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Review

Forensic implications of PCR inhibition—A review

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ABSTRACT

Polymerase chain reaction (PCR) is currently the method of choice for the identification of human remains in forensic coursework. DNA samples from crime scenes often contain co-purified impurities which inhibit PCR. PCR inhibition is the most common cause of PCR failure when adequate copies of DNA are present. Inhibitors have been routinely reported in forensic investigations of DNA extracted from a variety of templates. Humic compounds, a series of substances produced during decay process have been considered as the materials contaminating DNA in soil, natural waters and recent sediments. Those compounds have been frequently assigned as PCR inhibitors. The current report reviews the characteristics of PCR inhibition, including the proposed mechanisms of inhibition, detection methods and the available technologies to remove or overcome the inhibitory activities.

Contents

1. Introduction

While continuing to address issues related to forensic population studies [1], research has been focused on methodological problems in forensic context, including postmortem DNA degradation [2], tissue preservation [3], repair strategies [4], and contamination [5]. One less explored topic in forensic DNA technology is the co-existence of polymerase chain reaction (PCR) inhibitors with DNA [6]. PCR is currently the method of choice for *in vitro* amplification of DNA molecules for genetic analysis in forensic DNA identification, through different strategies including short tandem repeats (STRs) [7], Mini-STRs [8], and sequence analysis of mitochondrial DNA (mtDNA) [9].

PCR inhibition is the most common cause of PCR failure when adequate copies of DNA are present. It remains a great challenge in molecular analysis of biological remains recovered from the environment. Theoretically, inhibitory activities may affect every component of PCR reaction including the template DNA, the nucleotides, the amplification primers, Mg^{2+} and the polymerase enzyme [10,11]. Furthermore, co-existing impurities can interfere with cell lysis during DNA extraction [12,13], or degrade or capture nucleic acids [14]. Severe inhibition can lead to the loss of alleles from the larger STR loci, or complete false-negative results, which is a pattern similar and often mistakenly attributed to severe template degradation [2]. A slight to moderate inhibition can result

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in a minor loss of alleles and misestimating of the affected sample's DNA quantity [15]. The latter has potential consequences for downstream applications such as STR analysis [16]. Inhibitors have been routinely reported in forensic investigations of DNA extracted from clothing [17], hair [18], skeletal muscles [19], blood stains [20], urine [21], feces [22], buccal swabs [23], body fluid aspirates [24], formaldehyde fixed tissue [25], dyes from latent fingerprint processings [26], quids [27], bone [28,29], and soil [30].

The failure of PCR from samples recovered from the environment has been attributed, most notably, to a brown substance contaminating DNA preparations [31–34]. The substance is intractable to removal by ordinary technologies, and may interfere with molecular technologies other than PCR [35]. It appears as a blurred blue fluorescent when illuminated by UV light on DNA electrophoresis products [36,37]. The substance can also block fluorescence detection in real-time PCR systems [38] and the formation of primer dimers in the PCR reaction [28].

Humic compounds have been determined to be as at least one of the PCR inhibitors contaminating DNA in soil, natural waters and recent sediments [39–41]. Other kinds of intrinsic and extrinsic chemicals have also been reported as inhibitors to PCR, including collagen type I [36], chemically altered carbohydrates [42], porphyrines catabolism residues [43], coexisting non-human [44,45] or excessive DNA [46], heavy metals found with the bone samples from mass graves, most commonly, iron, copper, cadmium and lead [45], gold nanoparticles [47], bile salts [48], complex polysaccharides in feces [22], heme in blood [20], proteinases in milk [10], urea in urine [21] and chondroitin sulfate chains in skeletal material [49]. Using high amounts of DNA extracts as template has significantly inhibited the PCR amplification process [50].

Skeletal remains retain the capacity of strong protection of their macromolecular composition for longer periods, even to reach to archaeological record in favorable conditions. DNA fragments from a 2400 year old Egyptian mummy have been cloned and characterized [51]. The longer duration of DNA survival in bone as compared to soft tissues increases the potential of its exposure to decay reactions. PCR inhibition phenomenon from bone specimens has been reported in ancient as well as modern DNA studies [52,53].

2. Proposed mechanisms of PCR inhibition

While the effect of the presence of inhibitors is well known, the mechanisms of PCR inhibition are often unclear. Based on simulated studies, co-purified inhibitors in nucleic acid extracts may not affect all PCR reactions with different products equally [54]. Designing real-time PCR experiments with variable amplicon lengths, melting temperatures and primer sequences, Opel et al. determined that certain primers with a higher melting temperature are less affected by inhibition [55].

Although the inhibitory activities may affect different components of PCR reaction, research has been mostly focused on the function of the polymerase enzymes. The polymerase can be degraded by proteinases [10], denatured by phenol [56] or detergents [57], and inhibited by blocking of the active site by the inhibitor, which is the reversible effect of heme [20].

The inhibitory effect of divalent ions (Ca^{2+} and Mg^{2+}) was more pronounced than that of monovalent ions (K^+ and Na^+), with Ca^{2+} being the most inhibitory. The polymerase most sensitive to K^+, Mg^{2+}, and Na^+ was Ultma from Thermatoga maritima, whereas the polymerases AmpliTaq Gold and Taq were most sensitive to Ca^{2+} [58]. Ca^{2+} ion concentrations above 3 mM have been found to be inhibitory to the amplification capacity of AmpliTaq DNA polymerase possibly through competition with the Mg^{2+} ion [58]. This inhibitory effect was reversed by increasing the Mg^{2+} ion concentration in the reaction mixture [59]. The effects of collagen on Taq and Pwo have been partially reversed by the addition of Mg^{2+}, suggesting that collagen sequesters Mg^{2+} or affects Mg^{2+}–polymerase interaction [60]. The addition of K^+ ions at concentrations higher than 75 mM was completely inhibitory to amplification of DNA by AmpliTaq [61], and increasing the concentration of Mg^{2+} ions to 15 mM has been found to inhibit the polymerase ability of Taq DNA polymerase [62].

2.1. Maillard reaction

Humic compounds are dark-colored, amorphic and highly stable compounds [63], produced in decay process through the Maillard reaction [64]. Termed after its inventor Louis-Camille Maillard in early 1900s [65], the Maillard reaction is a biochemical aging process, referring to as nonenzymatic browning reactions between amines and carbonyl compounds, especially reducing sugars such as glucose or glucose 6-phosphate (G-6-P). At the early stages, however, the reaction may not display visible brown coloration of the products. With time, these adducts are dehydrated to form yellow brown fluorescent compounds that can cross-link proteins [66]. The Maillard reaction is strongly affected by conditions such as heating, moisture, pH, type of sugar present and possibly predisposed through condensation reactions between sugars and amino acids [67].

Depending on the soil type, humic compounds can make up 5.0–7.63 mg/g of soil [35]. They can be extracted by alkali and then fractionated into humic (HA) and fulvic (FA) acids through acidification [68]. Reportedly, 0.2–1 of soil HAs were extracted with nucleic acids from soils containing 1–1.97 total organic carbon [35]. Notably, 0.08 μg/ml HAs is sufficient to inhibit the most sensitive Taq polymerase, and 0.5–17 μg/ml will inhibit restriction enzymes [35]. Considering the charge to mass ratio closeness to DNA and their structural heterogeneity [69], high molecular weight humic compounds feature the most recalcitrant impurity in DNA purification procedures. The amount and composition of humic compounds that contaminate DNA preparations may depend on the DNA extraction technique applied [70].

HAs have physicochemical properties similar to the phosphate groups of the sugar-phosphate backbone of DNA [71], so that they can compete with DNA for adsorption sites during the purification steps [72]. HAs may chelate with magnesium ions required by the Taq polymerase [73,74]. As with DNA, humic compounds carry a negative charge [75]. Electrophoretically, humic compounds, mostly, will move through the gel faster than genomic DNA because of their lower mass to charge ratio [76].

Free DNA in soil rapidly adsorbs and binds on HAs especially in acidic conditions [77,78]. This binding provides protection against degradation by nucleases and retains the capacity to transform cells [79]. Sand-adsorbed DNA was 100 times more resistant against DNase I as compared to free DNA in solution [80].

Maillard products however, may retain DNA-breaking activity [81,82] or entrapping DNA and making it inaccessible to polymerase enzyme [83]. HAs and other oligomeric compounds with free phenolic groups (e.g., Tannins) oxidize to form quinones, which covalently bind to and inactivate DNA polymerase [84]. The level of inhibition for those compounds is directly related to the amount present in the reaction. Complete inhibition of the TaqMan real-time PCR assay occurs at concentrations greater than 1.4 ng per 25 mL reaction of tannins. Concentrations below 1.4 ng per 25 mL reaction will also affect amplification efficiencies and confound real-time quantitation estimates [16]. Real-time PCR studies on HAs and melanin effects indicate a sequence specific manner of PCR inhibition through binding to and inactivating at least a portion of available DNA template, whereas collagen and

hematin affect taq enzyme activity. Tannic acid (an agent mostly found in leather) follows both DNA inactivation through direct binding and taq polymerase inhibition [55].

Based on real-time PCR studies, the inhibition mechanism of HAs can be classified as uncompetitive inhibition, where the inhibitor binds to enzyme–substrate complex making it unreactive. The result is a decrease in V_{max} (the maximum initial velocity that the enzyme can achieve) and an equivalent decrease in K_m (the substrate concentration at which 1/2 V_{max} is achieved), without changes in the K_m/V_{max} ratio value. Moreover, HA shifts the DNA melting temperature point, T_m, to higher temperatures [39].

