Current Trends in Forensic Genetics

Simple and highly effective DNA extraction methods from old skeletal remains using silica columns

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Authors compared various silica-based DNA concentration/extraction methods using quantitative real-time PCR to assess DNA yield and removal of PCR inhibitors and presented a simple and highly effective DNA extraction method for genotyping of old skeletal remains. The need for this study was to limit DNA typing failures mainly in skeletal remains due to procurement of small quantity of endogenous DNA, likelihood of DNA degradation, possible presence of PCR inhibitors and risk of contamination.

Authors used skeletal remains for genomic DNA, artificially prepared degraded DNA along with, PCR inhibitors like porcine hematin and humic acid, real-time PCR to determine DNA concentration and STR analysis. DNA concentration/purification methods were assessed by two commercially available kits i.e., QIAamp mini spin columns and QIAquick spin column and another modified method with QIA blood maxi column. DNA concentration/purification methods were tested with known concentrations of PCR inhibitors at varies concentrations 20, 50 and 100μM of hematin and 2.5, 12.5, 25.0 and 50.0 ng/μl of humic acid. Three methods (A,B,C) of demineralization procedure after cryogenic grinding were tested to assess DNA extraction from old skeletal remains. QIAamp mini spin columns showed higher DNA recovery because of the larger binding surface increasing adsorption by more number of silica particles. At highest concentration of humic acid the QIAamp mini kit showed increased C_T value (>28.0). Method C in which organic grinding and complete demineralization with high concentrations of EDTA is followed by DNA extraction procedure using QIAamp blood maxi spin columns and buffers from the QIAquick PCR purification kit showed more efficient results in DNA extraction. Therefore, this method increases the possibility of obtaining authentic DNA profiles from highly degraded samples, thereby contributing to solve forensic cases dealing with skeletal remains. Authors developed a simple and highly effective method for DNA extraction from old skeletal remains in terms of high-quality DNA recovery and PCR inhibitor removal by optimizing the combination of silica columns and buffers.

Room temperature DNA preservation of soft tissue for rapid DNA extraction: An addition to the disaster victim identification investigators toolkit?

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In case of calamity a massive number of bodies will be recovered showing DNA fragmentation, decomposition and putrefaction due to the tropical climate of the area and present a different challenge to disaster victim identification teams. DNA profiling is proven useful in allowing identification and re-association of fragmented, burnt or decomposed corpses that would be difficult or impossible using traditional techniques. Usually samples collected for DNA identification are usually stored at -20°C to halt the degradation processes but DNA extraction from these samples must be defrosted, removed from the container, dissected, weighed, macerated and then should be digested for 1-3 hours.

Authors have examined the ability of two buffer solutions; Lysis storage and transportation (LST) buffer and the Oragene DNA self-collection kit to preserve DNA present in fresh muscle tissue at various room temperatures ranging from 16° to 30.5°C with an average of 24.2°C over a period of 52 weeks. On DNA quantification using real-time PCR both buffer solutions have shown sufficient DNA preservation over a 12-month period of storage at room temperature to allow DNA profiling, which was successfully performed when 5-1000 mg muscle tissue was stored in each solution. Oragene collection pots are superior to LST buffer in recovery of high DNA yield. The quality of DNA recovered from tissue stored in LST buffer is not significantly reduced compared with that recovered from samples stored in Oragene collection pots. Also yield of DNA per mg of tissue stored was greater when samples were stored in 1 ml rather than 5 ml LST also, better suited to the preservation of small (<100 mg) amounts of tissue. Authors concluded both buffer solutions could be used as an alternative to freezing of samples for DNA preservation over a 12-month period of storage at room temperature.

