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Authentication of forensic DNA samples

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ABSTRACT

Over the past twenty years, DNA analysis has revolutionized forensic science, and has become a dominant tool in law enforcement. Today, DNA evidence is key to the conviction or exoneration of suspects of various types of crime, from theft to rape and murder. However, the disturbing possibility that DNA evidence can be faked has been overlooked. It turns out that standard molecular biology techniques such as PCR, molecular cloning, and recently developed whole genome amplification (WGA), enable anyone with basic equipment and know-how to produce practically unlimited amounts of *in vitro* synthesized (artificial) DNA with any desired genetic profile. This artificial DNA can then be applied to surfaces of objects or incorporated into genuine human tissues and planted in crime scenes. Here we show that the current forensic procedure fails to distinguish between such samples of blood, saliva, and touched surfaces with artificial DNA, and corresponding samples with *in vivo* generated (natural) DNA. Furthermore, genotyping of both artificial and natural samples with Profiler Plus® yielded full profiles with no anomalies. In order to effectively deal with this problem, we developed an authentication assay, which distinguishes between natural and artificial DNA based on methylation analysis of a set of genomic loci: in natural DNA, some loci are methylated and others are unmethylated, while in artificial DNA all loci are unmethylated. The assay was tested on natural and artificial samples of blood, saliva, and touched surfaces, with complete success. Adopting an authentication assay for casework samples as part of the forensic procedure is necessary for maintaining the high credibility of DNA evidence in the judiciary system.

1. Introduction

The current forensic procedure that deals with DNA evidence starts at the crime scene where biological samples such as blood and saliva stains are detected, identified, documented, collected, and transferred to the forensic laboratory. In the laboratory, DNA is extracted and quantified, usually by real time PCR amplification of the hTERT locus (Quantifiler®) or similar targets [1]. Following quantification, about 1 ng of the DNA is used for a profiling reaction, in which 9–15 highly polymorphic short tandem repeat (STR) loci and the sex-typing marker amelogenin are genotyped. The loci are usually chosen from a standard set of core loci such as the 13 Combined DNA Index System (CODIS) loci. A detailed description of the forensic procedure is provided in Text S1.

The DNA profile of every person is considered unique (except for identical twins) [2], and consequently, this "DNA fingerprint" is used in police investigations to link between a crime scene and a specific individual, who is either a suspect in the case, or identified by an automatic search of the database (e.g. CODIS). In recent years, DNA evidence has become the "gold standard" of forensic testing, and is an invaluable tool for the criminal justice community [3–7]. The high credibility of DNA evidence in court stems from the fact that it uses a statistical approach based on population genetics and empirical testing [8], in contrast to other types of forensic evidence, such as ballistics, blood-spatter analysis, and fiber analysis, which rely on expert judgment and have limited connection to established science [7]. It is even considered to be more reliable than eyewitness evidence, which is known to suffer from a relatively high rate of errors [8].

The use of DNA recovered at crime scenes as evidence in court relies on the implicit assumption that the DNA is genuine—originating from natural biological material. However, as we show here, this assumption may not necessarily be true: DNA with any desired genetic profile can easily be synthesized *in vitro* using common [9,10], and recently developed [11,12] biological techniques, integrated into genuine human tissues or applied to surfaces of objects, and then planted in crime scenes. When the current forensic procedure is applied to objects or human tissues that contain synthesized DNA, it fails to recognize the artificial origin of the sample, and the resulting profile is indistinguishable from a genuine DNA profile. Nevertheless, we demonstrate

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that natural and artificial samples can be differentiated based on differential methyaltion patterns. Methylation is an epigenetic chemical modification of DNA, occurring in mammals in the form of a methyl group ($-CH_3$) that is enzymatically added to the C5 position of cytosine in some CpG dinucleotides [13]. DNA methylation is believed to inhibit gene expression in animal cells, probably by affecting chromatin structure [14]. In the human genome 70–80% of all CpGs are methylated, while unmethylated CpGs are grouped in clusters called "CpG islands" [15].

2. Materials and methods

2.1. Collection of biological tissues

Samples of blood, dry saliva stains on absorbent paper, skin scrapings, hair, and smoked cigarette butts were collected from volunteers. Informed consent was obtained from all participants recruited into the study. DNA from these samples was extracted and quantified as described in Section 2.6.

2.2. CODIS allele library

For construction of the library, individual alleles of CODIS STRs and the hTERT locus were amplified from pooled DNA (Control Human Genomic DNA of the GenomePlex WGA2 kit, Sigma–Aldrich) by separate PCR reactions (primers and conditions as described in Section 2.9). Amplified fragments were purified (QIAquick PCR purification kit, QIAGEN), and cloned into the pGEM-T-Easy vector (Promega). Plasmid DNA was purified by the QIAprep Spin Miniprep kit (QIAGEN) and quantified (Nanodrop 1000, Thermo Scientific). For genotyping of cloned alleles, the PowerPlex16 (Promega) kit was used. Genotyping was performed in a high throughput manner by simultaneously genotyping 10–15 clones (from different CODIS loci) in a single PowerPlex16 reaction. In the resulting library each element is a microcentrifuge tube with trillions of copies of a single allele (for example, one element is allele 11 of locus D8S1179, while another is allele 12 of D8S1179, and likewise for the other CODIS loci). We note that 1 fg of plasmid in the library contains ~160 copies of its cloned allele—the same copy number that is present in ~1 ng of a haploid genome.

