Int J Legal Med (2001) 114 : 285–287

SHORT COMMUNICATION

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Less is more – length reduction of STR amplicons using redesigned primers

Received: 6 January 2000 / Accepted: 26 April 2000

Abstract PCR primers closely flanking the repeat region were redesigned to reduce the amplicon length of the selected STRs down to approximately 100 bp for the shorter alleles (loci HumTH01, D10S2325, DYS19 and DYS391). Highly degraded DNA (e.g. formalin-fixed tissue) and very low amounts of DNA could be more successfully typed using the new redesigned primers compared to the established sequences generating longer amplicons.

Keywords STRs · Primers · Degradation · DNA

Introduction

The investigation of biological stains often requires the analysis of degraded, sometimes even highly degraded material. The use of PCR technology, especially STR systems, has been accompanied by a significant increase in the success rates of DNA typing because only relatively short fragments of intact DNA are necessary (Brinkmann 1992).

The aim of our study was to look for efficient STRs that are robust and informative for successful typing of low amounts of highly degraded DNA. In such cases, it may be useful to select new primer sequences closely flanking the repetitive region.

The approach was to reduce the amplicon length of selected STRs down to 100 bp for the shorter alleles of the polymorphism. We focused on four systems that show a relatively low number of repeats: TH01, which is one of the most widely used systems among the forensically established STRs, D10S2325 containing a pentameric repeat array of 7–15 for the most frequent alleles and the two tetrameric Y-chromosomal STRs DYS19 and DYS391.

TH01	Urquhart et al. 1995	154 bp - allele 5 -> 80 bp
D10	Lee et al. 1998	118 bp - allele 7 -> 96 bp
Y391	Kayser et al. 1997	279 bp - allele 9 -> 122 bp
Y19	Roewer et al. 1992	186 bp - allele 13 -> 100 bp

Fig. 1 An overview of the established primer sequences and the redesigned primers

PCR typing with the commonly used TH01 primer pair generates amplicon lengths in the range 154–178 bp (alleles 5–11; Fig. 1). We selected new primer sequences that bind closer to the repeat region and lead to a significant reduction of the amplicon lengths (80–104 bp). The redesigned new primers for the pentameric STR D10S2325 resulted in an amplicon length of 96 bp for allele 6. The allele nomenclature according to the number of repeats (6–17) was determined after sequencing (Wiegand et al. 1999, heterozygosity rate approximately 90% in Caucasians).

Primer redesigning enabled a reduction of the amplicon length down to 122 bp for the shortest allele 9 of DYS391 and 100 bp for allele 13 of DYS19 (Fig. 1).

Material and methods

The conditions for DNA amplification and electrophoresis (according to Wiegand et al. 1999) were 1–20 µl template DNA extracted in 200 µl Chelex (buffer supplemented with 10 µg BSA, Sigma, Germany), 0.5 U of Taq polymerase (Serac, Germany), 0.2 µM of each primer and 100 µM of each nucleotide in a total volume of 25 µl (old and new primer sequences for HumTH01, D10S2325, DYS19 and DYS391 are marked in Fig. 2).

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D10S2325

CTC ACG AAA GAA GCC TTC TGA AGC CT $(TCTTA)_n$ TTG GGG GAG GCG
GAC TCC GTC ACC CAG GCT GGA ATG CAG TGC GTG ATC TCT CAG CTC,

HumTH01

GTG GGC TGA AAA GCT CCC GAT TAT, CCA GCC TGG CCC ACA CAG TCC CCT
GTA CAC AGG GCT TCC GAG TGC AGG TCA CAG GGA ACA CAG ACT CCA TGG
TG $(AATG)_n$ AGG GAA ATA AGG GAG GAA CAG GCC AAT GGC AAT CAC,

DYS19

CTA CTG AGT TTC TGT TAT AGT, GTT TTT TAA TAT ATA TAT AGT ATT ATA TAT
ATA GTG TTA TAT ATA TAT AGT GTT TTA $(GATA)_3$ GGTA $(GATA)_n$ TAG TGA
CAC TCT CCT TAA CCC AGA TGG ACT CCT, TGT CCT CAC TAC ATG CCA T,

DYS391

CTA TTC ATT CAA TCA TAC ACC CAT ATC TGT CTG TCT GT $(CTAT)_n$ CTG CCT
ATC TGC CTG CCT ACC TAT CCC TCT ATG GCA ATT GCT TGC AAC CAG GGA
GAT TTT ATT CCC AGG AGA TAT TTG GCT ATG TCT GAC AAC AAT TTT TTT
GGT TGT CAC AAA TGG GAT GAA TGT TAC TGG CAT CTG GTG GGT GGA GCC
CAG AGA TGC TGC TCA ACA CCC TAC AGT GCA CAA GAC, AGA CCC ACC ACA
AAG AAT C,