2.1.1. DNA glycation

In addition to co-purified Maillard products affecting DNA amplification reactions, the amino groups of nucleic acids can serve as substrates for modification by reducing sugars [85]. *In vitro* incubation of individual nucleotides or purified DNA with reducing sugars at 37 °C produced moieties with similar absorbance and spectral properties as the products that form during the nonenzymatic glycosylation of proteins [86].

G-6-P, and glucose at a much slower rate, form adducts with the primary amino groups of bases. With time, these adducts undergo chemical rearrangement that can labilize the glycosidic bond between the purine and the deoxyribose. This leads to depurination and followed by β-elimination and strand scission [66]. The glycation process modifies the nitrogenous base of DNA molecules which block PCR and require repair procedures ahead of PCR. Fig. 1 displays the nonenzymatic glycosylation products of DNA.

Although characteristically a slow process, Maillard reaction modifications occur as integral function of time and sugar concentration [87]. Four days incubation of single and double stranded DNA at 37 °C with high concentrations of either glucose or G-6-P was sufficient to produce absorbance changes in the 300–400-nm range that are similar to those described for the nonenzymatic browning of proteins [66]. There was no significant reaction of the reducing sugar with thymidine. This demonstrates the requirement for a free amino group on the nucleotide for the reaction to occur [88]. The reaction occurred at a faster rate with single stranded DNA than with double stranded DNA.

DNA retains double stranded structure in postmortem tissue. A reactive intermediate has been found to be formed when reducing sugars were incubated with lysine or polyamines. These intermediates can react rapidly with either single or double stranded DNA to cross-link the amine to the DNA [89]. Polyamines such as spermine, spermidine, putrescine and cadaverine are normally produced in postmortal tissue through microorganismal metabolism of biological material. They can possibly serve as substrates for the formation of the reactive intermediate agents. PCR inhibition reportedly affects the analysis of DNA extracted from degraded samples (e.g. ancient DNA) more than that of optimal samples (e.g. modern DNA) [90]. This phenomenon might be explained by advanced glycation of DNA molecules which is a time dependent reaction.

Coprolites, in ancient DNA studies were shown to contain large amounts of cross-links between reducing sugars and other components, including DNA, hence producing Maillard products [4]. DNA amplification from the coprolite was reportedly possible only after sugar-derived cross-links had been resolved by N-phenacyl thiazolium bromide (PTB) [91,92]. However, the effectiveness of PTB has been challenged at least in some cases where different components were responsible for PCR inhibition and most notably removable by silica extractions [90].

3. DNA quantitation

Further to their PCR inhibitory effects, humic compounds may also hamper DNA quantitation of the environmental samples. HAs possess high absorption coefficients in the ultraviolet (UV) spectral range, which strongly impairs nucleic acid quantitation by UV spectrophotometry and often leads to an overestimation of DNA concentrations [93]. In contrast to UV spectrophotometry, fluorescent assays are more sensitive and specific for DNA. However, standard H 33258, Picogreen (PG) and SYBR Green (SG) assays were also strongly influenced by the presence of HAs [69]. Spectroscopic scans suggest binding affinities of HAs to (SG) in DNA quantitation studies [93].

Interferences by HAs fluorescence will be less prominent with the SG or the PG assays, where excitation is preferentially performed at 485 nm and emission is determined at about 522 nm, in contrast to the H 33258 assay where excitation and emission detections take place at lower wavelengths. Therefore it may be of interest to apply DNA-specific dyes with an excitation at even longer wavelengths than PG or SG [69]. Diluting the sample and increasing the SG concentration has been found beneficial in reducing the concentration and so the influence of HAs on DNA quantitation [69].

Quantitative PCR technologies have been recently employed for DNA quantitation purposes. Absolute quantitation can be achieved using a standard curve, constructed by amplifying serial dilutions of known amounts of target DNA in a parallel group of reactions as references [94]. This method requires the assumption of uniform reaction efficiency between standards and unknown samples. This assumption does not hold in the presence of inhibitory activities [95]. Even slight variations in amplification efficiency between samples due to unequal activity of inhibitors can significantly affect the accuracy of template quantitation [96]. Stahlberg et al. measured amplification performances using a dilution series of each sample [97]. This is the best way to reduce the negative effects of inhibitors on DNA quantitation, when satisfactory removal of inhibitors is not achievable. However, dilution of samples containing inhibitors, would affect PCR efficiency and therefore the calculated quantitations through attenuation of inhibitory activities.

4. Detection of PCR inhibition

The presence of PCR inhibitory substances can be studied indirectly by monitoring the PCR product(s) quantity and quality through a few technologies including gel electrophoresis [98], dot blots [99], high-pressure liquid chromatography [100] and calorimetric assays [101]. PCR inhibition can be identified from amplification failures of the internal standards and monitored semi-quantitatively by observing the PCR failures of a series of known amounts of the internal standard [102].

Quantitative real-time PCR is the method of choice to monitor PCR inhibitors through calculating PCR efficiency [16], or more classically by incorporating an exogenous internal positive control (IPC) fragment co-amplified in the same multiplex reaction along genomic products [103,104]. Some commercially available systems such as the Quantifiler Human DNA Quantitation Kit (Applied Biosystems, Foster City, CA, USA) have been based on this method [105]. In the presence of inhibitory activities on real-time amplification plot, reactions cross the detection threshold at later cycles and the exponential phase slopes decrease. Suppressed amplification efficiencies also have a negative effect on the linear phase and as a result, samples with partial inhibition reach lower plateau fluorescence values at the end of the reaction [16].

Differential susceptibility to PCR inhibition between assays has been reported [54], therefore the effects of inhibitors on the IPC may not be predictive of those on other targets in the assay [106]. The efficiencies of the two reactions, i.e., the PCR of the studied gene and the PCR of the housekeeping or IPC motifs, are not

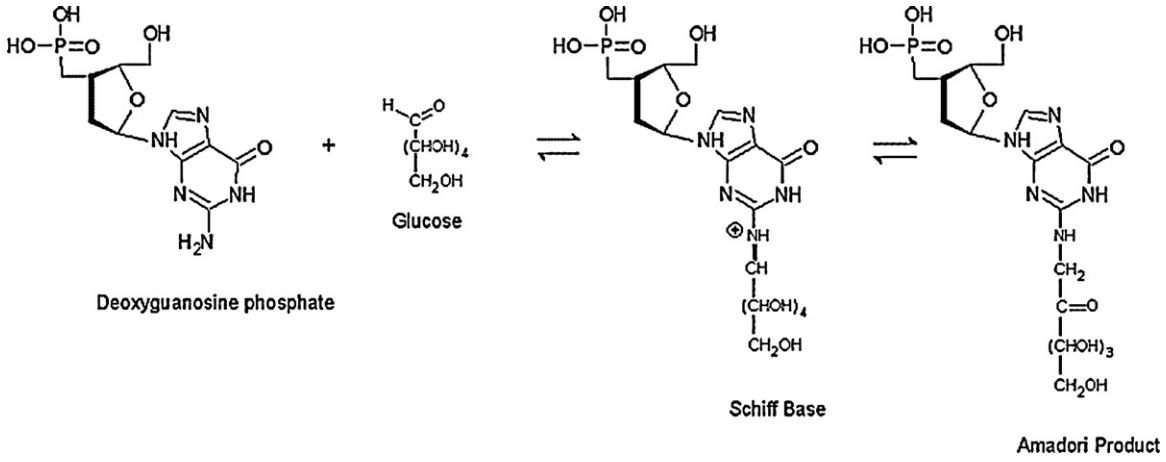

Fig. 1. DNA nonenzymatic glycosylation products.

affected to the same degree in the standard sample as compared to the test samples [97].

5. Overcoming PCR inhibition

Procedures that circumvent PCR inhibition need to be developed if PCR is to be successfully applied to the environmental samples. Following the detection of inhibition, some laboratories use routine inhibitor trouble-shooting strategies including extensive dilution [107], bovine serum albumin (BSA) [108], heat-soaked PCR [109], hot start PCR [110], and extra polymerase enzymes [111].

5.1. Extraction/purification protocols

The choice of extraction/purification protocol impacts the quantity and quality of DNA yield [112]. EDTA containing methods can release PCR inhibitors from the soil into solution [34,102]. EDTA treatment of bone samples resulted in co-extraction of an excess of proteins and other biomolecules in their DNA extract possibly through release of extracellular matrix components [113]. These substances originating from bone may act as endogenous PCR inhibitors [49]. Boiling, has been effective with urine samples [114] and cervical specimens [115]. Aqueous two-phase systems [116], density gradient centrifugation [117], enrichment media [118], filtration [119], proteinase inhibitors [38], phytase for bovine fecal specimens [120], immunological techniques [121], magnetic capture hybridization [122,123] and electroelution [124,76] have also been used to improve PCR. Furthermore, employment of immobilized sequence specific oligos followed by direct sequence analysis has been reported for DNA typing in forensic casework [125].

Considering the high weight range of humic compounds, between 20 and 65 of the DNA was reportedly lost during the electroelution procedures [76]. This might be problematic in some forensic caseworks with degraded templates. HAs are known to contain phenyl groups [126]. Due to their ability to absorb phenolic compounds, polyvinylpolypyrrolidone (PVPP) has been proposed for incorporation into purification protocols [127,128,84]. PVPP removes HAs with phenolic groups from crude DNA extracts via hydrogen bounding and formation of PVPP–phenolic complexes [129]. Inclusion of an extra extraction method or additional steps to remove possible PCR inhibitors would have increased the amplification success [130]. Pre-PCR Centricon 100 dialysis/concentration [131], using agarose embedded DNA preparations followed by washing with a lysis solution, which takes advantage of the size difference between DNA macromolecules and smaller sized inhibitor molecules [132], gel filtration electrophoresis [41,133,49,134], DNA precipitation with isopropanol [37] or 10 polyethylene glycol 8000 [135]. A variety of gel-packed columns and affinity beads are also used to remove inhibitors from DNA extracts. Sephadex G-50 chromatography was used to remove "a reddish brown contaminant" from DNA extracts of old Chilean human mummies [136].

