) **"Direct" protocol:** A small amount of sample (bone powder resp. post-decalcification material) was mixed with the respective 1x reaction mix and PCR was started immediately thereafter.

"Dilution" protocol: A small aliquot of sample (bone powder resp. post-decalcification material) was placed into 1x TE buffer (pH 8.0) supplemented with DNARelease® Additive (Finnzymes). After 15 seconds of vortexing, samples were incubated at 75°C for 5 minutes and then at 96°C for 2 minutes. Subsequent to centrifugation, 4 µl of the supernatant was used as template for PCR.

Results

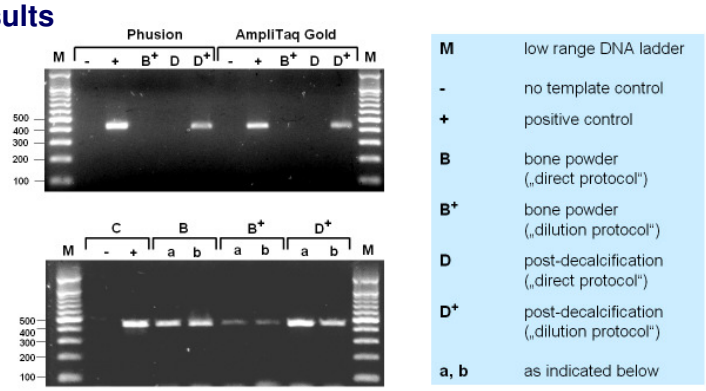

Fig. 2. Analysis of PCR products by agarose gel electrophoresis.

Upper gel (direct PCR): Samples were analysed by PCR (controls; C) or direct PCR. Amplification products (443 bp; mtDNA/HV-1) were separated on an agarose gel (1%).

Lower gel (nested PCR): 2 µl aliquots from a first run of PCR (direct PCR with the Phusion® Human Sample (Beta) kit; a/b: different amounts of sample) were further analysed in a second run of nested PCR under the same conditions. Amplification products were separated on an agarose gel (1%; shown above), subsequently purified and the integritiy of the PCR products was confirmed by DNA sequencing (data not shown).

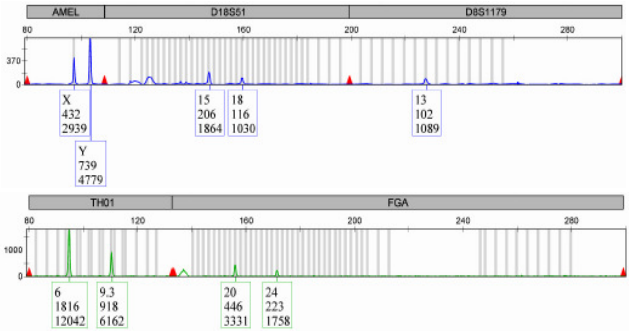

Fig. 3. Nuclear short tandem repeat (STR) typing by multiplex PCR. A 10 µl aliquot from post-decalcification material treated according to the "dilution" protocol was subjected to multiplex PCR applying the PowerPlex® S5 kit (Promega). PCR products were separated by capillary electrophoresis (ABI PRISM 310 Genetic Analyzer, Applied Biosystems) and analysed with the GeneMapper software v3.2 (Applied Biosystems).

Discussion

Herein we demonstrate that direct PCR is a possible means for mtDNA analysis of post-decalcification material when applying the "dilution" protocol. The same type of sample may also be used for STR typing by multiplex PCR. By combining direct PCR and nested PCR we were further able to perform mtDNA profiling of human bone powder without the need of prior DNA extraction or even decalcification. We conclude that the direct PCR approach may constitute a real option, especially when screening large numbers of sample material (e.g. mass graves) or when given time-windows are narrow. However, due to the inherent risk of creating artefacts by nested PCR, it is advisable to be cautious and to confirm results by analysing proper DNA extracts in parallel before drawing final conclusions.

Acknowledgements

We thank Ingo Nagler (Biozym Biotech Trading GmbH, Austria) for providing Phusion®, Phire® and Phusion® Human Sample (Beta) test kits.

References

[1] www.finnzymes.com/directpcr

[2] Davoren J, et al. (2007) Highly Effective DNA Extraction Method for Nuclear Short Tandem Repeat Testing of Skeletal Remains from Mass Graves. Croat Med J. 48:478-85.

[3] Parson W, et al. (1998) Population data for 101 Austrian Caucasian mitochondrial DNA d-loop sequences: Application of mtDNA sequence analysis to a forensic case. Int J Legal Med 111:124–132.