2.3. In vitro synthesis of DNA

Artificial DNA was synthesized by one of the following methods:

PCR: For the sample whose profile is shown in Fig. 1, the 10 loci included in the Profiler Plus® kit (Applied Biosystems) were amplified separately from 1 ng of DNA extracted from a cigarette butt smoked by 'N400' (PCR conditions were as described in Section 2.9; primer sequences are in Text S3). Individual amplified fragments were purified (QIAquick PCR purification kit, QIAGEN), quantified (Nanodrop 1000, Thermo Scientific), diluted about a million fold (depending on the concentration of the specific amplicon), and combined in a single test tube. For the sample whose profile is shown in Fig. 2, 1 ng of 'N222' DNA (extracted from a saliva stain on absorbent paper) was used as template in a single PCR reaction using the Profiler Plus® primer mix. A 1:1000 dilution of the PCR reaction was used for generating the artificial sample.

WGA: Whole genome amplification was performed by multiple displacement amplification [16] with the Repli-g Midi kit (QIAGEN) using 10 ng of natural DNA as template.

Assembly from CODIS allele library: For assembling profiles using the CODIS allele library, equal quantities of alleles (cloned into plasmids) in the desired profile were picked from the library and combined in a single tube.

2.4. Generation of mock forensic samples

For generating artificial touch DNA samples, *in vitro* synthesized DNA was applied directly to the surface of the object and allowed to dry. For generating artificial blood samples, red blood cells were isolated from whole blood by centrifugation (1500 × *g*, 10 min), and mixed with *in vitro* synthesized DNA. Drops of the red

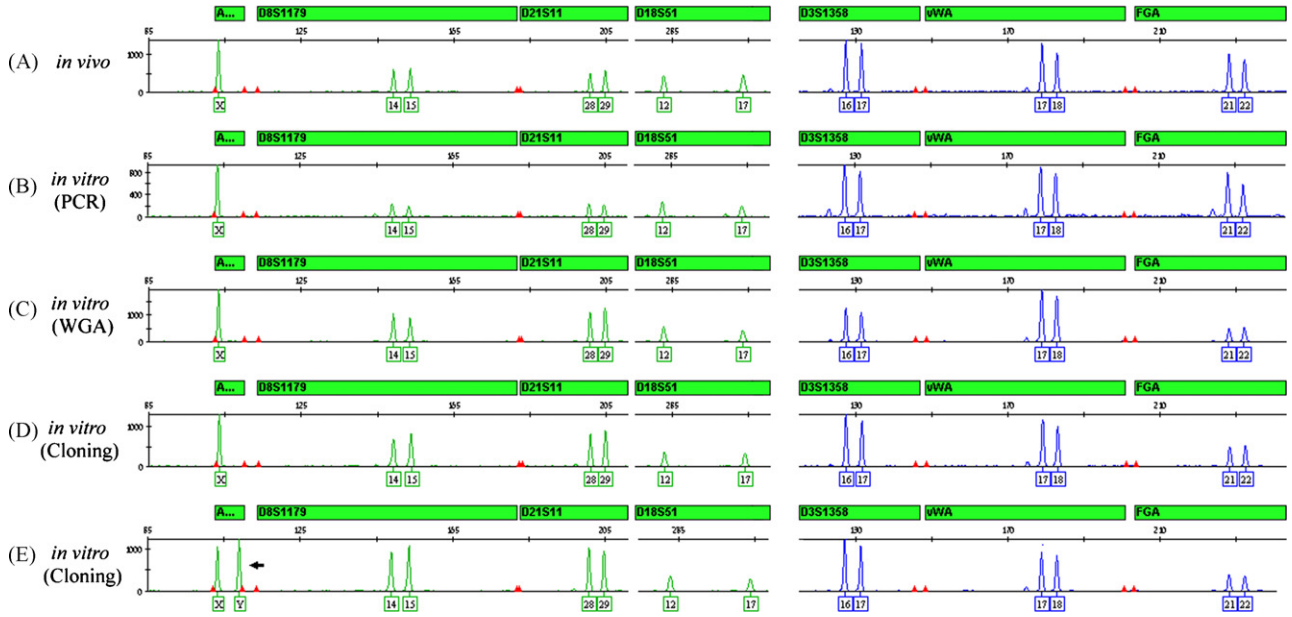

Fig. 1. Profiles of *in vivo*- and *in vitro*-synthesized DNA are indistinguishable. (A) Profile of natural DNA obtained from the saliva of female donor 'N400'. (B–D) Artificial profiles of 'N400' obtained from DNA that was synthesized *in vitro* by three different methods: PCR (B), WGA (C), and assembly from a library of cloned CODIS alleles (D). (E) Artificial profile of 'male-N400', which is identical to the profile of 'N400' at all loci, except for the Amelogenin locus. This profile was created by adding a cloned Y allele (indicated by arrow) to the mix used to generate the profile in (D). In A–E partial profiles are depicted; full profiles are provided in Text S2.