Influence of template DNA degradation on the genotyping of SNPs and STR polymorphism from forensic material

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Detection of single nucleotide polymorphisms (SNPs) and short tandem repeat (STR) polymorphisms by PCR is widely used to analyze degraded DNAs in forensic science. The success of DNA analysis from human remains largely depends on the quality of the template DNA. In this study analysis of degraded DNA were done using two SNPs (HLA-DQA1 and ABO) and two STR polymorphisms (VWA and CD4) by SSCP gel or denaturing gel electrophoresis, using two kinds of degraded DNA samples (teeth and blood stains contaminated with saliva) derived from the same person and investigated the influence of template DNA degradation on genotyping.

In this study, one set of teeth and cotton or gauze patches which usually absorb blood and saliva known as "blood stain samples" were dried at room temperature immediately after extraction of teeth in order to minimize DNA degradation. The other set of teeth and cotton patches in contrast were stored in a damp environment for 1-2 weeks. The tooth samples collected were highly decayed teeth, remaning roots, teeth with infected root canals, root-canal-treated teeth, or teeth broken in the course of extraction. The DNA isolation from dried gauze were done in 1-2 weeks after collection of samples and that from teeth were performed within 1-7 months after tooth extraction and latter following two weeks, DNA isolation from 27 teeth were done in 2-6 months after extraction, 34 teeth were done seven years after extraction, and that from 47 teeth were done nine years after extraction.

The investigators found that, as the degradation of DNA proceeded, unbalanced amplification of alleles occurred in the analysis of both SNPs and STRs, followed by allele drop, and further by loss of amplification. Non-target allelic products of STRs were amplified from highly degraded DNA samples; however, false allelic products of SNPs were not amplified from them. Amplification efficiency increased in proportion to the decrease of PCR target size, but reduction of the PCR target size also increased the chances of amplifying contaminating DNA, especially in highly degraded DNA specimen. It was found that, unbalanced amplification of heterozygotic alleles as the first sign of DNA degradation. When misleading PCR amplification pattern are observed, it should be considered for the degree of degradation of amplifiable DNA.

DNA yield, DNA quality and bite registration from a dental impression wafer

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Significant quantities of DNA can be recovered from saliva and teeth, but although DNA analysis is a powerful and accurate tool for identifying humans, the methods for recovering DNA from teeth have not been efficient or cost-effective.

In this study, boys and girls with mixed dentition who ranged from age 7 to 12 years formed the study group. Sample collection was done in four steps. In the first step bite registration was done using dental impression wafer, in second step saliva was obtained by making patients to rinse with 10ml of mouth wash for 15 seconds, third step buccal mucosa cells were collected by twisting cytology brush. Finally a maxillary alginate impression was made for each patient. Genomic DNA was extracted and quantified.

The results showed that use of the dental impression wafer resulted in significantly lower DNA yields than did the mouthwash and buccal swab method in Quant- it Pico green DNA yield. But in RT-PCR DNA yield the dental impression wafer method yielded significantly lower RT-PCR measurements than did the mouthwash and buccal swab methods. But there was no significant correlation between all 3 methods. It can be concluded that dental impression wafers captured DNA but not in high quantities.

A new method to extract dental pulp

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Extraction and detection of bacterial DNA from dental pulp exposed to laboratory environment, could yield host bacterial DNA that is contaminated with environmental bacteria. The current technique to recover the dental pulp out of its cavity relies upon longitudinal opening of the tooth resulting in large exposure of the material to the laboratory environment thus potentially leading to dental pulp contamination. Alternatively, dental pulp can be drilled off the dental pulp cavity after a small bore hole had been made to expose the dental pulp cavity.

In the present study the authors developed a new protocol by encasing decontaminated tooth into sterile resin, extracting DNA into the dental pulp chamber itself and

decontaminating PCR reagents by filtration and double restriction enzyme digestion. 16S rDNA-based detection was used to assess the bacterial contamination. Bacterial identifications were all confirmed by amplification and sequencing of specific rpoB sequence. They identified mainly two groups of bacteria, aerobic gram-negative bacteria presumably responsible for blood-borne infection and oral flora species associated with periodontal infection. Most of the detected gram-negative bacteria are known to cause bacteremia in humans and are not found in the safe or diseased periodontal tissue. Their results validated the postulate that it is possible to retrieve bacterial DNA in the dental pulp during a bacteremia.

This protocol was highly effective in eliminating background DNA contamination, while preserving the sensitivity of the assay. They concluded that the new protocol prevented laboratory contamination of the dental pulp and allowed the detection of bacteria responsible for dental pulp colonization from blood and periodontal tissue. Only 10% of such samples contained 16S rDNA. Thus it provides a new tool for the retrospective diagnostic of bacteremia by allowing the universal detection of bacterial DNA in animal and human, contemporary or ancient tooth.

Such protocol can be recommended for decontaminating PCR reagents for routine use and could be further applied to the identification of host DNA in forensic medicine and anthropology.

Personal identification by DNA analysis of samples from dental prostheses made of acrylic resin

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The procedures and possibilities for personal identification by DNA analysis of samples from dental prostheses made of acrylic resin were investigated. Acrylic resin pieces dipped in the whole saliva for a moment, dried in air and left for atleast two months at room temperature retained saliva stains that could be used as materials for DNA analysis for personal identification. The investigators found that the amounts of DNA extracted from 0.5x0.5x0.1 cm resin pieces did not correlate with the period of time the prostheses were left at room temperature or the period of time they were used in the oral cavity. Sex determination by amplification of segments of the amelogenin gene and typing of the 184bp fragment in the D4S43 locus was possible from the entire extracted DNA. Hence, resin prostheses used in the oral cavity and left at room temperature for as long as approximately 200 days could be used for DNA extraction followed by DNA analysis.

Optimization of DNA recovery from tooth brushes

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In human identification, the victim's toothbrush is an invaluable personal item as the deposited cellular material contains DNA from which a reference profile can be produced. The profile obtained then allows direct comparison to be made with the profile from the unidentified body. In this study, the minimum number of bristle bundles that would generate a complete DNA profile, and the minimum period of usage for a toothbrush to retain enough cells for genotyping were investigated. Two commonly used DNA extraction methods (DNA Mini Kit and Chelex1 100) were also tested to explore the efficiency of these protocols in recovering DNA from toothbrushes.

Data from the quantitative real-time PCR revealed that QIAamp1 DNA Mini Kit performed better at yielding DNA in terms of purity, quantity, and quality than Chelex1 100. It was also found that, with a suitable method of recovery, comparison of the number of detectable STR loci among the samples suggested that five bristle bundles is the minimum amount of starting material for DNA extraction from toothbrushes. DNA samples from five bundles of bristles from all of the toothbrushes generated complete profiles.

Based on the experimental results, a general guideline concerning the appropriate extraction method and the quantity of the starting material for the analysis of DNA from toothbrushes could be suggested.

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