Fig. 2 Old and new primer sequences for HumTH01, D10S2325, DYS19 and DYS391 (Old primer sequences are marked with a line under the sequence, new primers are marked with a line above the sequence. The repeat region is in capital letters)

The new PCR primers and annealing temperatures were as follows:

- D10S2325 annealing temperature 64 °C
  - Primer 1. 5′-CTCACGAAAGAAGCCTTCTGA
  - Primer 2. 5′-ATTCCAGCCTGGGTGACGGA
- TH01 annealing temperature 61 °C
  - Primer 1. 5′-GTCACAGGGAACACAGACTC
  - Primer 2. 5′-ATTCCCATTGGCCTGTTCCT
- DYS19 annealing temperature 54 °C
  - Primer 1. 5′-GTGTTATATATATATAGTGTTTTA
  - Primer 2. 5′-GGTTAAGGAGAGTGTCACTA
- DYS391 annealing temperature 54 °C
  - Primer 1. 5′-CTATTCATTCAATCATACACCCA
  - Primer 2. 5′-GTTGCAAGCAATTGCCATAGAG

PCR (30 cycles) was carried out in a Biometra PC thermocycler (Göttingen, Germany). The amplified alleles were separated by optimized high resolution polyacrylamide gel electrophoresis (Wiegand et al. 1999).

Results

A comparison of the allelic ladder ranges for the redesigned primers shows a clearly optimized electrophoretic separation, for example the TH01 alleles 9.3 and 10 (Fig. 3).

If the DNA was not highly degraded, no relevant differences in the sensitivity of typing could be seen for TH01. In contrast, for D10S2325, although the length reduction was only 22 bp, clear differences in the sensitivity of typing and susceptibility to degradation were found due

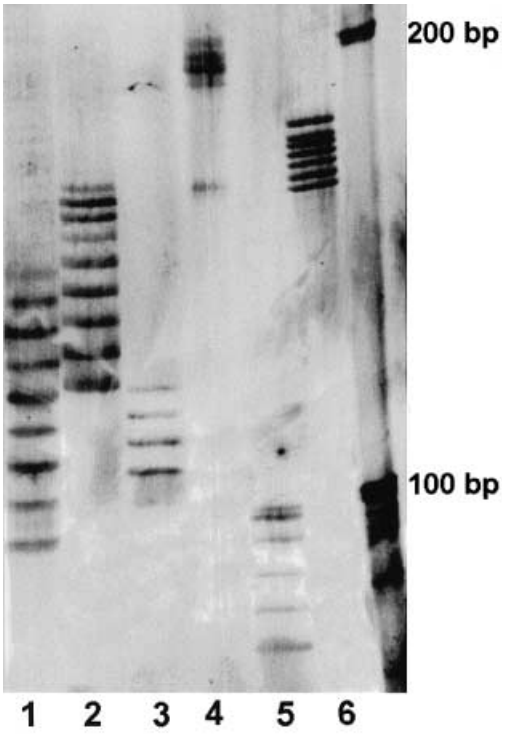

Fig. 3 A comparison of the allelic ladder ranges for the established and redesigned primers D102325, DYS19 and TH01. For D10S2325 short range amplicons (*lane 1* alleles 7–15) show a length reduction by 22 bp compared to D10S2325 long range (*lane 2* alleles 7–15). DYS19 short range (*lane 3* alleles 14–17); length reduction by 86 bp compared to DYS19 long range (*lane 4* alleles 14–17); TH01 short range (*lane 5* alleles 6, 7, 8, 9, 9.3 and 10); long range amplicons (*lane 6* alleles 5, 6, 7, 8, 9, 9.3 and 11)

