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ORIGINAL ARTICLE

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# D18S535, D1S1656 and D10S2325: three efficient short tandem repeats for forensic genetics

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**Abstract** Three short tandem repeat (STR) polymorphisms characterized by PCR product length < 175 bp were investigated. D18S535 and D1S1656 contained a 4 bp unit as basic repeat motif, D10S2325 a 5 bp unit. The heterozygosity rates were 0.76 (D18S535), 0.88 (D10S2325) and 0.90 (D1S1656), leading to a combined discrimination power of 0.9999. In contrast to D10S2325 and D18S535, which showed a homogeneous repeat array without any variation in the repeat motifs, repeat length and sequence variation was found for D1S1656. Robust typing results could be observed for all three STRs using highly degraded DNA.

**Key words** Short tandem repeats · Population genetics · Sequencing data

## Introduction

Robust typing results, high sensitivity and low susceptibility to degraded DNA are main criteria for the selection of short tandem repeat (STR) systems, which may be added to an established STR package. Based on these criteria, systems with short fragment lengths (< 175 bp), low susceptibility to PCR slippage artifacts (also known as stutter bands; Walsh et al. 1996) and highly reproducible typing results were tested. A further relevant point of selection criteria was the heterozygosity rate, which should be > 75% leading to a substantial information content.

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D18S535 and D1S1656 are STRs containing 4 bp repeat units as basic repeat motifs while D10S2325 shows a pentameric repeat array.

## Material and methods

Population sample: unrelated German Caucasians from the area around Halle/Saale. DNA extraction: Chelex-extracted DNA was used as described previously (Wiegand et al. 1993 a).

PCR reagents and thermocycler see Wiegand et al. (1998). PCR primers and conditions:

D18S535 primer 1: 5′ – TCA TGT GAC AAA AGC CAC AC, primer 2: 5′ – AGA CAG AAA TAT AGA TGA GAA TGC CA (Lareu et al. 1998 a); D1S1656 primer 1: 5′ – GTG TTG CTC AAG GGT CAA CT (Lareu et al. 1998 b); primer 2: 5′ – GAG AAA TAG AAT CAC TAG GGA ACC; D10S2325 primer 1: 5′ – CTC ACG AAA GAA GCC TTC TG; primer 2: 5′ – GAG CTG AGA GAT CAC GCA CT (Lee et al. 1998); amplification protocol: 94° C – 1 min, 61° C – 1 min, 72° C – 1 min; 30 cycles.

Electrophoretical separation: the amplified alleles were resolved by high resolution polyacrylamide gel electrophoresis according to Wiegand et al. (1993 a, b) with the following modifications to improve the resolution of the PCR products:

thinner gels were prepared (0.45 mm instead of 0.75 mm) and the polyacrylamide/crosslinker (piperazindiacrylamide; Biorad, Germany) relation was changed to 7% C and 3.7% T respectively.

STR sequencing: isolation of silver stained fragments, subsequent Taq-cycle-sequencing and sequence analysis was performed as described previously (Brinkmann et al. 1998 a).

Statistical analysis: test for heterogeneity between populations: R × C contingency table (G. Carmody, Ottawa, Canada) discrimination power according to Jones (1972); Hardy-Weinberg equilibrium: exact test (Guo and Thompson 1992, HWE 3.0 software from C. Puers, Münster, Germany).

## Results and discussion

### Sequence data

D18S535 (Lareu et al. 1998 a) shows a conserved 4 bp and D10S2325 (Lee et al. 1998) a conserved 5 bp array (Table 1). Two point mutations were found in the 3′ flanking region of D10S2325. The sequence of alleles 7 and 8 is 5′ FR – $(TCTTA)_n$ TTG GGG GAG **G**CG G**A**C – 3′ FR

**Table 1** Sequencing data of the repeat array, allele definition and PCR product lengths of three STRs

| STR system | Consensus sequence (FR = flanking region) | Allele nomenclature | Fragment length |
|---|---|---|---|
| D18S535 | 5′FR – $(GATA)_n$ – 3′FR | 9–16 | 130–158 bp |
| D1S1656 | 5′FR – $(TAGA)_n(TGA)_{0-1}(TAGA)_n(TAGG)_{0-1}(TG)_5$ – 3′FR | 9–19.3 | 125–168 bp |
| D10S2325 | 5′FR – $(TCTTA)_n$ – 3′FR | 6–17 | 113–168 bp |

