

Whole genome amplification and real-time PCR in forensic casework

Giardina E, Pietrangeli I, Martone C, Zampatti S, Marsala P, Gabriele L, Ricci O, Solla G, Asili P, Arcudi G, Spinella A, Novelli G
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WGA (Whole Genome Amplification) in forensic genetics can eliminate the technical limitations arising from low amounts of genomic DNA (gDNA). However, it has not been used to date because any amplification bias generated may complicate the interpretation of results.

This paper was aimed at assessing the applicability of MDA to forensic SNP genotyping by performing a comparative analysis of genomic and amplified DNA samples. A 26-SNPs TaqMan panel specifically designed for low copy number (LCN) and/or severely degraded genomic DNA was typed on 100 genomic as well as amplified DNA samples.

Aliquots containing 1, 0.1 and 0.01 ng each of 100 DNA samples were typed for a 26- SNPs panel. Similar aliquots of the same DNA samples underwent multiple displacement amplification (MDA) before being typed for the same panel. Genomic DNA samples showed 0% PCR failure rate for all three dilutions, whilst the PCR failure rate of the amplified DNA samples was 0% for the 1 ng and 0.1 ng dilutions and 0.077% for the 0.01 ng dilution. The genotyping results of both the amplified and genomic DNA samples were also compared with reference genotypes of the same samples obtained by direct sequencing. The genomic DNA samples showed genotype concordance rates of 100% for all three dilutions while the concordance rates of the amplified DNA samples were 100% for the 1 ng and 0.1 ng dilutions and 99.923% for the 0.01 ng dilution. Moreover, ten artificially-degraded DNA samples, which gave no results when analyzed by current forensic methods, were also amplified by MDA and genotyped with 100% concordance.

The suitability of MDA material for forensic SNP typing was investigated.

Comparative analysis of amplified and genomic DNA samples showed that a large number of SNPs could be accurately typed starting from just 0.01 ng of template. It was found that the MDA genotyping call and accuracy rates were only slightly lower than those for genomic DNA.

Such efficiency and accuracy of SNP typing of amplified DNA suggest that MDA can also generate large amounts of genome-equivalent DNA from a minimal amount of input DNA. These results show that MDA material is suitable for

SNP based forensic protocols and in general when samples fail to give interpretable STR results.

Different dental tissues as source of DNA for human identification in forensic cases

Malaver PC, Yunis JJ
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The identification of human remains can be carried out by means of anthropological methods, dental structure analysis and DNA-based analytical methods. DNA analysis remains the only method of identification in cases with very small, fragmented or mixed forensic material or teeth.

Analysis of DNA from human remains can be carried out from genetic material obtained from bones or teeth. The teeth are the hardest tissue in the human body because of the dental enamel, which makes them resistant to adverse conditions degrading the DNA, such as humidity, high temperature, and the action of fungus and bacteria. The pulp and dentin can be used as a source of DNA whereas dental cement as a source of DNA has not been tested yet. Although the soft pulp tissue is protected by hard tissues (e.g., dentin), it is affected by putrefaction until its total decomposition. When teeth are extracted and stored at environmental temperature, a rapid dehydration of the pulp tissue occurs. Also, when teeth are loose inside the dental alveoli, humidity and bacterial action destroy the pulp. In other instances, when no humidity is present, dry atmosphere makes ideal conditions for the mummification of soft tissues. The cellular cement is characterized by the presence of numerous lacunae occupied by cementocytes, which are less exposed to external damages, especially to chemical or bacterial degradation.

The aim of this study was to determine if and in what degree different dental tissues – pulp, dentin, or cement could serve as a source of DNA for human identification in forensic casework.

A total of 20 teeth were obtained from unidentified bodies buried at the Central Cemetery in Bogotá in 1995 and exhumed in 2000. The tissues from three teeth obtained after surgery were used as controls. The pulp cavity was exposed after cutting each tooth with a high-speed handpiece. The pulp was removed and processed separately from the dentin and cement. The dentin and cement were obtained by drilling with a high-speed handpiece and placed into separate tubes. DNA was extracted from mineralized tissues after a short decalcification step with EDTA. The DNA was quantified by dot-blot hybridization with D17Z1 probe, a primate- and human-specific alpha satellite DNA for the D17Z1 locus. Polymerase chain reaction was carried out

for the hypervariable control region between nucleotides 29 and 408 bp (HV2 region) of the mitochondrial DNA, followed by gel electrophoresis to evaluate the amount and efficiency of the amplification.

The pulp yielded the strongest amplification signals. The signals for dentin and cement were very similar because of the presence of cells of the periodontal ligament in the cement. The results of the amplification of the HV2 region of the mitochondrial DNA showed that dentin and cement acted as protective factors for the cells and allowed the conservation of the DNA.

Cementoblasts and odontoblasts located within the cement and dentin are surrounded by the mineral matrix of the dental structure and thus protected from any environmental degradation forces, which makes them suitable sources for the DNA analysis.

DNA extraction from human saliva deposited on skin and its use in forensic identification procedures

Anzai-Kanto E, Hirata MH, Hirata RDC, Nunes FD, Melani RFH, Oliveira RN
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The dental forensic field includes the identification of unknown human remains as part of a crime or disaster investigation. It also includes the analysis of stains and organic liquids from the buccal cavity or its contents, bite mark comparison, investigation of trauma and oral injuries such as personal injury cases and dental malpractice. In criminal investigations, one of the fundamental requirements is that the victim and aggressor be positively identified.