Authentication of forensic DNA samples

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In recent years DNA evidence has become the "gold

standard" of forensic testing and is an invaluable tool for criminal justice community. The possibility that DNA evidence can be faked and planted in crime scenes is normally overlooked. Artificial DNA can be applied to surface objects or incorporated into genuine human tissues and planted in crime scenes but, current forensic procedure fails to distinguish between such samples of blood, saliva and touched surfaces with artificial DNA and corresponding samples with *in vivo* generated DNA.

Authors developed an authentication assay which distinguishes between natural and artificial DNA based on the fact that *in vitro* synthesized DNA is completely unmethylated and *in vivo* generated DNA contains loci that are completely and consistently methylated. Sodium bisulphite converts all unmethylated cytosines to uracils leaving methylated cytosines unaffected. PCR amplification was done on a set of genomic loci containing one reference CODIS locus (FGAref) and four non-CODIS loci (NT18, ADD6, MS53, SW14). Authors applied the DNA authentication assay to 20 mock forensic samples-10 with natural DNA, 10 with artificial DNA and a negative control sample without DNA. All samples with natural DNA showed successful amplification of all loci and the FGAref amplicon was present in all samples. Samples with artificial DNA failed to amplify the four non-CODIS loci. These samples were therefore determined to be non-authentic. Few whole genome amplification (WGA) synthesized DNA showed amplification for all loci similar to natural DNA. Natural DNA showed complete methylation of all CPG positions in NT18 and ADD6 and no methylation in any of the CPG positions in MS53 and SM14 where as WGA synthesized samples showed complete lack of methylation in all loci. Authors presented an authentication assay for casework sample as part of the forensic procedure is necessary for maintaining in high credibility of DNA evidence in the judiciary system.

Reliable genetic identification of burnt human remains

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In cases of mass disasters involving generation of high temperatures, suicidal burning, car accidents, domestic fire or in cases where fire may have been set in order to conceal a crime forensic analysis is dealt with burnt bodies of which only bones or bone fragments are available for reliable genetic identification. Extremely charred bodies frequently render highly degraded DNA which hampers STR analysis. Also, PCR inhibitors will be coextracted during DNA isolation of burnt bodies e.g., gasoline, melted plastic or textiles infiltrated into the bone during incineration.

Authors investigated 13 human bodies in various degrees of burn based on color grades which deduce the approximate burning temperature: a yellowish or brownish discoloration correlates with temperatures of 200-300°C, black with temperatures of 300-350°C, grey with temperatures of 550-600°C, and white with temperatures higher than 650°C. Also authors aimed to investigate whether original DNA can be successfully be extracted from differently burnt bones and can it be reproducibly analyzed using STR profiling and mtDNA sequencing. A total of 71 compact bones were analyzed: 9 are well-preserved, 18 are semi-burnt, 26 are black-burnt, 10 are blue-grey burnt and 8 are blue-grey-white burnt. From these samples DNA extraction was done and DNA quality was tested using a GeneAmp PCR system 2700 developed for highly degraded DNA. All samples of well preserved and semi-burnt bones gave a full screening profile. Black-burnt bones showed a variable pattern ranging from a full profile to a complete allelic drop out. Blue-grey and blue-grey-white bones resulted in negative results. According to authors negative PCR results were most probably caused by either highly degraded or even a total lack of template DNA. STR analysis was performed using AmpFlSTR identifiler blue-grey burnt bones was successful in few instances whereas typing of blue-grey-white burnt bones succeeded only sporadically probably due to bone adherent soft tissue. Amplification of mitochondrial DNA was done using two HVI-specific mitochondrial fragments (220 bp and 439 bp). Later PCR products were separated on ethidium bromide-stained 2% agarose gels and quantified on a Geldoc EQ system, amplicon amounts of about 10 ng were judged as sufficient for sequencing. Well-preserved and semi-burnt bones yielded sufficient quantities of both HVI fragments whereas only the smaller 220 bp HVI fragment was detectable in case of black burnt, blue-grey burnt and blue-grey-white burnt bones. Finally they concluded that analysis of mitochondrial DNA is more promising as it is present in higher copy numbers per cell than nuclear DNA in case of burnt sample.

