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Research paper

# Prediction of autosomal STR typing success in ancient and Second World War bone samples

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ABSTRACT

Human-specific quantitative PCR (qPCR) has been developed for forensic use in the last 10 years and is the preferred DNA quantification technique since it is very accurate, sensitive, objective, time-effective and automatable. The amount of information that can be gleaned from a single quantification reaction using commercially available quantification kits has increased from the quantity of nuclear DNA to the amount of male DNA, presence of inhibitors and, most recently, to the degree of DNA degradation. In skeletal remains samples from disaster victims, missing persons and war conflict victims, the DNA is usually degraded. Therefore the new commercial qPCR kits able to assess the degree of degradation are potentially able to predict the success of downstream short tandem repeat (STR) typing. The goal of this study was to verify the quantification step using the PowerQuant kit with regard to its suitability as a screening method for autosomal STR typing success on ancient and Second World War (WWII) skeletal remains. We analysed 60 skeletons excavated from five archaeological sites and four WWII mass graves from Slovenia. The bones were cleaned, surface contamination was removed and the bones ground to a powder. Genomic DNA was obtained from 0.5 g of bone powder after total demineralization. The DNA was purified using a Biorobot EZ1 device. Following PowerQuant quantification, DNA samples were subjected to autosomal STR amplification using the NGM kit. Up to 2.51 ng DNA/g of powder were extracted. No inhibition was detected in any of bones analysed. 82% of the WWII bones gave full profiles while 73% of the ancient bones gave profiles not suitable for interpretation. Four bone extracts yielded no detectable amplification or zero quantification results and no profiles were obtained from any of them. Full or useful partial profiles were produced only from bone extracts where short autosomal (Auto) and long degradation (Deg) PowerQuant targets were detected. It is concluded that STR typing of old bones after quantification with the PowerQuant should be performed only when both Auto and Deg targets are detected simultaneously with no respect to [Auto]/[Deg] ratio. Prediction of STR typing success could be made according to successful amplification of Deg fragment. The PowerQuant kit is capable of identifying bone DNA samples that will not yield useful STR profiles using the NGM kit, and it can be used as a predictor of autosomal STR typing success of bone extracts obtained from ancient and WWII skeletal remains.



## 1. Introduction

Determination of the quantity of DNA recovered from forensic samples is a critical step in the overall DNA typing process. A number of DNA quantification tests were used before quantitative real-time PCR (qPCR) was implemented in forensics [45,44,37,1,38,15,28]. Based on the qPCR amplification of specific targets, the concentration of amplifiable DNA can be measured with a high degree of accuracy and sensitivity [35,11]. The most advanced qPCR assays amplify four targets simultaneously and, besides the possibility of determining the ratio of female to male DNA, also provide information on the quality of the DNA within the sample by determining the degree of DNA degradation and the presence of inhibitors [39,16,27]. Most forensic DNA laboratories

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utilize commercial qPCR kits (Quantifiler, Investigator Quantiplex, Quantifiler Duo and Plexor HY) for detecting and quantitating biological evidence. The recently developed Quantifiler Trio and PowerQuant also provide information on the degree of DNA degradation [43,14,31]. Protocols for commercial qPCR assays are straightforward, labour-saving and amenable to automation, all considerations for routine forensic application [39].

The primary purpose of DNA quantification in forensic casework is to determine the appropriate amount of DNA template to include in PCR amplification of STR loci, since the commercial STR amplification kits are sufficiently robust only within a defined range of template quantities (mostly 0.5–1 ng of template DNA). Too much DNA leads to artefacts in STR profiles such as off-scale (pull-up peaks), split peaks (due to incomplete adenylation) and enhanced stutter, while too little may produce stochastic effects such as allele drop-out. The extreme sensitivity of the qPCR assay could be used for identifying DNA samples with quantities below STR reaction sensitivity and therefore to forego analysis of samples less likely to amplify, saving both time and money. Several laboratories that have investigated Quantifiler and Investigator Quantiplex performance on buccal swabs [8,40] and on contact DNA samples [22] found that interpretable STR typing results could be obtained even when a zero or "undetected"quantitative value is observed. In the majority of those samples there was insufficient data produced for statistical assessment or CODIS upload (called alleles could be used only for exclusionary purposes) but, in any case, some samples have been described where full profiles were obtained. To decide whether or not to proceed with STR typing of low template DNA samples after DNA quantification, a validation study should be performed in each laboratory [5].