**Table 2** STR allele frequency data from Halle area, North East Germany. The nomenclature is given according to the number of repeats. n = number of individuals. H = heterozygosity rate; MEC = mean exclusion chance; DP = power of discrimination

| STR individuals | D18S535 $n = 150$ | D1S1656 $n = 150$ | D10S2325 $n = 190$ |
|---|---|---|---|
| Allele | | | |
| 6 | | | 0.003 |
| 7 | | | 0.137 |
| 8 | | | 0.055 |
| 9 | 0.086 | 0.003 | 0.113 |
| 10 | 0.016 | | 0.142 |
| 11 | 0.022 | 0.080 | 0.116 |
| 12 | 0.205 | 0.120 | 0.176 |
| 13 | 0.325 | 0.073 | 0.124 |
| 14 | 0.203 | 0.102 | 0.082 |
| 15 | 0.133 | 0.105 | 0.047 |
| 15.3 | | 0.103 | |
| 16 | 0.010 | 0.073 | 0.003 |
| 16.3 | | 0.045 | |
| 17 | | 0.036 | 0.003 |
| 17.3 | | 0.160 | |
| 18 | | 0.017 | |
| 18.3 | | 0.070 | |
| 19.3 | | 0.013 | |
| H | 0.76 | 0.90 | 0.88 |
| MEC | 0.58 | 0.78 | 0.74 |
| DP | 0.91 | 0.97 | 0.96 |

while for the longer alleles 9 to 16 the following two transitions could be detected: 5′ FR – $(TCTTA)_n$ TTG GGG GAG ACG GGC – 3′ FR.

D1S1656 (Lareu et al. 1998b) is characterized by a more complex repeat structure containing variable numbers of 3 and 4 bp motifs leading to 1 bp differences for alleles > 15 (Table 2).

D1S1656 and D10S2325 allele frequencies are below 0.2 while for D18S535 the dominant allele 13 exceeds 0.3. In relation to the allele distribution D1S1656 shows the highest heterozygosity rate, followed by D10S2325 and D18S535. The combined power of discrimination (DP) for the three STRs is 0.9999. No deviation from Hardy-Weinberg equilibrium was found ($p > 0.05$).

## Population genetic studies

Comparisons of population genetic data for D1S1656 (Fig. 1 a) from two different German subpopulations (Halle area, Münster area) and a subpopulation from NW Spain showed no significant differences between the two German subpopulations ($p > 0.05$) but significant differences between the German subpopulations and the Spanish population data (Contingency test according to Carmody, Ottawa, Canada; Wiegand et al. 1998). For D18S535 no significant differences were found comparing German data for Halle area and NW Spain (Fig. 1 b).

Considering studies with STRs of variant levels of polymorphism among different ethnic groups (Brinkmann et al. 1998 a), one can explain the similarity of allele frequency data in D18S535 according to a lower degree of polymorphism caused by lower mutation rates in comparison to D1S1656. Higher polymorphic systems with 4 bp repeats as basic repeat motifs such as HumFIBRA and HumACTBP2 show mutation rates > 0.4% leading to an

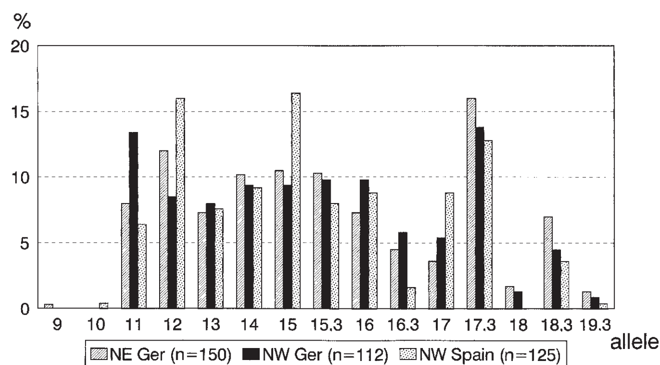


**Fig. 1 a** D1S1656 allele frequency profiles: comparison of two German (North East and North West Germany) and a Spanish (North West Spain) subpopulation. n = number of individuals

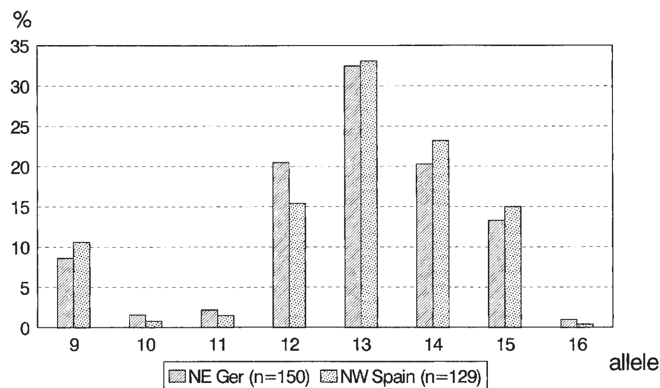


**Fig. 1 b** D18S535 allele frequency profiles: comparison of a German (North East Germany) and a Spanish (North West Spain) subpopulation. n = number of individuals