Sex-specific fluorescent labelling of cells for laser microdissection and DNA profiling

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Laser microdissection (LMD) is currently the method of choice for single-cell isolation and widely used in research. LMD can also greatly improve the recovery of male DNA from unfavorable mixtures of male and female cells. Male- and female-specific florescent labels are used for hybridization inorder to isolate single male and female cell from a mixture of cells. With this study authors wanted to test whether DNA profiling of LMD isolated, fluorescent hybridized cells could be used for successful short tandem repeat profiling.

Five microliters of blood samples from both male and female

individuals were spread over a poly-l-lysine-coated slides. Few smeared slides were used directly and few were air-dried for 1 week in order to simulate forensic casework. Later cells were transferred from one slide to another by two transfer techniques one using a presoaked swab and other by pipetting. Pepsin solution was added for enzyme digestion and later subjected to hybridization. In order to obtain different fluroscent signals Y-specific spectrum green label was used and X-specific spectrum orange label was used. After successful hybridization female cells showed two red signals whereas male cells showed one green and one red signal. From the stained slides 10, 20, 30 and 40 cells were isolated using SL μCut LMD system (MMI). After cell isolation DNA was extracted using QIAamp DNA microkit and subjected to multiplex PCR using AmpF1STR SGM Plus kit and PCR products were analysed on an ABI PRISM 3100 Avant capillary electrophoresis system. Results showed successful hybridization with both native and dried blood cells with male and female cells showing different signals. By means of LMD isolation of hybridized cells a full STR profile was obtained from samples containing atleast 30 cells. Samples with 20 cells yielded a partial profile with one or two locus drop-outs. Thus, this method would appear to be suitable in forensic stain investigation.

Validation of SRY marker for forensic casework analysis

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Determination of gender for a forensic DNA sample at times can be informative in various forensic investigations, especially in sexual assault cases. Sex determination is routinely performed by amplifying a region of amelogenin by the PCR. Owing to the discrepancy in the structural variability within the Y chromosome, several studies have shown that amelogenin gender test is not always correct with true male gender determination in forensic casework or in prenatal diagnosis. To reduce the potential interpretation difficulties authors have investigated using genetic markers lying in the sex-determination region Y (SRY) on the Y chromosome. This study was undertaken by the authors to perform some validation studies including repeatability, sensitivity, gender specificity and mixture studies on SRY marker for use in forensic cases. Quantification of DNA was conducted using the Quantifiler Human DNA Quantification Kit and DNA Typing was done by combining the amplified product with the Genscan-500 ROX internal line standard and loaded on an ABI Prism 310 genetic analyser.

Repeatability of DNA samples obtained from buccal swabs taken from 115 unrelated male individuals showed successful amplification and typing of SRY marker. On capillary electrophoresis samples yielded sizes of 94.44±0.07 bp (base pair), 94.47±0.18 bp and 94.36±0.06 bp. These lengths in bp are slightly lower than the known 96-bp size of the amplicon. No null allele was observed, SRY marker enables precise and repeatable results for male gender determination as a single system. Sensitivity studies showed reliable male gender determination for routine forensic samples with a DNA quantity as low as 125 pg. Gender-specificity was performed using two female DNA samples, which cross-reacted with SRY marker assay in a singleplex amplification but this multiplex assay failed to produce detectable SRY product from female control DNA even at concentrations of 5 and 10 ng/μL, this data support that SRY primer is highly specific for the SRY gene on the Y chromosome. Mixture studies were carried out in a multiplex reaction. The presence of a high background of female DNA in a sample had no impact on amplification of SRY marker down to the tested ratio of 1:16. Therefore mixtures with low amounts of male DNA with high concentrations of female DNA can be typed with the SRY male gender assay. The authors concluded that SRY is a sensitive and reliable male gender marker.

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