Centrifugation of extracted DNA through a spin column packed with Sepharose 4B removed HAs more efficiently than either Sephadex G-200 or G-50 columns [137]. Furthermore, Thiopropyl Sepharose 6B beads have been useful in removing inhibitory textile dyes from DNA extracts [138]. These technologies however may reduce DNA yield.

Water soluble polyvinylpyrrolidone is sometimes incorporated into agarose gels to prevent the migration of humic compounds with DNA [139,140]. This is a time consuming method and also may reduce the yield of DNA [72]. As a cationic surfactant, cetyltrimethyl-ammonium bromide (CTAB) has both a hydrophobic group and a positively charged group [141]. CTAB forms insoluble complexes with denatured proteins, polysaccharides and cell debris [142] and binds to DNA mainly by electrostatic interaction [143].

Numerous techniques have been proposed for the further purification of soil DNA, including dialysis against large volumes of solvents or CsCl density centrifugation [144,145], hydroxyapatite chromatography [146], ion-exchange chromatography [35], binding to glass beads [144], selective precipitations [128], elution from agarose gels [147,148], and the use of commercial spin columns and filters [149,137,150]. Some of these treatments although simple, imply the loss of various amounts of the original sample. Dimethyl sulfoxide (DMSO) has been shown to increase PCR and reverse transcriptase-PCR yields, presumably by stabilizing nucleic acid complexes and improving primer annealing efficiency [151,152]. The effects of polyamines in stabilizing nucleic acids structure has been confirmed [153], however, the precise mechanism of spermidine in alleviating PCR inhibition is yet to be determined [154].

Considering the two facts associated with inhibitory material, Bourke et al. [32] developed a method to remove the inhibitors. Firstly, the most problematic inhibitors bind and therefore hyper-stabilize double stranded DNA. Secondly, the inhibitors mostly have smaller molecular size compared to DNA. Bourke et al. developed a procedure that detaches inhibitors from DNA and releases them into solution by denaturing DNA with 0.4 mM NaOH. Passing through a Microcon-100, DNA retains on the membrane while smaller sized inhibitors pass through. The renatured DNA is then used as template in PCR amplification [32]. Denaturing

conditions through NaOH treatment would release intercalated inhibitors and that denaturing washes would allow for their removal. In an effort to increase the rate of success for highly fragmented templates, Kemp et al. substituted Microcon-30s for Microcon-100s. This made it possible to type the mtDNA of some samples that previously failed to amplify [90]. However, the NaOH protocol is not advised when the quantity of DNA is limited, since the treatment results in significant loss of DNA [45]. It must be noted that humic compounds are also removable in alkaline conditions [64]. Chelex extraction method also provides alkaline conditions. However, the effectiveness of Chelex extraction method as compared to organic extraction methods is controversial [155,129]. This method which is effective in removal of heavy metals [156] has not been considered as an effective method in elimination of PCR inhibitors.

5.1.1. Diluting DNA extract

The most common employed technique to circumvent inhibition is diluting DNA extract sufficient enough to eliminate the inhibition [53,138]. A protocol has been suggested for determining appropriate dilutions [157]. This technology although effective especially for mtDNA analysis, may not be the best choice in case of highly degraded templates as DNA is already in low copy numbers increasing the probability of failures or miscoding lesions [158]. Starting DNA figures below 100 pg genomic DNA (about 15–17 diploid copies of nuclear DNA markers such as autosomal STRs) in forensic coursework are considered as low copy numbers [15], below which the authenticity of DNA profiles requires further assessments [159,160].

5.2. Amplification facilitators

The addition of amplification facilitators has been found to improve the specificity of PCR [161,61,162]. The possible mechanism of facilitators with protein structures such as BSA and single stranded DNA binding T4 gene 32 protein (GP32) [163], may be through binding to and therefore inactivating the inhibitors [38]. BSA has been noted by a few studies for reversal of PCR inhibition [20,163,108]. The binding efficiency of albumin at least in some cases may explain its ability to reduce the amplification inhibition [164]. BSA is not highly effective in reducing inhibition caused by bilirubin and NaCl [163]. Betaine has been suggested to increase PCR specificity and product yield [165] and to increase the thermal stability of proteins [166]. Carrying both positive and negative charges at pH close to neutrality, PCR facilitation activity of Betaine has been suggested to be due to its ability to destabilize GC-rich DNA sequences [167].

5.3. Polymerase enzymes

Using extra polymerase enzyme may be useful in case of inhibitors targeting that enzyme [28,168]. However, extra polymerase enzyme may increase the chance of non-specific amplifications. Different polymerases exhibit different properties with regard to inhibition and facilitation [38]. Tth DNA polymerase and hot-start Taq variants (particularly Ex Taq HS) were found resistant to higher concentrations of inhibitors from skeletal materials [111]. Some DNA polymerases from *T. aquaticus* and other species can have a greater tolerance for inhibitory substances including HAs [111]. Some DNA polymerases have a greater tolerance for inhibition. Recently, genetically engineered DNA polymerases have been shown to tolerate high concentrations of inhibitors [169].

The DNA polymerase least affected by inhibition from HAs and FAs is pfu DNA polymerase, followed by KlenTaq LA and RealTaq DNA polymerases [170]. Selecting appropriate polymerase [58,171] or employing a blend of inhibitor tolerant DNA polymerase–buffer systems [172], makes it possible to more efficiently amplify nucleic acids in the presence of inhibitory material, or in reactions containing PCR facilitators [38]. This strategy would be more beneficial in case of sample shortages, where extra purification steps can lead to DNA loss.

The enzymatic properties of polymerases (e.g., nuclease activity, fidelity, extension rates, and processivity; the average number of nucleotides added before disassociation of the enzyme) vary widely among families and species of origin [173].

5.4. mtDNA analysis

In case of PCR inhibition, mtDNA analysis is considered as a good alternative to nuclear DNA identification measures. mtDNA exists within cytoplasmic mitochondria as a separate small circular genome of about 16,569 base pairs. Every mitochondrion may contain more than one copy of mitochondrial DNA [174]. Dependent on cell type, mammalian cells host 80–680 mitochondria and 200–1700 mtDNAs, or about 2.6 DNAs per mitochondrion [175]. The amounts of amplifiable mtDNA were approximately 100–1000 times higher than those of nuclear DNA in the skin and ribs [102]. mtDNA has become the method of choice for working on skeletal remains such as bones [176–180] and teeth [181–183] as well as fingernails [184] and shed hairs [185] where nuclear DNA testing might be less successful. Sequence analysis of hypervariable regions of mtDNA is used in highly degraded samples where STR analysis is not possible. This is however a time-consuming process, and due to the haploid, non-Mendelian nature of mtDNA inheritance, the data are not as powerful for identification purposes as a full 13-locus STR match [186].

5.5. Chaotropic/silica based extraction methods

Generally, it is difficult to predict which types of inhibitors are present in any given sample. Therefore it is desirable to develop a generalized robust technique able to sufficiently remove as many of them as possible, regardless of the template source and methods of DNA extraction [32]. Chaotropic salt induced adsorption of DNA to silica is among the most commercialized methods for purifying DNA from cell homogenates [187–193]. Silica-based extraction method showed better performance in removal of inhibitors and extraction of DNA in nuclear STR typing from degraded bone samples than a commonly used phenol/chloroform method [189]. Silica membranes were found to be effective for a variety of samples [24].

Yang et al. found a silica-based column (Qiaquick) extraction more effective than a standard phenol/chloroform extraction in removing inhibition from extracts of human remains dating to 5000 years BP [194].

In natural conditions DNA is a hydrated macromolecule, with 8–10 tightly bound water molecules per nucleotide residue [195]. The silanol groups on the silica surface are also hydrogen bound to water through one or more hydration layers [196]. With the addition of high-ionic-strength chaotropic salts to the reaction, this relatively ordered structure of water molecules of the hydrate shell is destroyed. Chaotropic ions saturate the silica membrane with positive charges and also create a hydrophobic environment under which, the silica selectively binds duplex DNA [80]. In the presence of silica, water and sufficient concentration of chaotropic salt in controlled pH, duplex DNA adsorbs to silica chiefly due to electrostatic and hydrophobic interaction [197].

Three contributing forces control duplex DNA adsorption to silica including shielded intermolecular electrostatic forces, dehydration of the DNA and silica surface and intermolecular hydrogen bond formation in the DNA silica contact layer [198].

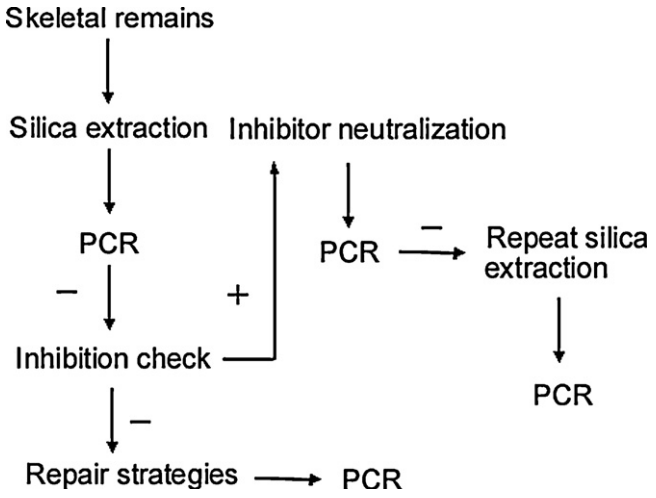

Fig. 2. Proposed protocol for PCR amplification of skeletal remains. Inhibitor neutralization strategies include: heat-soaked PCR, hot start PCR, BSA, extra Taq, NaOH treatment and extensive dilution.