Forensic dentistry contributes to the forensic process by either a direct comparison of the deceased's dentition with that of known dental records or by enabling a profile of the individual regarding age at death, sex and possible racial ancestry in order to narrow down the search for a possible victim.

Saliva is usually deposited in bite marks found in many homicides, assault and other criminal cases. In the present study, saliva obtained from volunteers was deposited on skin and recovered for DNA extraction and typing in order to evaluate its usefulness for practical case investigation and discuss the contribution of forensic dentistry to saliva DNA typing. Twenty saliva samples were collected from different donors and used as suspects' samples. Five of these samples were randomly selected and deposited (250 µl) on arm skin. Saliva was collected from skin using the double swab technique. DNA from saliva and skin-deposited saliva samples was extracted by the phenol-chloroform method. DNA samples were amplified by PCR for DNA typing using a set of 15 STRs. The recovery of DNA from saliva deposited in the

skin was 14 to 10 times lower than DNA quantity from saliva samples. DNA typing was demonstrated in 4 of 5 deposited saliva samples. These results indicate that standardized procedures used for DNA collection and extraction from skin-deposited saliva can be used as a method to recover salivary DNA in criminal cases. This study suggests that the analysis of saliva deposited on skin be incorporated into a criminal investigation since it may have great discriminatory power.

Extracting evidence from forensic DNA analyses: Future molecular biology directions

Budowle B, van Daal A
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DNA molecular biology tools have enhanced the capability of forensic science to characterize biological evidence and enabled reliable typing of extremely minute quantities of DNA with a resolving power such that the number of evidence-sample contributors can be reduced to a few individuals, if not just one source. Even with the forensic DNA field's maturity, there still are a number of areas where improvements can be made. The study addresses the gaps that need to be filled in forensic biology, some of which it identifies as the need to improve the current limits of typing samples of low quantity and quality; improving the efficiency of sample recovery and extraction; converting current STRs to mini-STRs; selecting and validating a variety of SNPs for different applications; enhancing multiplexing; developing automation for high throughput; developing expert systems for data interpretation; developing sequencing capabilities for screening microorganism genomes and field testing.

Forensically relevant SNP classes

Budowle B, van Daal A
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Genetic typing of forensic samples, using genetic markers is used to characterize an evidentiary sample or identify an unknown person. The study describes new genetic markers, which have been developed, namely Single Nucleotide Polymorphisms (SNPs). The SNP markers help analyze forensic samples that contain too little template DNA or the ones which are too degraded. They further find great use in providing investigative lead value in cases without a suspect. The SNP markers can be divided into four categories:

- *Identity testing SNPs*- for individualization; requiring high heterozygosity and a low coefficient of inbreeding (*F_{st}*) (i.e., low population heterozygosity).
- *Lineage informative SNPs*- Sets of tightly linked SNPs that function as haplotype markers to identify missing persons through kinship analyses.
- *Ancestry informative SNPs*- for establishing high probability of an individual's biogeographical ancestry

to indirectly infer some phenotype characteristics for investigative lead value; requiring low heterozygosity and high FST values.

- *Phenotype informative SNPs*- for establishing high probability that an individual has a particular phenotypic characteristic, such as skin colour, hair colour or eye colour for investigative lead value.

The study discusses the applications of these different types of SNPs.

Improved forensic DNA analysis through the use of alternative DNA polymerases and statistical modeling of DNA profiles

Hedman J, Nordgaard A, Rasmusson B, Ansell R, Rådström P

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DNA evidence, is central to many legal proceedings. Diagnostic DNA analysis is often limited by components that interfere with the amplification, the so called PCR

inhibitors.

PCR inhibitory substances may generate blank or incomplete DNA profiles. Extensive DNA purification helps to rid the samples of these inhibitors, although these procedures increase the risk of DNA loss. The study analyzes the potential of several DNA polymerase-buffer systems. The DNA amplification kits presently in use are the AmpFISTR SGM Plus kits with the DNA polymerase Ampli taq gold as the gold standard. Alternative DNA polymerase-buffer systems using Bio-X-Act Short, Ex Taq Hot Start or Pico Maxx High Fidelity, can improve the quality of forensic DNA analysis and efficiently circumvent PCR inhibition in DNA evidence samples.

Dhivya K, Gaurav Ghosh

Post Graduate Students,

Department of Oral & Maxillofacial Pathology,

Meenakshi Ammal Dental College & Hospitals, India

E-mail: drgauravghosh@gmail.com

Retraction of article

The Journal of Forensic Dental Sciences announces the retraction of the article titled "**Denture marking: An introduction and Review**" authored by Thillam V. Padmanabhan and Rajiv K. Gupta appeared in Jan-June 2009/ Vol 1 / Issue 1 / page 11-16 for the act of plagiarism.

The original article was published by Raymond Richmond and Iain A Pretty in Journal of Forensic Sciences, and the details are as follows- Contemporary methods of labeling Dental Prostheses-A Review of literature, September 2006, Vol 51, No.5, page 1120-26.

The editorial team of Journal of Forensic Dental Sciences sincerely apologize Raymond Richmond and Iain A Pretty for the publication of this article. The editorial team strongly condemns plagiarism and those indulging in plagiarism will be banned for three years from publishing in the Journal of Forensic Dental Sciences.