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A preliminary assessment of the effect of PreCR™ DNA repair treatment on mixture ratios in two person mixtures

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ABSTRACT

In this study, DNA extracted from known buccal samples was combined into two component mixture samples. These were subjected to UV exposure prior to their amplification with the Promega PowerPlex® 16HS amplification kit, and subsequent capillary electrophoresis on the ABI 3130xl instrument. Damaged samples were subjected to enzymatic repair treatment and retested to assess the amount of repair. Data showed that there is fidelity associated with the application with profile concordance after its use, and a corresponding increase in the amount of recovered alleles post damage. Results also showed changes in the stochastic relationship between mixture components that appear to be induced by the repair process itself. The mixture ratios of DNA samples were altered from an approximate original 1:3 ratio, to a ratio of 1:2 or greater. This variation can have a significant effect regarding the ability to reliably de-convolute DNA mixtures that have been subjected to the repair process.

1. Introduction

Enzymatic "cocktails" have been developed that mirror the in vivo repair mechanisms associated with cellular DNA damage and its repair [1]. The PreCR™ cocktail, made by New England BioLabs, was developed as a treatment kit to repair damaged regions allowing for STR analysis [1]. Initial studies performed to assess the efficacy and fidelity of the PreCR™ system focused on single source samples [2,3].

The nature of forensic specimens often exposes them to environmentally harsh conditions and various degradative factors. This is most often the case with biological specimens [4]. It has been observed that these conditions have direct consequences on the ability to obtain useful interpretative information [5,6].

Previous work has established that many different forms of biomolecules are susceptible to degradation [7]. Proteins, as well as nucleic acids, have all been found to degrade, lose conformation, and subsequently, lose function when left over time. These degradative processes are accelerated when exposed to various environmental insults [8,9]. Some of these are, but are not necessarily limited to; prolonged ultraviolet light exposure, increased temperature, water damage, and various forms of chemical/enzymatic damage [10–13]. In the case of damage to DNA in forensic evidentiary specimens, each of these has had deleterious effects on the ability to develop an interpretable profile. The ability to derive a DNA profile from these samples is often severely hindered if not impossible [6,18,21].

In many instances the inability to detect a profile is directly related to the nature of the damage sustained. The actions of various enzymes such as DNase's destroy the ability of the molecule to act as template for new DNA material.

Polymerase chain reaction testing has proven itself to be a great benefit to the majority of the biosciences [14,15]. This simple procedure, as conceptualized by Mullis [16], revolutionized countless areas within molecular biology. One area reaping huge dividends pursuing this form of testing has been forensic DNA analysis. This method has led to the testing of very small biological samples, while also providing high levels of discriminatory information via short tandem repeat (STR) analysis [18].

It was determined, however, that even though the testing of smaller sized amplicons (ranging from approximately 100 bp to 400 bp) allowed for increased chances of sample survival and degradation resistance compared to older methods, they were still not immune to damage [17–19]. The nature of the PCR relies on the utilization of existing DNA strand material to act as a "template" for newly synthesized strands. In instances where that material is compromised/degraded, the reaction cannot proceed. This may be attributed to sequence damage either in the primer region associated with a locus, or

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within the amplicon itself, preventing the polymerase from replicating the DNA sequence(s).

Over the last several years research attempts have been made to try to address this problem [20–22] by creating an effective way to repair DNA damage allowing for the successful typing of evidence samples. Nelson, et al. conducted a study that attempted to address this area [2]. This work touched on the nature of damage as well as possible mechanisms on how to repair it. Relying on a combination of enzymes normally utilized in vivo (i.e. ligases, topoisomerases, etc.), they established the potential viability for forensic sample usage.

Recent work by Diegoli, et al. in 2011 [3] looked at treatment of these damage situations incorporating a DNA repair enzyme "cocktail" mix. This mix, known as PreCR™ Repair Mix, is produced by New England BioLabs (Ipswich, MA) [1] Their study assessed the effectiveness of the kit in treating UV damaged specimens while also trying to establish optimal conditions for forensic usage; how much template was required to yield a usable result, and what combination of reaction mix components and treatment/incubation times yielded the best results.

Their study indicated that for most forensic specimens, using one quarter of the prescribed reaction mix concentration and a standard 20 min incubation period (as per the manufacturer) yielded amounts of repair to the damaged specimens that led to increased DNA profile detection. Damage was induced by UV exposure over a range of 30 to 120 s. All samples utilized 1 ng of template DNA for subsequent STR amplification [3].

An important observation was made when the amplification reactions took place. The normal treatment protocol required two separate amplification reactions using much more in the way of starting template. It was determined that by cutting down template amount as well as performing the repair incubation prior to the addition of STR primers, higher percentages of recovered peak heights were obtained from the damaged specimens [3].