Silica based purification methods, have been demonstrated to be efficient procedure in removal or attenuation of the inhibition [43,199] and are also amenable to automation [200] and miniaturization [201]. Kemp et al. proposed repeating silica extraction for further removal of inhibitors in case of PCR failure [90].

Due to its high DNA saturation value, Guanidinium Isothiocyanate is the most efficient chaotropic agent in DNA adsorption. This salt is also known to efficiently lyse cells and denature proteins [43].

Fig. 2 demonstrates the proposed steps for amplification of DNA especially from skeletal remains.

In the case of direct involvement of DNA nitrogenous bases in Maillard reaction, the binding between the nitrogenous base and sugars will make the base unrecognizable for the polymerase enzyme. The only effective choice would be possibly DNA repair. DNA repair technologies are beyond the scope of this report. Excision and repair enzymes have been introduced to facilitate molecular analysis of compromised samples [202].

References

[1] Shepard, E.M. Herrera,R.J. Iranian STR variation at the fringes of biogeographical demarcation, Forensic Sci. Int. 158 (2–3) (2006) 140–148.
[2] Alaeddini, R. Walsh, S.J. Abbas,A. Forensic implications of genetic analyses from degraded DNA—a review, Forensic Sci. Int. Genet. 4 (3) (2010) 148–157.
[3] Parsons, T.J. Weedn,V.W. Chemical and ultrastructural aspects of decomposition, in: HaglundW.D. , SorgM.H. (Eds.), Forensic Taphonomy: The Post-mortem Fate of Human Remains, Robert B. Stern, CRC Press, Inc., 1997, pp. 93–108.
[4] Vasan, S. Zhang, X. Kapurniotu, A. Bernhagen, J. Teichberg, S. Basgen, J. Wagle, D. Shih, D. Terlecky, I. Bucala, R. Cerami, A. Egan, J. Ulrich,P. An agent cleaving glucose-derived protein crosslinks in vitro and in vivo, Nature 382 (6588) (1996) 275–278.
[5] Longo, M.C. Berninger, M.S. Hartley,J.L. Use of uracil DNA glycosylase to control carry-over contamination in polymerase chain reactions, Gene 93 (1) (1990) 125–128.
[6] Wilson,I.G. Inhibition and facilitation of nucleic acid amplification, Appl. Environ. Microbiol. 63 (10) (1997) 3741–3751.
[7] Holland, M.M. Fisher, D.L. Lee, D.A. Bryson, C.K. Weedn,V.W. Short tandem repeat loci: application to forensic and human remains identification, EXS 67 (1993) 267–274.
[8] Butler, J.M. Shen, Y. McCord,B.R. The development of reduced size STR amplicons as tools for analysis of degraded DNA, J. Forensic Sci. 48 (5) (2003) 1054–1064.
[9] Sullivan, K.M. Hopgood, R. Gill,P. Identification of human remains by amplification and automated sequencing of mitochondrial DNA, Int. J. Leg. Med. 105 (2) (1992) 83–86.
[10] Powell, H.A. Gooding, C.M.C.M. Garrett, S.D. Lund, B.M. McKee,R.A. Proteinase inhibition of the detection of listeria monocytogenes in milk using the polymerase chain reaction, Lett. Appl. Microbiol. 18 (1994) 59–61.
[11] Saulnier, P. Andremont,A. Detection of genes in feces by booster polymerase chain reaction, J. Clin. Microbiol. 30 (1992) 2080–2083.
[12] Jacobsen, C.S. Rasmussen,O.F. Development and application of a new method to extract bacterial DNA from soil based on separation of bacteria from soil with cation-exchange resin, Appl. Environ. Microbiol. 58 (1992) 2458–2462.
[13] Simon, M.C. Gray, D.I. Cook,N. DNA extraction and PCR methods for the detection of listeria monocytogenes in cold-smoked salmon, Appl. Environ. Microbiol. 62 (1996) 822–824.
[14] Lienert, K. Fowler,J.C.S. Analysis of mixed human/microbial DNA samples: a validation study of two PCR AMP-FLP typing methods, BioTechniques 13 (1992) 276–281.
[15] Butler,J.M. Forensic DNA Typing: Biology, Technology and Genetics of STR Markers, 2nd ed., Elsevier Academic Press, New York, 2005.
[16] Kontanis, E.J. Reed,F.A. Evaluation of real-time PCR amplification efficiencies to detect PCR inhibitors, J. Forensic Sci. 51 (2006) 795–804.
[17] Larkin, A. Harbison,S. An improved method for STR analysis of bloodstained denim, Int. J. Leg. Med. 112 (6) (1999) 388–390.
[18] Eckhart, L. Bach, J. Ban, J. Tschachler,E. Melanin binds reversibly to thermostable DNA polymerase and inhibits its activity, Biochem. Biophys. Res. Commun. 271 (3) (2000) 726–730.
[19] Belec, L. Authier, J.R. Eliezer-Vanerot, M.C. Piedouillet, C. Ali Si Mohamed, A.S. Gherardi,R.K. Myoglobin as a polymerase chain reaction (PCR) inhibitor: a limitation for PCR from skeletal muscle tissue avoided by the use of thermus thermophilus polymerase, Muscle Nerve 21 (8) (1998) 1064–1067.
[20] Akane, A. Matsubara, K. Nakamura, H. Takahashi, S. Kimura,K. Identification of the heme compound copurified with deoxyribonucleic acid (DNA) from bloodstains, a major inhibitor of polymerase chain reaction (PCR) amplification, J. Forensic Sci. 39 (2) (1994) 362–372.
[21] Khan, G. Kangro, H.O. Coates, P.J. Heath,R.B. Inhibitory effects of urine on the polymerase chain reaction for cytomegalovirus DNA, J. Clin. Pathol. 44 (5) (1991) 360–365.
[22] Monteiro, L. Bonnemaison, D. Vekris, A. Petry, K.G. Bonnet, J. Vidal, R. Cabrita, J. Megraud,F. Complex polysaccharides as PCR inhibitors in feces: Helicobacter pylori model, J. Clin. Microbiol. 35 (4) (1997) 995–998.
[23] Beckett, S.M. Laughton, S.J. Pozza, L.D. McCowage, G.B. Marshall, G. Cohn, R.J. Milne, E. Ashton,L.J. Buccal swabs and treated cards: methodological considerations for molecular epidemiologic studies examining pediatric populations, Am. J. Epidemiol. 167 (10) (2008) 1260–1267.
[24] Boddinghaus, B. Wichelhaus, T.A. Volker, B. Bittner,T. Removal of PCR inhibitors by silica membranes: evaluating the amplicor mycobacterium tuberculosis kit, J. Clin. Microbiol. 39 (10) (2001) 3750–3752.
[25] Karlsen, F. Kalantari, M. Chitemerere, M. Johansson, B. Hagmar,B. Modifications of human and viral deoxyribonucleic acid by formaldehyde fixation, Lab. Invest. 71 (4) (1994) 604–611.
[26] Von Wurmb, N. Meissner, D. Wegener,R. Influence of cyanoacrylate on efficiency of forensic PCRs, Forensic Sci. Int. 124 (1) (2001) 11–16.
[27] Chiou, F.S. Pai, C.Y. Hsu, Y.P. Tsai, C.W. Yang,C.H. Extraction of human DNA for PCR from chewed residues of betel quid using a novel "pvp/ctab" method, J. Forensic Sci. 46 (5) (2001) 1174–1179.
[28] Fisher, D.L. Holland, M.M. Mitchell, L. Sledzik, P.S. Wilcox, A.W. Wadhams, M. Weedn,V.W. Extraction, evaluation, and amplification of DNA from decalcified and undecalcified united states civil war bone, J. Forensic Sci. 38 (1) (1993) 60–68.
[29] Sorensen, E. Hansen, S.H. Eriksen, B. Morling,N. Application of thiopropyl sepharose 6B for removal of PCR inhibitors from DNA extracts of a thigh bone recovered from the sea, Int. J. Leg. Med. 117 (4) (2003) 245–247.
[30] Braid, M.D. Daniels, L.M. Kitts,C.L. Removal of PCR inhibitors from soil DNA by chemical flocculation, J. Microbiol. Methods 52 (3) (2003) 389–393.
[31] Tsai, Y.L. Olson,B.H. Rapid method for direct extraction of DNA from soil and sediments, Appl. Environ. Microbiol. 57 (4) (1991) 1070–1074.
[32] Bourke, M.T. Scherczinger, C.A. Ladd, C. Lee,H.C. NaOH treatment to neutralize inhibitors of taq polymerase, J. Forensic Sci. 44 (5) (1999) 1046–1050.
[33] Yoshii, T. Tamura, K. Ishiyama,I. presence of a PCR-inhibitor in hairs, Nihon Hoigaku Zasshi 46 (5) (1992) 313–316.
[34] Watson, R.J. Blackwell,B. Purification and characterization of a common soil component which inhibits the polymerase chain reaction, Can. J. Microbiol. 46 (7) (2000) 633–642.
[35] Tebbe, C.C. Vahjen,W. Interference of humic acids and DNA extracted directly from soil in detection and transformation of recombinant DNA from bacteria and a yeast, Appl. Environ. Microbiol. 59 (8) (1993) 2657–3665.
[36] Scholz, M. Giddings, I. Pusch,C.M. A polymerase chain reaction inhibitor of ancient hard and soft tissue DNA extracts is determined as human collagen type i, Anal. Biochem. 259 (2) (1998) 283–286.
[37] Hanni, C. Brousseau, T. Laudet, V. Stehelin,D. Isopropanol precipitation removes PCR inhibitors from ancient bone extracts, Nucl. Acids Res. 23 (5) (1995) 881–882.
[38] Al-Soud, W.A. Radstrom,P. Effects of amplification facilitators on diagnostic PCR in the presence of blood,feces, and meat, J. Clin. Microbiol. 38 (12) (2000) 4463–4470.
[39] Sutlovic, D. Gamulin, S. Definis-Gojanovic, M. Gugic, D. Andjelinovic,S. Interaction of humic acids with human DNA: proposed mechanisms and kinetics, Electrophoresis 29 (7) (2008) 1467–1472.
[40] Preston,C.M. Applications of NMR to soil organic matter analysis: history and prospects, Soil Sci. 161 (1996) 144–166.
[41] Zhou, J. Bruns, M.A. Tiedje,J.M. DNA recovery from soils of diverse composition, Appl. Environ. Microbiol. 62 (2) (1996) 316–322.
[42] Paabo, S. Higuchi, R.G. Wilson,A.C. Ancient DNA and the polymerase chain reaction, the emerging field of molecular archaeology, J. Biol. Chem. 264 (17) (1989) 9709–9712.

