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Assessing PreCR™ repair enzymes for restoration of STR profiles from artificially degraded DNA for human identification☆

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ABSTRACT

Forensic scientists have used several approaches to obtain short tandem repeat (STR) profiles from compromised DNA samples, including supplementing the polymerase chain reaction (PCR) with enhancers and using procedures yielding reduced-length amplicons. For degraded DNA, the peak intensities of the alleles separated by electrophoresis generally decrease as the length of the allele increases. When the intensities of the alleles decrease below an established threshold, they are described as drop-outs, thus contributing to a partial STR profile. This work assesses the use of repair enzymes to improve the STR profiles from artificially degraded DNA. The commercial PreCR™ repair kit of DNA repair enzymes was tested on both purified DNA and native DNA in body fluids exposed to oxidizing agents, hydrolytic conditions, ultraviolet (UV) and ionizing radiation, and desiccation. The strategy was to restrict the level of DNA damage to that which yields partial STR profiles in order to test for allele restoration as opposed to simple allele enhancement. Two protocols were investigated for allele restoration: a sequential protocol using the manufacturer's repair procedure and a modified protocol reportedly designed for optimal STR analysis of forensic samples. Allele restoration was obtained with both protocols, but the peak height appeared to be higher for the modified protocol (determined by Mann–Kendall Trend Test). The success of the approach using the PreCR™ repair enzymes was sporadic; it led to allele restoration as well as allele drop-out. Additionally, allele restoration with the PreCR™ enzymes was compared with restoration by alternative, but commonly implemented approaches using Restorase™, PCRBoost™, bovine serum albumin (BSA) and the Minifiler™ STR system. The alternative methods were also successful in improving the STR profile, but their success also depended on the quality of the template encountered. Our results indicate the PreCR™ repair kit may be useful for restoring STR profiles from damaged DNA, but further work is required to develop a generalized approach.

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1. Introduction

In forensic investigations, DNA analysis plays a major role in human identification. However, evidence collected from a crime scene may have been exposed to environmental and/or chemical stresses that may produce lesions in the DNA. If the DNA has been damaged, the progression of the DNA polymerase during the polymerase chain reaction (PCR) may be prevented. PCR amplification of the short tandem repeat (STR) loci or of the mitochondrial hypervariable 1 (HV1) and HV2 regions are the major tools in human identification. Thus any DNA damage that halts the polymerase can result in a partial or full loss of the STR profile and make obtaining complete HVI and HV2 sequences difficult. The issues involved with analysis of degraded DNA have been collated and presented in a thorough review [1].

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DNA damage, such as double-strand breaks, single-strand nicks and modified bases, can occur from numerous processes. For instance, DNA in an aqueous environment can undergo a loss of base residues at high temperatures, leading to base modifications and chain scission [2]. Heat can induce oxidation of the nucleotides and produce base modifications such as 8-oxoguanine, which can result in mispairing [3]. Bleach, which is often used to clean bones and teeth of contaminating environmental DNA [4], produces oxidized residues but also fragments DNA [5]. Exposure to ultraviolet (UV) radiation can introduce lesions in the DNA that, if not repaired, can alter the DNA structure [6,7]. Hydrolytic degradation of DNA can even occur in the dry state [8]. DNA is also susceptible to fragmentation upon repeated freezing and thawing events, which is generally measured by the presence of nick translation [9]. Chemical stresses, such as changes in pH and the presence of metal radicals, can cause base aberrations while reactive aldehydes can produce non-repairable crosslinks [5]. A compilation of the common lesions expected from a variety of damage treatments is given in Supplementary Table S1.

In living cells, excision repair pathways can correct lesions in the DNA caused by either endogenous processes (e.g., oxidation) or exogenous agents (e.g., radiation). These repair mechanisms include enzymes such as: glycosylase to excise modified or mismatched bases [10], apurinic or apyrimidinic (AP) endonucleases to remove AP sites [5,11], DNA polymerase to fill in gaps, and ligase to seal nicks [12]. DNA exposed to the environment and certain chemicals, which is common in evidentiary samples, does not have the protection and benefit of these cellular processes since the cells are no longer living. In addition upon cell death, not only is the DNA is exposed to a cascade of enzymes that metabolize the macromolecule into fragments and precursor molecules [1], but it is also susceptible to metabolism by microorganisms. Highly fragmented DNA from nonliving cells may not be able to produce long STR amplification products required for full STR profiles. Similarly, DNA in archived evidence from unsolved crime cases and in stored extracts may suffer damage and be difficult to analyze [13].