The potential using of genetic information for human identification of skeletal remains is largely dependent on the quality of the evidentiary specimens (which itself depends heavily on the environmental conditions to which the remains had been exposed). In forensic genetic identification of disaster victims, missing persons and war conflict victims, skeletal remains are among the most challenging biological samples for successful STR typing due to the degradation, presence of inhibitors and possible contamination with modern DNA [19]. The poor quality of long-time stored ancient samples, e.g. historical skeletal remains, can be explained by the time dependent DNA degradation caused by various environmental (humidity, temperature, presence of microorganisms) and physical (ultraviolet light) factors [33]. The total number of genome copies and the number of available copies suitable for PCR in old bone samples are often not the equal because of the degradation, suggesting that the quality of DNA should also be evaluated. The methods for assessing both quantity and quality of DNA in challenged samples are therefore important for identifying skeletal remains. We have been carrying out genetic investigations of ancient skeletons from archaeological sites [51] and Second World War (WWII) victims from Slovenian mass graves [50,53,47,49]. Effective quantification screening method capable of identifying DNA samples that do not yield useful STR profiles is highly desirable, since STR genotyping contributes greatly to both the costs and the time associated with developing a DNA profile. Preserving the sample for single nucleotide polymorphism (SNP) or mtDNA analyses is also important. Next generation sequencing (NGS) with identity SNPs is today the best option for identifying degraded skeletons, but the technology is not yet in routine use in forensic laboratories. For ancient skeletal remains, NGS already provides strong advantages over previously used technologies [21].

The new, highly informative multiplex qPCR assay [32] PowerQuant System (Promega, Madison, WI, USA), that can simultaneously quantify total human nuclear DNA, male DNA, the extent of DNA degradation and presence of PCR inhibitors, was examined in the present study on ancient and WWII bone samples to predict the success of autosomal STR typing. The multicopy targets used in PowerQuant provide a much lower level of detection than single copy gene assays such as Quantifiler. To assess the level of DNA degradation, two regions of different lengths at the same autosomal multicopy locus were amplified with PowerQuant. The ratio of relative amounts of short autosomal (Auto) and long degradation (Deg) amplicons provided assessment of the quality of DNA through the quantitative estimate of the degree of DNA degradation ([Auto]/[Deg] ratio) in each sample. It was observed that, for degraded samples, there is more amplification of small target, resulting in a higher [Auto]/[Deg] ratio [16]. Quantification of short Auto and long Deg targets, and hence the [Auto]/[Deg] ratio, were used for predicting profiling success of old bone DNA samples.

## 2. Materials and methods

### 2.1. Skeletal remains samples

Sixty skeletons from five archaeological sites and four WWII mass graves, whose quantity and quality of extracted DNA had been determined using the PowerQuant System (Promega) and STR typing using the AmpF*l*STR NGM PCR Amplification Kit (Applied Biosystems, Foster City, CA, USA), were used to evaluate which parameters of the tested PowerQuant System (amount of short and long DNA fragments, [Auto]/[Deg] ratio and presence of inhibitors) could serve as predictors of autosomal STR typing success. The skeletal remains analysed had undergone various levels of environmental challenge due to the conditions to which they had been exposed. Some of the bodies were buried in soil and some thrown into karst caves, where they were not covered with soil but exposed to water run-off. Only femurs (one per each skeleton) were included in the study. All the ancient human bones belong to archaeologically secure, undisturbed graves. The bones and artefacts found together were thus deposited together, and their stratigraphic position is clear. The latter is all fundament for relative or absolute dating and for reliable interpretation of graves with all their contents. We analysed two skeletons from the 6th to 5th century BC archaeological site "Grofove njive" near Drnovo from Slovenia, six from the 4th AD century archaeological site Beljnjača" from Serbia, and seven from three 16th to 18th century archaeological sites from cemeteries associated with church or monastery complexes in Ptuj, Maribor and Ponikva from Slovenia (Table 1). Among WWII victim skeletons excavated from four mass graves where mass executions took place at the end of WWII [10,18], skeletons from the Konfin 2, Mačkovec, Babna gora and Mače were analysed (Table 1).

30 femurs from Konfin 2 mass grave, 13 from the Mačkovec grave, one from the Babna gora and one from the Mače grave were included. A total of 15 ancient and 45 WWII femur samples were evaluated in this study (Table 1).

For genetic investigations, an 8–10 cm fragment was taken from the compact cortical diaphysis of each femur and frozen at −20 °C until extracting the DNA. Buccal swabs on sterile cotton swabs were collected from the persons involved in the elimination databases for WWII mass graves and archaeological sites.

The research project was approved by the Medical Ethics Committee of the Republic of Slovenia (0120-87/2015-2; KME 117/05/15).

### 2.2. DNA extraction

The bone samples were cleaned by physical removal of the surface using a rotary sanding tool (Dremel, Racine, WI, USA)) and washed successively in 5% Alconox detergent (Sigma-Aldrich, St.