Available online at www.sciencedirect.com
ScienceDirect
Forensic Science International 166 (2007) 121–127
Forensic Science International
www.elsevier.com/locate/forsciint

A new sensitive short pentaplex (ShoP) PCR for typing of degraded DNA

C. Meissner [a,*], P. Bruse [a], E. Mueller [c], M. Oehmichen [a,b]

[a] Department of Forensic Medicine, Medical University of Luebeck, Kahlhorststrasse 31-35, 23562 Luebeck, Germany
[b] Department of Forensic Medicine, University of Kiel, Germany
[c] Bundeskriminalamt, Wiesbaden, Germany

Received 13 May 2005; received in revised form 18 April 2006; accepted 21 April 2006
Available online 30 June 2006

Abstract

Analysis of short tandem repeat makers has become the most powerful tool for DNA typing in forensic casework analysis. Unfortunately, typing of DNA extracted from telogen shed hairs, bones buried in the soil or from paraffin-embedded, formalin-fixed tissue often reveals no results due to the degradation of DNA. The reduction in size of the target fragments by development of new primers and their combination in multiplex approaches open a new field of DNA analysis. Here we present a new sensitive short pentaplex PCR including the loci amelogenin, TH01, VWA, D3S1358 and D8S1179. Validation tests of our new method included sensitivity, mixtures, human specificity, artificial degradation of DNA by DNase I and case work analysis on a panel of different forensic samples. The detection limit was 12.5 pg of human DNA, and mixtures of 50 pg in a total of 1000 pg were clearly detectable and revealed complete profiles. Only DNA extracts of human primates displayed a few signals, whereas other animal, fungal or bacterial DNA showed no signals. Our method proved extremely valuable in the analysis of artificially degraded DNA and in forensic cases, where only poorly preserved DNA was available. This approach and other similar methods can aid in the analysis of samples where allelic drop out of larger fragments is observed. It is highly recommended to develop more of these multiplexes to improve poor quality DNA typing.

Keywords: DNA typing; Short tandem repeats; Multiplex PCR; Forensic casework

1. Introduction

In the last decade, DNA typing of highly polymorphic STR (short tandem repeat) loci has become the most powerful tool for discrimination of individuals, because these loci are even stable in decomposed tissues [1]. In particular, the simultaneous amplification of multiple STR markers in the same PCR has found multiple applications even in cases with only minute amounts of DNA [2]. In these multiplex PCR assays usually fluorescent dyes are attached to the forward primer of different loci to allow discrimination of fragments of similar size [3]. The development of capillary electrophoresis enables the on-line detection of these labelled PCR amplicons with the advantage of high throughput, automatic operation and automated data acquisition [4]. Nevertheless there are a lot of forensic cases where DNA is highly degraded due to the quality of the sample itself [5] or environmental conditions [6,7]. These cases include skeletal remains buried in the soil [8], decomposed bodies [9], paraffin-embedded tissue [10] or shed telogen hairs [5]. The rate of decay of DNA depends to a large extend on the geochemical properties of the soil, the effects of the surrounding milieu, contamination with microorganisms and temperature [7,11,12]. Especially exposure of bone or teeth in damp environments seems to be crucial for successful DNA typing [6,13]. On the other hand, DNA degradation is reduced under permafrost conditions in arctic regions, facilitating analyses of remains up to 50,000 years old [11]. Nevertheless, ancient DNA extracted from bones up to more than 10,000 years old displays an average size between 100 and 150 bp and oxidative as well as hydrolytic damage making PCR amplification and DNA typing extremely difficult [11,14,15]. At worst, DNA can be degraded to such an extent that it is no longer suitable for demonstration of STR profiles [16]. Therefore, a lot of different approaches have been presented to

* Corresponding author. Tel.: +49 451 500 2752; fax: +49 451 500 2760.
E-mail address: meissner@rmed.mu-luebeck.de (C. Meissner).

0379-0738/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.forsciint.2006.04.014

improve the quality of DNA profiling of degraded DNA. These studies include the evaluation of different extraction methods [9], removal of special PCR inhibitors, as well as purification of extracts [17,18], a nested PCR method [19] and typing of standardized degraded DNA [12]. Recently an excellent review about the application of reduced size amplicons for reliable DNA typing of degraded DNA has been published. These miniplex PCRs are recommended in cases where allelic drop out and reduced sensitivity especially of larger alleles occurs [20]. So far, a few of these interesting miniplex PCRs have already been described for a variety of forensic samples [20,21] including shed telogen hairs [5]. Learning from lessons of degradation of DNA even over long periods of time we developed a multiplex PCR where none of the detectable alleles is more than 150 bp in length. Here we present a short pentaplex PCR (ShoP-PCR) approach which is highly suitable to obtain excellent results even in cases where DNA typing was unsuccessful using commercially available kits.