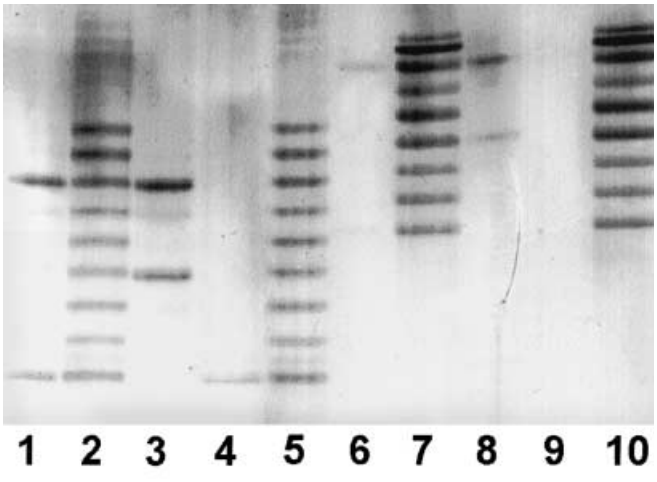

Fig. 4 D10S2325 typing of approximately 100 pg K562 cell line (Promega, USA; alleles 7 and 13; the K562-DNA concentration was determined by Promega and given on the tube; different aliquots which should contain 100 pg cell line DNA, leading to comparable typing results, were used; data not shown). *Lanes 1 and 6* K562, *lanes 3 and 8* mummified tissue, *lanes 4 and 9* formalin-fixed tissue, *lanes 2, 5, 7, 10* allelic ladder containing alleles 7–15. *Lanes 1–5* short range amplicons, *lanes 6–10* long range amplicons

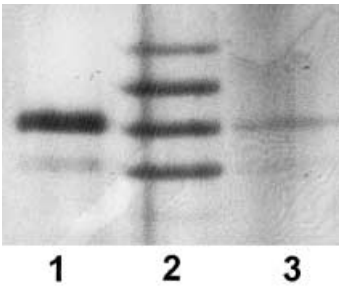

Fig. 5 DYS19 typing of epithelial cells from the hand of a male individual, which were transferred to the surface of a strangulation tool (200 µl Chelex-extracts, 10 µl were used for PCR). *Lane 2* allelic ladder 14–17, *lane 1* a DNA equivalent of approximately 100 nucleated cells, *lane 3* DNA amount from approximately five nucleated cells

to more efficient PCR conditions based on the new primer sequences (Fig. 4). A comparison of cell line K562 typing using 100 pg template DNA resulted in clearly visible bands for the shorter amplicons but weak bands for the long range alleles. Additionally, as an example for highly degraded DNA, mummified tissue from a neonate was investigated, which was found after a post-mortem interval of 6 years. DNA extracted from muscle resulted in higher PCR yields for the shorter amplicons. The second example of highly degraded DNA is formalin-fixed and paraffin-embedded biopsy tissue, which was relevant for a paternity analysis. The D10S2325 short range primers gave a detectable allele 7 compatible with the paternity hypothesis. In contrast, for D10S2325 long range primers no band could be detected.

For the locus DYS19, epithelial cells from the hands of male individuals were transferred to the surface of different tools and investigated in an experimental series (Wiegand et al. 2000). Prior to DNA extraction, a microscopic investigation was carried out and the number of epithelial cells, which should be transferred in our subsequent DNA extracts, was counted. A DNA amount equivalent to approximately five nucleated cells gave a weak but detectable band (Fig. 5).

Discussion

Other studies have shown that the reduction of STR amplicon lengths can be used to improve the typing of highly degraded DNA and in addition, a better electrophoretic separation can be obtained (Yoshida et al. 1997; Ricci et al. 1999). Considering forensically efficient STR systems, such as TH01 and D10S2325, this reduction of the amplicon length may be used to improve the chance of successful typing of a very low amount of DNA.

Pentameric STRs, such as D10S2325 and CD4 (Edwards et al. 1991), are less susceptible to slippage artefacts compared to tetrameric STRs which typically contain a longer and more homogeneous repeat array (e.g. HumVWA) and can further improve the reliability of typing (Brinkmann et al. 1998; Wiegand et al. 1999). Additionally, the inclusion of Y-chromosomal STRs may give a better chance of successful DNA typing, especially for mixtures containing male and female DNA (e.g. vaginal swabs in rape cases, epithelial cells in strangulation cases, Wiegand and Kleiber 1997).

Sometimes the aim of the reduction of the amplicon length requires compromises in the selection of the primer sequences. Usually, one should try to avoid secondary structures, such as hairpin loops, which reduce the efficiency of the PCR reaction (Robertson and Walsh-Weller 1997). Another criterion is the selection of primers with nearly identical T_m values. Computer programs may be helpful for the primer selection but empirical investigations are more relevant (Robertson and Walsh-Weller 1997).

It would also be possible to combine, for instance TH01 long range primers with DYS19 short range primers in a duplex PCR, but a more interesting approach for further investigations in this respect would be the use of short range primers labelled with different fluorescent dyes.

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