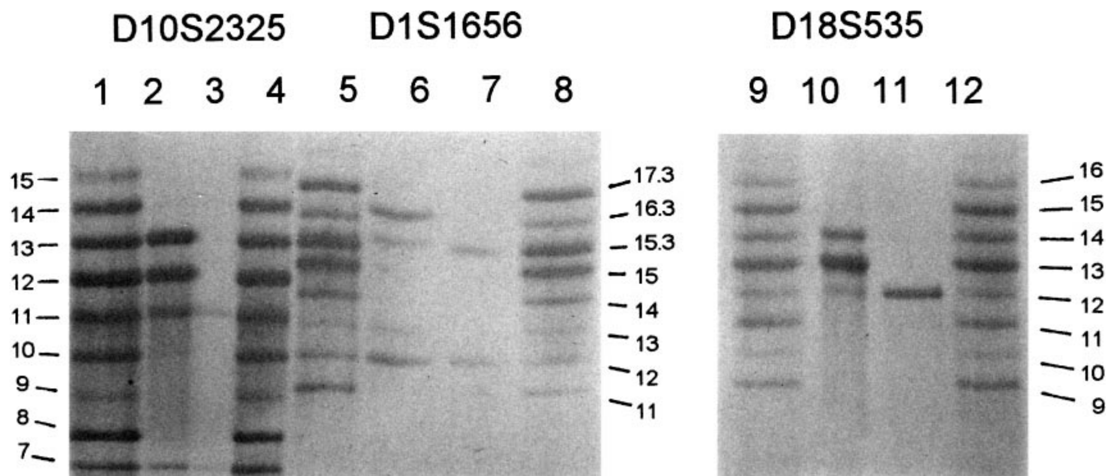


**Fig. 2a** PCR products of the STRs D10S2325, D1S1656 and D18S535 visualized after non-denaturing electrophoresis and subsequent silver staining. Lane 1,4 = allelic ladder for D10S2325 (alleles 7–15), D1S1656 (alleles 11,12,13,14,15,15.3,16.3,17.3) and D18S5353 (alleles 9–16). Lane 2, 6, 10 = mixed experimental bloodstain containing a 1:10 blood ratio of two different individuals (D10S2325 lane 2: alleles 7,11,12,13; D1S1656 lane 6: alleles 12,13,16,17; D18S535 lane 10: alleles 12,13,14). Lane 3, 7, 11 = highly degraded DNA extracted from a 10 years old femur (D10S2325 lane 3: alleles 7,11; D1S1656 lane 7: alleles 12,15.3; D18S535 lane 11: allele 12)

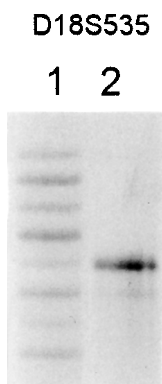


**Fig. 2b** D18S535 – Lane 1: allelic ladder; lane 2: DNA extracted from a 10-year-old femur using three times more DNA for PCR compared to Fig. 2a, lane 11; the higher amount of DNA led to a weak slippage band, 1 repeat shorter than the true homozygote pattern

increase in the evolution dynamic of ethnic diversity (Brinkmann et al. 1998b).

## Validity of typing

Using an improved electrophoretical separation method the 1 bp difference between longer alleles of D1S1656 can be reliably resolved using non-denaturing conditions and subsequent silver staining of the PCR products (Fig. 2a; lane 6, alleles 16 and 17).

The susceptibility for stutter bands was low for all three systems, but D10S2325 was less susceptible to slippage than D1S1656 and D18S535 (Fig. 2b).

There seem to be a correlation between the probability of polymerase slippage depending on the length of the repeat motifs, the homogeneity of the repeat stretch and increasing number of repeats. For tetrameric STRs with longer and homogeneous repeat stretches, higher mutation rates were found compared to shorter stretches or composed STRs (Walsh et al. 1996; Brinkmann et al. 1998b). Pentameric repeat arrays (D10S2325) seem to have a clearly lower risk of slippage artifacts due to the increased length of the repeat motif which is also well known for the STR HumCD4 (Edwards et al. 1991).

To prove whether STRs which are characterized by relatively short PCR products could improve the chance of successful typing of highly degraded DNA, sceletal remains were investigated. Compared to longer PCR products which failed to amplify highly degraded DNA such as HumACTBP2 (Polymeropoulos et al. 1992), typing was more robust and reliable using the present systems. Additionally, as expected, these STRs reached a typing sensitivity < 100 pg template DNA and enabled successful typing of experimental mixed stains using a 1 : 10 ratio (Fig. 2a); also typing of epithelial cells which were transferred from the hands of the suspect onto the neck of the victim during strangulation was successful for all three systems.

## Conclusion

PCR typing with these three STRs lead to reproducible and robust results especially for highly degraded DNA. Using non-denaturing electrophoretical conditions D10S2325 was the most suitable system for typing by side to side comparision with allelic ladders.

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