[43] Boom, R. Sol, C.J. Salimans, M.M. Jansen, C.L. Wertheim-van Dillen, P.M. van der, J. Noordaa, Rapid and simple method for purification of nucleic acids, J. Clin. Microbiol. 28 (3) (1990) 495–503.
[44] Paabo,S. Ancient DNA: extraction, characterization, molecular cloning, and enzymatic amplification, Proc. Natl. Acad. Sci. U.S.A. 86 (6) (1989) 1939–1943.
[45] Primorac,D. The role of DNA technology in identification of skeletal remains discovered in mass graves, Forensic Sci. Int. 146S (2004) S163–S164.
[46] Pillai, S.D. Josephson, K.L. Bailey, R.L. Gerba, C.P. Pepper,I.L. Rapid method for processing soil samples for polymerase chain reaction amplification of specific gene sequences, Environ. Microbiol. 57 (1991) 2383–2386.
[47] Wan, W. Yeow,J.T. The effects of gold nanoparticles with different sizes on polymerase chain reaction efficiency, Nanotechnology 20 (32) (2009) 325702.
[48] Lantz, P.G. Matsson, M. Wadstrom, T. Radstrom,P. Removal of PCR inhibitors from human fecal samples through the use of an aqueous two-phase system for sample preparation prior to PCR, J. Microbiol. Methods 28 (1997) 159–167.
[49] Alaeddini, R. Burgoyne,L.A. Endogenous inhibition of DNA amplification from human skeletal remains, in: The 18th International Symposium of the Forensic Sciences, Fremantle (Perth), Australia, 2006.
[50] Wurmb-Schwark, N.V. Schwark, T. Harbeck, M. Oehmichen,M. A simple duplex-pcr to evaluate the DNA quality of anthropological and forensic samples prior short tandem repeat typing, Leg. Med. 6 (2004) 80–88.
[51] Paabo,S. Molecular cloning of ancient egyptian mummy DNA, Nature 314 (6012) (1985) 644–645.
[52] Hagelberg, E. Bell, L.S. Allen, T. Boyde, A. Jones, S.J. Clegg,J.B. Analysis of ancient bone DNA: techniques and applications, Philos. Trans. R. Soc. Lond. B Biol. Sci. 333 (1268) (1991) 399–407.
[53] Imaizumi, K. Noguchi, K. Shiraishi, T. Sekiguchi, K. Senju, H. Fujii, K. Yoshida, K. Kasai, K. Yoshino,M. DNA typing of bone specimens-the potential use of the profiler test as a tool for bone identification, Leg. Med. (Tokyo) 7 (1) (2005) 31–41.
[54] Huggett, J.F. Novak, T. Garson, J.A. Green, C. Morris-Jones, S.D. Miller, R.F. Zumla,A. Differential susceptibility of PCR reactions to inhibitors: an important and unrecognised phenomenon, BMC Res. Notes 1 (2008) 70.
[55] Opel, K.L. Chung, D. McCord,B.R. A study of PCR inhibition mechanisms using real time PCR, J. Forensic Sci. 55 (1) (2010) 25–33.
[56] Katcher, H.L. Schwartz,I. A distinctive property of tth DNA polymerase: enzymatic amplification in the presence of phenol, BioTechniques 16 (1994) 84–92.
[57] Rossen, L. Norskov, P.P. Holmstromet, K. Rasmussen,O.F. Inhibition of PCR by components of food samples, microbial diagnostic assays and DNA-extraction solutions, Int. J. Food Microbiol. 17 (1992) 37–45.
[58] Al-Soud, W.A. Radstrom,P. Capacity of nine thermostable DNA polymerases to mediate DNA amplification in the presence of PCR-inhibiting samples, Appl. Environ. Microbiol. 64 (10) (1998) 3748–3753.
[59] Bickley, J. Short, J.K. McDowel, D.G. Parkes,H.C. Polymerase chain reaction (PCR) detection of listeria monocytogenes in diluted milk and reversal of PCR inhibition caused by calcium ions, Lett. Appl. Microbiol. 22 (1996) 152–158.
[60] Kim, S. Labbe, R.G. Ryu,S. Inhibitory effects of collagen on the PCR for detection of clostridium perfringens, Appl. Environ. Microbiol. 66 (3) (2000) 1213–1215.
[61] Innis, M.A. Myambo, K.B. Gelfand, D.H. Brow,M.A.D. DNA sequencing with thermus aquaticus DNA polymerase and direct sequencing of polymerase chain reaction-amplified DNA, Proc. Natl. Acad. Sci. U.S.A. 85 (1988) 9436–9440.
[62] Wiedbrauk, D.L. Werner, J.C.J.C. Drevon,A.M. Inhibition of PCR by aqueous and vitreous fluids, J. Clin. Microbiol. 33 (1995) 2643–2646.
[63] Borkovsky,O.K. Sources of organic matter in marine basins, Mar. Geol. 3 (1965) 5–31.
[64] Rocha, J.C. Rosa, A.H. Furlan,M. An alternative methodologyfor the extraction of humic substances from organic soils, J. Braz. Chem. Soc. 9 (1) (1998) 51–56.
[65] Maillard,L.C. Action des acides aminés sur les sucres: formation des mélanoidines par voie méthodique, C. R. Hebd. Séances Acad. Sci. 154 (1) (1912) 66–68.
[66] Bucala, R. Model, P. Cerami,A. Modification of dna by reducing sugars: a possible mechanism for nucleic acid aging and age-related dysfunction in gene expression, Proc. Natl. Acad. Sci. U.S.A. 81 (1984) 105–109.
[67] Abelson, P.H. Hare,P.E. Reactions of amino acids with natural and artificial humics and kerogens, in: Washington Yearbook, vol. 69, Carnegie Inst., 1971, pp. 327–334.
[68] Parsons,J.W. Isolation of humic substances from soils and sediments, in: FrimmelF.H. , ChristmanR.F. (Eds.), Humic Substances and Their Role in the Environment, John Wiley and Sons, Chichester, 1988, pp. 3–14.
[69] Zipper, H. Buta, C. Lammle, K. Brunner, H. Bernhagen, J. Vitzthum,F. Mechanisms underlying the impact of humic acids on DNA quantification by SYBR Green I and consequences for the analysis of soils and aquatic sediments, Nucl. Acids Res. 31 (7) (2003) e39.
[70] Steffan, R.J. Goksoyr, J. Bej, A.K. Atlas,R.M. Recovery of dna from soils and sediments, Appl. Environ. Microbiol. 54 (12) (1988) 2908–2915.
[71] Dong, D. Yan, A. Liu, H. Zhang, X. Xu,Y. Removal of humic substances from soil DNA using aluminium sulfate, J. Microbiol. Methods 66 (2006) 217–222.
[72] Roose-Amsaleg, E. Garnier-Sillam, C.L. Harry,M. Extraction and purification of microbial dna from soil and sediment samples, Appl. Soil Ecol. 18 (2001) 47–60.
[73] Tsai, Y.L. Olson,B.H. Detection of low numbers of bacterial cells in soils and sediments by polymerase chain reaction, Appl. Environ. Microbiol. 58 (2) (1992) 754–757.
[74] Muller-Wegener,U. Interaction of humic substances with biota, in: FrimmelF.H. , ChristmanR.F. (Eds.), Humic Substances and Their Role in the Environment, John Wiley and Sons, Inc., New York, 1988, pp. 179–192.