Within the last decade, researchers have investigated strategies to repair damaged DNA in non-living cells. Some of this effort was aimed at increasing the ability to sequence ancient DNA (aDNA), which is largely, but not exclusively characterized by modified residues (deaminated pyrimidines), but also fragmentation [14]. The strategies often involved treatment of aDNA with a mixture of repair enzymes, however the success of these repair treatments depends on the extent and type of DNA damage. Initially the only available source of repair enzymes was research laboratory-devised mixtures. Responding to the need for a commercial kit for the repair of damaged DNA, New England Biolabs (NEB) developed the PreCR™ Repair Mix, which includes a polymerase, ligase, endonucleases, and glycosylases. The suite of enzymes was developed to repair abasic sites, nicks, thymidine dimers, blocked 3′ends, oxidized guanine and pyrmidines and deaminated cytosines; however, it does not ligate double-stranded breaks. Considering the PreCR™ repair kit has the potential to repair multiple types of lesions in DNA (Supplementary Table S1), the kit is promising for forensic analysis of compromised samples, as the DNA damage may not be restricted to a specific type of lesion.

In this work, the PreCR™ Repair Mix was assessed for its ability to restore DNA profiles from forensically relevant, yet compromised samples. Preliminary studies were focused on optimizing the PreCR™ Repair Mix using purified human DNA, which was artificially degraded by several methods to obtain partial STR profiles. After optimization, the PreCR™ Repair Mix was tested with body-fluid samples of blood, saliva and semen from varying donors. These samples were subjected to various degradation treatments to determine if lost STR alleles can be restored using the PreCR™ Repair Mix. In addition to this, we evaluated Restorase™, a mixture of polymerases reported to improve analysis of compromised DNA [15], for repairing some degraded body fluid samples. Experiments were also performed to distinguish STR restoration by repair from STR improvement by general PCR enhancement, using two approaches: (1) PCRBoost™, a commercial product reported to enhance PCR; and (2) bovine serum albumin (BSA), which has been documented to relieve PCR inhibition [16] and promote successful genotyping from compromised forensic samples [17]. After either the repair or enhancement treatment of all samples, STRs were generated using the kits AmpFℓSTR® Identifiler® and AmpFℓSTR® Minifiler™, the latter of which may provide full STR profiles from compromised DNA without the addition of repair enzymes or enhancers [18].

2. Materials and methods

2.1. DNA source and sample collection

In this work, the term *purified-DNA* is used for genomic DNA that was purchased as a purified DNA standard or for samples which had been purified before use (i.e., DNA was no longer in whole cells). DNA was purchased from the American Type Culture Collection (HL-60 DNA; Manassas, VA), Promega Corp. (9947A Control DNA; Madison, WI) and Applied Biosystems (AB) (Raji Control DNA; Foster City, CA). *Native-DNA* is used for DNA obtained from body fluids that had undergone either artificial or environmental degradation. A range of body fluid samples were collected from a set of volunteer laboratory staff according to the FBI Institutional Review Board (IRB) privacy protection procedures. For saliva samples, donors were requested to refrain from eating, drinking, and teeth brushing 30 min prior to collection. Saliva was collected from five donors after they swished 5 mL of room temperature water around in their mouths for 30 s. This was repeated until 3–5 mL saliva was collected from each donor (stored in a 50 mL tube). Blood samples were collected from five donors using two approaches: (1) with a Contact-Activated Lancet (BD Microtainer, Franklin Lakes, NJ) and used immediately for preparing stains; and (2) using a Vacutainer Eclipse™ Blood Collection system with a tube containing EDTA as an anticoagulant. For semen samples, each donor was provided with a sterile, leakproof container. Both donors (n, 2) were instructed to refrigerate the sample instead of freezing it and to return the container to the laboratory within 24 h of collection. In addition, semen from three donors was purchased from the Fairfax Cryobank (Fairfax, VA). After collection, body-fluid samples were divided into small aliquots (to minimize freeze/thawing events) and were stored at 4 °C for immediate use or frozen, either at −20 °C (blood and saliva) or in liquid N_2 (semen), for long term storage.

2.2. Preparation of artificially damaged purified-DNA and native-DNA in body fluids

The objective of the various damage treatments was to generate partial-profile DNA as opposed to DNA with complete profile loss or DNA with all allele peaks present but with only reduced peak heights. With a partial-profile DNA substrate, lost alleles could theoretically be recovered upon repair, helping to distinguish restoration from PCR enhancement and general improvement in peak height. Purchased purified DNA and native DNA samples from donors were artificially damaged in the laboratory following strategies obtained from published procedures as described in Supplementary material 1 [2,19–25]. Samples were subjected to hydrolysis, oxidation by bleach and hydrogen peroxide (H_2O_2), UVC irradiation, gamma irradiation, desiccation and environmental conditions, as outlined in Table 1

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