2. Materials and methods

2.1. Primer design

Sequences of primers were designed using the Primer3 [22] and GeneFisher (http://bibiserv.techfak.uni-bielefeld.de/cgi-in/gf_submit?mode=STARTUP&sample=dna) software. Forward primers were labelled with fluorescent dyes as shown in Table 1.

2.2. PCR amplification

Pentaplex PCR was carried out in a 10 μl reaction mix containing 20 mM Tris–HCl (pH 8.4), 50 mM KCl, 200 μM each dNTP (dATP, dGTP, dCTP, dUTP), 2.2 mM $MgCl_2$, 500 μg/ml bovine serum albumin, 1% Tween 20, 200 nM each of amelogenin primer, 600 nM each of D8S1179 primer, 1000 nM each of vWA primer, 75 nM each of TH01 primer, 150 nM each of D3S1358 Primer, 1 U Platinum Taq DNA Polymerase (Invitrogen, Karlsruhe, Germany), 0.1 U UNG (MBI Fermentas, Leon-Rot, Germany) and a variable amounts of template DNA. To avoid contaminating PCR products samples were digested with UNG (Uracil-DNA Glycosylase) prior to amplification by incubation at 37 °C for 5 min. The cycling profile of the ShoP-PCR in a GeneAmp PCR system 2400 (Applied Biosystems, Darmstadt, Germany) was 95 °C for 11 min (initial incubation), 96 °C for 2 min, followed by 10 cycles of denaturation for 30 s at 94 °C, annealing for 30 s at 60 °C and extension for 45 s at 70 °C and then by 18–22 cycles of denaturation for 30 s at 90 °C, annealing for 30 s at 60 °C and extension for 45 s at 70 °C. This was followed by a final elongation step of 90 min at 60 °C. At the end of the PCR reaction, the temperature was kept at 4 °C. Ramping time between annealing and extension was carefully adjusted between 0.3 and 0.5 °C/s.

2.3. Signal detection

One to two microliters of each PCR product was mixed with 0.5 μl GeneScan-400HD (ROX) internal lane standard (Applied Biosystems, Darmstadt, Germany) and 14.5 μl of deionized formamide (Sigma, Taufkirchen, Germany). The mixture was subjected to heat denaturation in the PCR thermocycler for 3 min at 96 °C. After cooling on ice the samples were injected electrocinetically for 5 s. Detection was performed on a 310 ABI Prism Genetic analyzer according to the manufacturers recommendations (Applied Biosystems, Darmstadt, Germany). Fragment sizes and amount of PCR products were determined automatically applying GeneScan Analysis Software 3.1 (Applied Biosystems, Darmstadt, Germany).

2.4. Validation procedures

2.4.1. Primer specificity

Buccal swabs were collected from 200 unrelated healthy volunteers and DNA was immediately extracted using the Chelex 100 method [23]. For comparison 200 human DNA profiles obtained by the ShoP-PCR were compared with profiles applying the SGM und PowerPlex 16 system of the same individuals.

To test human specificity DNA from various animals (cat, dog, pig, horse, cow, mouse, rat, frog, fish, sheep, rabbit, guinea pig, goat, deer, black deer, fox, pigeon and herring) and the three closest related primates (gorilla, orangutan, chimpanzee) was isolated. DNA extraction was performed using the QIAamp

Table 1
Description, repeat number of allelic ladder components, allelic size range and primer sequences of the ShoP-PCR loci

System	GenBank® accession	Repeat numbers of allelic ladder components	Size range of allelic ladder components	Primers (5′ → 3′)	Labelled with
Amelogenin	M55418 and M55419	3 bp difference	X: 79 bp, Y: 82 bp	CCTTTGAAGTGGTACCAGAGCA (forward), GCATGCCTAATATTTTCAGGGAA (reverse)	HEX
D8S1179	GO8710	7–18	83–127 bp	TTTTTGTATTTCATGTGTACATTCG (forward), TCCTGTAGATTATTTTCACTGTGG (reverse)	6-FAM
VWA	M25858	10–22	93–141 bp	GAATAATCAGTATGTGACTTGGATTGA (forward), GATGATAAATACATAGGATGGATGGA (reverse)	HEX
TH01	D00269	4–9, 9.3, 10–11, 13.3	68–107 bp	GCCTGTTCCTCCCTTATTTCC (forward), AGGTCACAGGGAACACAGACTC (reverse)	NED
D3S1358	11449919	12–20	113–145 bp	ACTGCAGTCCAATCTGGGTGAC (forward), GAAATCAACAGAGGCTTGCATG (reverse)	NED

Sequences of the D8S1179 forward primer have been already published [38].