[75] Elfarissi, F. Pefferkorn,E. Fragmentation of kaolinite aggregates induced by ion-exchange reactions within adsorbed humic acid layers, J. Colloid Interface Sci. 221 (1) (2000) 64–74.
[76] Kallmeyer, J. Smith,D.C. An improved electroelution method for separation of DNA from humic substances in marine sediment DNA extracts, FEMS Microbiol. Ecol. 69 (2009) 125–131.
[77] Crecchio, C. Stotzky,G. Binding of DNA on humic acids: effects on transformation of bacillus subtilis and resistance to DNase, Soil Biol. Biochem. 30 (8/9) (1998) 1061–1067.
[78] Tsai, Y.L. Olsen,B.H. Rapid method for separation of bacterial dna from humic substances in sediments for polymerase chain reaction, Appl. Environ. Microbiol. 58 (1992) 2292–2295.
[79] Vettori, C. Paffetti, D. Pietramellara, G. Stotzky, G. Gallori,E. Amplification of bacterial DNA bound on clay minerals by the random amplified polymorphic DNA (RAPD) technique, Ecology 20 (1996) 251–260.
[80] Romanowski, G. Lorenz, M.G. Wackernagel,W. Adsorption of plasmid DNA to mineral surfaces and protection against DNAse i, Appl. Environ. Microbiol. 57 (4) (1991) 1057–1061.
[81] Hiramoto, K. Kato, T. Kikugawa,K. Generation of DNA-breaking activity in the maillard reaction of glucose–amino acid mixtures in a solid system, Mutat. Res. 285 (2) (1993) 191–198.
[82] Hiramoto, K. Kido, K. Kikugawa,K. DNA breaking by Maillard products of glucose–amino acid mixtures formed in aqueous systems, J. Agric. Food Chem. 42 (1994) 689–694.
[83] Poinar, H.N. Hofreiter, M. Spaulding, W.G. Martin, P.S. Stankiewicz, B.A. Bland, H. Evershed, R.P. Possnert, G. Paabo,S. Molecular coproscopy: dung and diet of the extinct ground sloth nothrotheriops shastensis, Science 281 (5375) (1998) 402–406.
[84] Young, C. Burghoff, R.L. Keim, L.G. Minak-Bernero, V. Lute, J.R. Hinton,S.M. Poly1vinylpyrrolidone-agarose gel electrophoresis purification of polymerase chain reaction-amplifiable DNA from soils, Appl. Environ. Microbiol. 59 (1993) 1972–1974.
[85] Bucala, R. Model, P. Russel, M. Cerami,A. Modification of DNA by glucose 6-phosphate induces DNA rearrangements in an escherichia coli plasmid, Proc. Natl. Acad. Sci. U.S.A. 82 (1985) 8439–8442.
[86] Ikan,R. The Maillard Reaction: Consequences for the Chemical and Life Sciences, 1st ed., John Wiley and Sons, New York, 1996.
[87] Monnier, V.M. Cerami,A. Detection of nonenzymatic browning products in the human lens, Biochim. Biophys. Acta 760 (1) (1983) 97–103.
[88] Lee, A.T. Cerami,A. In vitro and in vivo reactions of nucleic acids with reducing sugars, Mutat. Res. 238 (1990) 185–191.
[89] Lee, A.T. Cerami,A. The formation of reactive intermediate(s) of glucose 6-phosphate and lysine capable of rapidly reacting with dna, Mutat. Res. 179 (2) (1987) 151–158.
[90] Kemp, B.M. Monroe, C. Smith,D.G. Repeat silica extraction: a simple technique for the removal of PCR inhibitors from DNA extracts, J. Archaeol. Sci. 33 (12) (2006) 1680–1689.
[91] Poinar, H.N. Hofreiter, M. Spaulding, W.G. Martin, P.S. Stankiewicz, B.A. Bland, H. Evershed, R.P. Possnert, G. Paabo,S. Molecular coproscopy: dung and diet of the extinct ground sloth nothrotheriops shastensis, Science 281 (5375) (1998) 402–406.
[92] Poinar, H. Kuch, M. McDonald, G. Martin, P. Paabo,S. Nuclear gene sequences from a late pleistocene sloth coprolite, Curr. Biol. 12 (2003) 1150–1152.
[93] Bachoon, D.S. Otero, E. Hodson,R.E. Effects of humic substances on fluorometric DNA quantification and DNA hybridization, J. Microbiol. Methods 47 (2001) 73–82.
[94] Rutledge, R.G. Cote,C. Mathematics of quantitative kinetic PCR and the application of standard curves, Nucl. Acids Res. 31 (16) (2003) e93.
[95] Guescini, M. Sisti, D. Rocchi, M.B. Stocchi, L. Stocchi,V. A new real-time PCR method to overcome significant quantitative inaccuracy due to slight amplification inhibition, BMC Bioinformatics 9 (2008) 326.
[96] Ramakers, C. Ruijter, J.M. Deprez, R.H. Moorman,A.F. Assumption-free analysis of quantitative real-time polymerase chain reaction (PCR) data, Neurosci. Lett. 339 (1) (2003) 62–66.
[97] Stahlberg, A. Aman, P. Ridell, B. Mostad, P. Kubista,M. Quantitative real-time PCR method for detection of b-lymphocyte monoclonality by comparison of κ and λ immunoglobulin light chain expression, Clin. Chem. 49 (1) (2003) 51–59.
[98] Sambrook, J. Russell,D.W. Molecular Cloning, A Laboratory Manual, 3rd ed., CSHL Press, 2001 pages 5.55–5.59.
[99] Saiki, R.K. Walsh, P.S. Levenson, C.H. Erlich,H.A. Genetic analysis of amplified DNA with immobilized sequence-specific oligonucleotide probes, Proc. Natl. Acad. Sci. USA 86 (1989) 6230–6234.
[100] Katz, E.D. Dong,M.W. Rapid analysis and purification of polymerase chain reaction products by high-performance liquid chromatography, BioTechniques 8 (1990) 546–555.
[101] Mantero, G. Zonaro, A. Albertini, A. Bertolo, P. Primi,D. Dna enzyme immunoassay: general method for detecting products of polymerase chain reaction, Clin. Chem. 37 (1991) 5422–5429.
[102] Imaizumi, K. Miyasaka, S. Yoshino,M. Quantitative analysis of amplifiable DNA in tissues exposed to various environments using competitive PCR assays, Sci. Justice 44 (4) (2004) 199–208.
[103] Swango, K.L. Timken, M.D. Chong, M.D. Buoncristiani,M.R. A quantitative PCR assay for the assessment of DNA degradation in forensic samples, Forensic Sci. Int. 158 (1) (2006) 14–26.
[104] Swango, K.L. Hudlow, W.R. Timken, M.D. Buoncristiani,M.R. Developmental validation of a multiplex qPCR assay for assessing the quantity and quality of nuclear DNA in forensic samples, Forensic Sci. Int. 170 (16) (2007) 35–45.
[105] Green, R.L. Roinestad, I.C. Boland, C. Hennessy,L.K. Developmental validation of the quantifiler real-time PCR kits for the quantification of human nuclear DNA samples, J. Forensic Sci. 50 (4) (2005) 809–825.