The overall results of the Diegoli, et al. study were promising in pointing out the potential usage of the PreCR™ Repair Mix for forensic DNA casework, but remained limited in its scope. As stated in the paper, more work needed to be conducted to assess the kit's full functionality with other types and patterns of damage, as well as various inhibitors and their effects [3].

For these reasons, additional assessment of the repair of DNA fragments is warranted. Assessing repair characteristics associated with various sample treatments contributes to determining the methods reliability and usage. This becomes critical when applied to mixture samples. Effective mixture deconvolution; the separation of the mixture into its contributor's components, is based upon the relationship of each contributor's peak heights to one another [26]. If those relationships are altered or influenced by a repair mechanism or process, those effects need to be characterized so that they can be accounted for in the interpretation step.

2. Materials & methods

2.1. Ethics statement

All biological samples used in this study were obtained as per the University of New Haven's IRB guidelines.

All funding for reagents and consumables was provided by the University of New Haven. The use of the various reagents in this paper is in no way a commercial endorsement, nor did the manufacturers provide any funding for their use.

2.2. Samples

Buccal swabs were obtained from two female donors for mixture preparation purposes. One donor was designated sample "A" and the other sample "B". The swabs were extracted using the QIAgen DNA Mini extraction kit, utilizing spin column enrichment following the manufacturer's suggested protocol [23].

The samples were quantitated on the Applied Biosystems 7500 Sequence Detection System (SDS software v. 1.2.3) with the Quantifiler® quantification kit. This consisted of the following conditions: a 25 µL sample volume (2 µL sample + 23 µL reaction master mix), 9600 Emulation, Manual C_T threshold of 0.200, utilizing Auto baseline function. Thermal cycling parameters were: 1 rep at 95 °C/10 min (Stage 1), and 40 reps at 95 °C/15 s, 60 °C/1 min (Stage 2).

The profile in sample "A" was 28 total alleles; sample "B" 30 total alleles.

2.3. Mixture sample preparation

Different mixture ratios were examined to assess treatment effects on sample stoichiometry. Alteration to the sample's mixture ratio could have direct implications on the sample's interpretation. The mixture ratios assessed were 1:1 and 1:3. This provided data sets representative of mixture ratios encountered in casework where, depending upon the ratio encountered, it would determine possible separation viability. Based on the quantification results, dilutions of each buccal swab extract were prepared to approximate a 1 ng/µL working stock solution for each mixture component. In each mixture one component was held at a constant template amount while varying the other (1:1 versus 1:3). The volumes of extract for each component used were adjusted to provide a total of 1 ng of DNA template for damage/repair purposes.

Sample aliquots were prepared by taking 2 µL of the mixed component extract solution and adding it to 33 µL of dH_2O for a total of 35 µL. The entire sample at this point was then damaged. Subsequently, each sample aliquot was then split in half after a brief vortex to ensure template homogeneity throughout. One half (17.50 µL) of the mixed sample went through the repair process, while the remaining half did not. This allowed for a direct comparison of the effects of treatment vs. non-treatment on a homogenously prepared sample, attempting to keep any potential sample preparation variations minimized. Each aliquot incorporated a total of 1 ng of DNA template for any subsequent repair processing, amplification, and profile detection.

The prepared mixture samples were initially analyzed prior to their induced damage and/or repair treatment. The mixture ratios (MR) initially created using quantitation data were confirmed by assessing the generated profile's component peak height ratios (PHR). Samples appearing to have MR's outside of the 1:1 and 1:3 relationship were remixed to attain the appropriate levels in the corresponding mixture ratios.

Mixture sample damage was then induced using a Spectrolinker XL-1500 UV Crosslinker (Spectronics Corp.). The sample exposure time used for this study was 30 s, at 15 watts of power, 254 nm. Each tube subjected to UV irradiation was laid flat on its side to ensure a larger tube/sample exposure surface area. This damage mimicked the effect of exposure to sunlight, frequently encountered by physical evidence submitted for forensic analysis. The damage most associated with these exposures is the formation of intra-strand thymine dimer crosslinks, as well as single stranded nicks [8,9].

2.4. PreCR™ repair process

PreCR™ Repair Mix cocktail was obtained from New England BioLabs. (Ipswich, MA). For a listing of all of the components in the reaction mix, see the accompanying insert sheet provided by the manufacturer.

Each sample requires 5 µL of PreCR™ Repair Mix along with 2.5 µL of NAD+ [3].

After preparation of the damaged samples with the repair enzyme cocktail, they were incubated at 37 °C for 20 min in an ABI 9700 Thermal Cycler.

Undamaged mixture samples prepared from the same stock dilutions were also run as repair treated and non-repair treated references