[106] King, C.E. Debruyne, R. Kuch, M. Schwarz, C. Poinar,H.N. A quantitative approach to detect and overcome PCR inhibition in ancient DNA extracts, BioTechniques 47 (5) (2009) 941–949.
[107] Alonso, A. Andelinovic, S. Martin, P. Sutlovic, D. Erceg, I. Huffine, E. de Simon, L.F. Albarran, C. Definis-Gojanovic, M. Fernandez-Rodriguez, A. Garcia, P. Drmic, I. Rezic, B. Kuret, S. Sancho, M. Primorac,D. DNA typing from skeletal remains: evaluation of multiplex and megaplex STR systems on DNA isolated from bone and teeth samples, Croat. Med. J. 42 (3) (2001) 260–266.
[108] Oikarinen, S. Tauriainen, S. Viskari, H. Simell, O. Knip, M. Virtanen, S. Hyoty,H. PCR inhibition in stool samples in relation to age of infants, J. Clin. Virol. 44 (3) (2009) 211–214.
[109] Ruano, G. Pagliaro, E.M. Schwartz, T.R. Lamy, K. Messina, D. Gaensslen, R.E. Lee,H.C. Heat-soaked PCR: an efficient method for DNA amplification with applications to forensic analysis, BioTechniques 13 (2) (1992) 266–274.
[110] Kermekchiev, M.B. Tzekov, A. Barnes,W.M. Cold-sensitive mutants of taq DNA polymerase provide a hot start for PCR, BioTechniques 31 (21) (2003) 6139–6147.
[111] Eilert, K.D. Foran,D.R. Polymerase resistance to polymerase chain reaction inhibitors in bone, J. Forensic Sci.. 54 (2009) 1001–1007.
[112] Viltrop, T. Krjutskov, K. Palta, P. Metspalu,A. Comparison of dna extraction methods for multiplex polymerase chain reaction, Anal. Biochem. 398 (2) (2010) 260–262.
[113] Evison, M.P. Smillie, D.M. Chamberlain,A.T. Extraction of single-copy nuclear DNA from forensic specimens with a variety of postmortem histories, J. Forensic Sci. 42 (6) (1997) 1032–1038.
[114] Van Vollenhoven, P. Heyns, C.F. de Beer, P.M. Whitaker, P. Van Helden, P.D. Victor,T. Polymerase chain reaction in the diagnosis of urinary tract tuberculosis, Urol. Res. 24 (1996) 107–111.
[115] Verkooyen, R.P. Luijendijk, A. Huisman, W.M. Goessens, W.H. Kluytmans, J.A. van Rijsoort-Vos, J.H. Verbrugh,H.A. Detection of PCR inhibitors in cervical specimens by using the AMPLICOR chlamydia trachomatis assay, J. Clin. Microbiol 34 (1996) 3072–3074.
[116] Lantz, P.G. Tjerneld, F. Borch, E. Hahn-Hagerdal, B. Radstrom,P. Enhanced sensitivity in PCR detection of listeria monocytogenes in soft cheese through use of an aqueous two-phase system as a sample preparation method, Appl. Environ. Microbiol. 60 (1994) 3416–3418.
[117] Lindqvist, R. Norling, B.B. Lambertz,S.T. A rapid sample preparation method for detection of food pathogens based on buoyant density centrifugation, Appl. Microbiol. 24 (1997) 306–310.
[118] Wernars, K. Delfgou, E. Soentoro, P.S. Notermans,S. Successful approach for detection of low numbers of enterotoxigenic escherichia coli in minced meat by using the polymerase chain reaction, Appl. Environ. Microbiol. 57 (1991) 1914–1919.
[119] DiMichele, L.J. Lewis,M.J. Rapid, species-specific detection of lactic acid bacteria from beer using the polymerase chain reaction, J. Am. Brew. Chem. Soc. 51 (1993) 63–66.
[120] Thornton, C.G. Passen,S. Inhibition of PCR amplification by phytic acid, and treatment of bovine fecal specimens with phytase to reduce inhibition, J. Microbiol. Methods 59 (2004) 43–52.
[121] Fluit, A.C. Terensma, R. Visser, M.J.C. Aarsman, C.J.M. Poppelier, M.J.J.G. Keller, B.H.I. Klapwijk, P. Verhoef,J. Detection of listeria monocytogenes in cheese with the magnetic immuno-polymerase chain reaction assay, Appl. Environ. Microbiol. 59 (1993) 1289–1293.
[122] Jacobsen,C.S. Microscale detection of specific bacterial DNA in soil with a magnetic capture-hybridization and PCR amplification assay, Appl. Environ. Microbiol. 61 (9) (1995) 3347–3352.
[123] Yankson, K.K. Steck,T.R. Strategy for extracting dna from clay soil and detecting a specific target sequence via selective enrichment and real-time (quantitative) PCR amplification, Appl. Environ. Microbiol. 75 (18) (2009) 6017–6021.
[124] Chandler, D.P. Schreckhise, R.W. Smith, J.L. Bolton,H. Jr., Electroelution to remove humic compounds from soil DNA and RNA extracts, J. Microbiol. Methods 28 (1997) 11–19.
[125] Gabriel, M.N. Calloway, C.D. Reynolds, R.L. Primorac,D. Identification of human remains by immobilized sequence-specific oligonucleotide probe analysis of mtDNA hypervariable regions I and II, Croat. Med. J. 44 (3) (2003) 293–298.
[126] Hedges,J.I. Polymerization of humic substances in natural environments, in: FrimmelF.H. , ChristmanR.F. (Eds.), Humic Substances and Their Role in the Environment, John Wiley and Sons, Chichester, UK, 1988, pp. 45–58.
[127] Berthelet, M. Whyte, L.G. Greer,C.W. Rapid, direct extraction of DNA from soils for PCR analysis using polyvinylpolypyrrolidone spin columns, FEMS Microbiol. Lett. 138 (1991) 17–22.
[128] Trevors, J.T. Lee, H. Cook,S. Direct extraction of DNA from soil, Microb. Releases 1 (1992) 111–115.
[129] Sutlovic, D. Gojanovic, M.D. Andelinovic,S. Rapid extraction of human DNA containing humic acid, Croat. Chem. Acta 80 (1) (2007) 117–120.
[130] Hoff-Olsen, P. Mevag, B. Staalstroem, E. Hovde, B. Egeland, T. Olaison,B. Extraction of DNA from decomposed human tissue, an evaluation of five extraction methods for short tandem repeat typing, Forensic Sci. Int. 105 (1999) 171–183.
[131] Jung, J.M. Comey, C.T. Baer, D.B. Budowle,B. Extraction strategy for obtaining DNA from bloodstains for PCR amplification and typing of the HLA-DQ alpha gene, Int. J. Leg. Med. 104 (3) (1991) 145–148.
[132] Moreira,D. Efficient removal of PCR inhibitors using agarose-embedded DNA preparations, Nucl. Acids Res. 26 (13) (1998) 3309–3310.
[133] Harry, M. Gambier, B. Bourezgui, Y. Garnier-Sillam,E. Evaluation of purification procedures for dna extracted from organic rich samples: interference with humic substances, Analysis 27 (1999) 439–442.
[134] Hou, W. Lian, B. Rothenberg,S. Two electrophoreses in different pH buffers to purify forest soil DNA contaminated with humic substances, Afr. J. Biotechnol. 9 (16) (2010) 2401–2407.
[135] LaMontagne, M.G. Michel, F.C. Jr., Holden, P.A. Reddy,C.A. Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis, J. Microbiol. Methods 49 (2002) 255–264.
[136] Rogan, P.K. Salvo,J.J. Molecular genetics of pre-columbian south american mummies, Mol. Evol. 122 (1990) 223–234.
[137] Jackson, C.R. Harper, J.P. Willoughby, D. Roden, E.E. Churchill,P.F. A simple, efficient method for the separation of humic substances and DNA from environmental samples, Appl. Environ. Microbiol. 63 (12) (1997) 4993–4995.
[138] Shutler, G.G. Gagnon, P. Verret, G. Kalyn, H. Korkosh, S. Johnston, E. Halverson,J. Removal of a PCR inhibitor and resolution of DNA STR types in mixed human-canine stains from a five year old case, J. Forensic Sci. 44 (3) (1999) 623–626.
[139] Liu, W. Marsh, T.L. Cheng, H. Forney,L.J. Characterization of microbial diversity by determining terminal restriction fragment length polymorphisms of genes encoding 16S rRNA, Appl. Environ. Microbiol. 63 (1997) 4516–4522.
[140] Boivin-Jahns, V. Ruimy, R. Bianchi, A. Daumas, S. Christen,R. Bacterial diversity in deep-subsurface clay environment, Appl. Environ. Microbiol. 62 (1996) 3405–3412.
[141] Zheng, H.Z. Pang, D.W. Lu, Z.X. Zhang, Z.L. Xie,Z.X. Combing DNA on CTAB-coated surfaces, Biophys. Chem. 112 (1) (2004) 27–33.
[142] Saano, A. Tas, E. Pippola, S. Lindstrom, K. Van Elsas,J.D. Extraction and analysis of microbial dna from soil, in: Van ElsasJ.D. , TrevorsJ.T. (Eds.), Nucleic Acids in the Environment: Methods and Applications, Springer-Verlag, Heidelberg, Germany, 1995 pp. 49–67.
[143] Liu, Q. Li, J. Tao, W. Zhu, Y. Yao,S. Comparative study on the interaction of DNA with three different kinds of surfactants and the formation of multilayer films, Bioelectrochemistry 70 (2) (2007) 301–307.
[144] Porteous, L.A. Armstrong,J.L. Recovery of bulk DNA from soil by a rapid, small-scale extraction method, Curr. Microbiol. 22 (1991) 345–348.
[145] Leff, L.G. Dana, J.R. McArthur, J.V. Shimkets,L.J. Comparison of methods of DNA extraction from stream sediments, Appl. Environ. Microbiol. 61 (1995) 1141–1143.
[146] Ogram, A. Sayler, G.S. Barkay,T. The extraction and purification of microbial DNA from sediments, J. Microbiol. Methods 7 (2–3) (1987) 57–66.
[147] Hilger, A.B. Myrold,D.D. Method for extraction of frankia DNA from soil, Agriculture, Ecosystems and Environment 34 (1–4) (1991) 107–113.
[148] Porteous, L.A. Armstrong,J.L. A simple mini-method to extract dna directly from soil for use with polymerase chain reaction amplification, Curr. Microbiol. 27 (2) (1993) 115–118.
[149] Borneman, J. Skroch, P.W. O'Sullivan, K.M. Palus, J.A. Rumjanek, N.G. Jansen, J.L. Nienhuis, J. Triplett,E.W. Molecular microbial diversity of an agricultural soil in wisconsin, Appl. Environ. Microbiol. 62 (6) (1996) 1935–1943.
[150] Picard, C. Ponsonnet, C. Paget, E. Nesme, X. Simonet,P. Detection and enumeration of bacteria in soil by direct dna extraction and polymerase chain reaction, Appl. Environ. Microbiol. 58 (9) (1992) 2717–2722.
[151] Shen, W.H. Hohn,B. DMSO improves PCR amplification of DNA with complex secondary structure, Trends Genet. 8 (7) (1992) 44–47.
[152] Sidhu, M.K. Liao, M.J. Rashidbaigi,A. Dimethyl sulfoxide improves RNA amplification, BioTechniques 21 (1) (1996) 44–47.
[153] Higashi, K. Terui, Y. Inomata, E. Katagiri, D. Nomura, Y. Someya, T. Nishimura, K. Kashiwagi, K. Kawai, G. Igarashi,K. Selective structural change of bulged-out region of double-stranded RNA containing bulged nucleotides by spermidine, Biochem. Biophys. Res. Commun. 370 (4) (2008) 572–577.
[154] Kikuchi, A. Sawamura, T. Kawase, N. Kitajima, Y. Yoshida, T. Daimaru, O. Nakakita, T. Itoh,S. Utility of spermidine in PCR amplification of stool samples, Biochem. Genet. 48 (5–6) (2010) 428–432.
[155] Walsh, P.S. Metzger, D.A. Higuchi,R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material, Biotechniques 10 (4) (1991) 506–513.
[156] Juniper, S.K. Cambon, M-A. Lesongeur, F. Barbier,G. Extraction and purification of DNA from organic rich subsurface sediments (ODP Leg 169S), Mar. Geol. 174 (2001) 241–247.
[157] Lalueza-Fox,C. Analysis of ancient mitochondrial dna from extinct aborigines from tierra del fuego-patagonia, Anc. Biomol. 1 (1996) 43–54.
[158] Ye, J. Ji, A. Parra, E.J. Zheng, X. Jiang, C. Zhao, X. Hu, L. Tu,Z. A simple and efficient method for extracting DNA from old and burned bone, J. Forensic Sci. 49 (4) (2004) 754–759.
[159] Taberlet, P. Griffin, S. Goossens, B. Questiau, S. Manceau, V. Escaravage, N. Waits, L.P. Bouvet,J. Reliable genotyping of samples with very low DNA quantities using PCR, Nucl. Acids Res. 24 (16) (1996) 3189–3194.
[160] Poinar,H.N. The top 10 list: criteria of authenticity for DNA from ancient and forensic samples, Prog. Forensics Genet. 9 (2003) 575–579.
[161] Sarkar, S. Kapelner, G. Sommer,S.S. Formamide can dramatically improve the specificity of PCR, Nucl. Acids Res. 18 (1990) 6465.
[162] Wu, J.R. Yeh,Y.C. Requirement of a functional gene 32 product of bacteriophage T4 in UV, J. Virol. 12 (1973) 758–765.
[163] Kreader,C.A. Relief of amplification inhibition in PCR with bovine serum albumin or T4 gene 32 protein, Appl. Environ. Microbiol. 62 (1996) 1102–1106.
[164] Curry, S. Mandelkow, H. Brick, P. Franks,N. Crystal structure of human serum albumin complexed with fatty acid, Nat. Struct. Biol. 5 (1998) 827–835.
[165] Frackman, S. Kobs, G. Simpson, D. Storts,D. Betaine and DMSO: enhancing agents for PCR, Promega Notes 27 (1998).
[166] Santoro, M.M. Liu, Y. Khan, S.M. Hou, L.X. Bolen,D.W. Increased thermal stability of proteins in the presence of naturally occurring osmolytes, Biochemistry 31 (1992) 5278–5283.

[167] Rees, W.A. Yager, T.D. Korte, J. Von Hippel,P.H. Betaine can eliminate the base pair composition dependence of DNA melting, Biochemistry 32 (1993) 137–144.
[168] Edwards, C.J. MacHugh, D.E. Dobney, K.M. Martin, L. Russell, N. Horwitz, L.K. McIntosh, S.K. MacDonald, K.C. Helmer, D. Tresset, A. Vigne, J.D. Bradley,D.G. Ancient DNA analysis of 101 cattle remains: limits and prospects, J. Archaeol. Sci. 31 (2004) 695–710.
[169] Kermekchiev, M.B. Kirilova, L.I. Vail, E.E. Barnes,W.M. Mutants of Taq DNA polymerase resistant to PCR inhibitors allow DNA amplification from whole blood and crude soil samples, Nucl. Acids Res. 37 (2009) e40.
[170] Matheson, C.D. Gurney, C. Esau, N. Lehto,R. Assessing PCR inhibition from humic substances, Open Enzyme Inhib. J. 3 (2010) 38–45.
[171] Hedman, J. Nordgaard, A. Rasmusson, B. Ansell, R. Radstrom,P. Improved forensic DNA analysis through the use of alternative DNA polymerases and statistical modeling of DNA profiles, BioTechniques 47 (5) (2009) 951–958.
[172] Hedman, J. Nordgaard, A. Dufva, C. Rasmusson, B. Ansell, R. Radstrom,P. Synergy between DNA polymerases increases polymerase chain reaction inhibitor tolerance in forensic DNA analysis, Anal. Biochem. 405 (2) (2010) 192–200.
[173] Pavlov, A. Pavlova, N. Kozyavkin, S. Slesarev,A. Recent developments in the optimization of thermostable DNA polymerases for efficient applications, Trends Biotechnol. 22 (5) (2004) 253–260.
[174] Bogenhagen, D. Clayton,D.A. The number of mitochondrial deoxyribonucleic acid genomes in mouse l and human hela cells, quantitative isolation of mitochondrial deoxyribonucleic acid, J. Biol. Chem. 249 (24) (1974) 7991–7995.
[175] Robin, E.D. Wong,R. Mitochondrial DNA molecules and virtual number of mitochondria per cell in mammalian cells, J. Cell. Physiol. 136 (3) (1988) 507–513.
[176] Nelson, K. Melton,T. Forensic mitochondrial dna analysis of 116 casework skeletal samples, J. Forensic Sci. 52 (3) (2007) 557–561.
[177] Andelinovic, S. Sutlovic, D. Erceg Ivkosic, I. Skaro, V. Ivkosic, A. Paic, F. Rezic, B. Definis-Gojanovic, M. Primorac,D. Twelve-year experience in identification of skeletal remains from mass graves, Croat. Med. J. 46 (4) (2005) 530–539.
[178] Seo, Y. Uchiyama, T. Shimizu, K. Takahama,K. Identification of remains by sequencing of mitochondrial DNA control region, Am. J. Forensic Med. Pathol. 21 (2) (2000) 138–143.
[179] Lutz, S. Weisser, H.J. Heizmann, J. Pollak,S. mtDNA as a tool for identification of human remains, identification using mtDNA, Int. J. Leg. Med. 109 (4) (1996) 205–209.
[180] Holland, M.M. Fisher, D.L. Mitchell, L.G. Rodriquez, W.C. Canik, J.J. Merril, C.R. Weedn,V.W. Mitochondrial DNA sequence analysis of human skeletal remains: identification of remains from the vietnam war, J. Forensic Sci. 38 (3) (1993) 542–553.
[181] Mornstad, H. Pfeiffer, H. Yoon, C. Teivens,A. Demonstration and semi-quantification of mtDNA from human dentine and its relation to age, Int. J. Leg. Med. 112 (2) (1999) 98–100.
[182] Yamada, Y. Ohira, H. Iwase, H. Takatori, T. Nagao, M. Ohtani,S. Sequencing mitochondrial DNA from a tooth and application to forensic odontology, J. Forensic Odontostomatol. 15 (1) (1997) 13–16.
[183] Ginther, C. Issel-Tarver, L. King,M.C. Identifying individuals by sequencing mitochondrial DNA from teeth, Nat. Genet. 2 (2) (1992) 135–138.
[184] Cline, R.E. Laurent, N.M. Foran,D.R. The fingernails of mary sullivan: developing reliable methods for selectively isolating endogenous and exogenous DNA from evidence, J. Forensic Sci. 48 (2) (2003) 328–333.
[185] Melton, T. Nelson,K. Forensic mitochondrial DNA analysis: two years of commercial casework experience in the united states, Croat. Med. J. 42 (3) (2001) 298–303.
[186] Butler, J.M. Levin,B.C. Forensic applications of mitochondrial DNA, Trends Biotechnol. 16 (4) (1998) 158–162.
[187] Lee, H.Y. Park, M.J. Kim, N.Y. Sim, J.E. Yang, W.I. Shin,K.J. Simple and highly effective dna extraction methods from old skeletal remains using silica columns, Forensic Sci. Int. Genet. 4 (5) (2010) 275–280.
[188] Vanek, D. Saskova, L. Koch,H. Kinship and y-chromosome analysis of 7th century human remains: novel dna extraction and typing procedure for ancient material, Croat. Med. J. 50 (3) (2009) 286–295.
[189] Davoren, J. Vanek, D. Konjhodzic, R. Crews, J. Huffine, E. Parsons,T.J. Highly effective DNA extraction method for nuclear short tandem repeat testing of skeletal remains from mass graves, Croat. Med. J. 48 (4) (2007) 478–485.
[190] Rohland, N. Hofreiter,M. Comparison and optimization of ancient dna extraction, BioTechniques 42 (2007) 343–352.
[191] Nagy, M. Otremba, P. Kruger, C. Bergner-Greiner, S. Anders, P. Henske, B. Prinz, M. Roewer,L. Optimization and validation of a fully automated silica-coated magnetic beads purification technology in forensics, Forensic Sci. Int. 152 (1) (2005) 13–22.
[192] Hanselle, T. Otte, M. Schnibbe, T. Smythe, E. Krieg-Schneider,F. Isolation of genomic dna from buccal swabs for forensic analysis, using fully automated silica-membrane purification technology, Leg. Med. (Tokyo) 5 (Suppl 1) (2003) S145–S149.
[193] V.V. Padhye, C. York, A. Burkiewiez, Nucleic acid purification on silica gel and glass mixture. United States patent US 5,658,548, Promega Corporation, August 1997.
[194] Yang, D.Y. Eng, B. Waye, J.S. Dudar, J.C. Saunders,S.R. Technical note: improved dna extraction from ancient bones using silica-based spin columns, Am. J. Phys. Anthropol. 105 (1998) 539–543.
[195] Lindahl,T. The croonian lecture, 1996: endogenous damage to DNA, Philos. Trans. R. Soc. Lond. B Biol. Sci. 351 (1347) (1996) 1529–1538.
[196] Pashley, R.M. Kitchener,J.A. Surface forces in adsorbed multilayer of water on quartz, J. Colloid Interface Sci. 71 (1979) 491–500.
[197] Parida, S.K. Dash, S. Patel, S. Mishra,B.K. Adsorption of organic molecules on silica surface, Adv. Colloid Interface Sci. 121 (2006) 77–110.
[198] Melzak, K.A. Sherwood, C.S. Turner, R.F.B. Haynes,C.A. Driving forces for DNA adsorption to silica in perchlorate solutions, J. Colloid Interface Sci. 181 (1996) 635–644.
[199] Hoss, M. Paabo,S. DNA extraction from Pleistocene bones by a silica based purification method, Nucl. Acids Res. 21 (1993) 3913–3914.
[200] Montpetit, S.A. Fitch, I.T. O'Donnell,P.T. A simple automated instrument for DNA extraction in forensic casework, J. Forensic Sci. 50 (3) (2005) 343–352.
[201] Poeckh, T. Lopez, S. Fuller, A.O. Solomon, M.J. Larson,R.G. Adsorption and elution characteristics of nucleic acids on silica surfaces and their use in designing a miniaturized purification unit, Anal. Biochem. 373 (2) (2008) 253–262.
[202] Kovatsi, L. Nikou, D. Triantaphyllou, S. Njau, S.N. Voutsaki, S. Kouidou,S. DNA repair enables sex identification in genetic material from human teeth, Hippokratia 13 (3) (2009) 165–168.