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# A Dutch population study of the STR loci D21S11 and HUMFIBRA

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**Abstract** To introduce a duplex PCR system consisting of the STR loci D21S11 and HUMFIBRA in forensic identity testing we analysed a Dutch Caucasian database of 205 individuals. The combined power of discrimination of the two loci is 0.9978 and there was no evidence for linkage equilibrium between the two loci (p = 0.91). However, we noticed departure from Hardy-Weinberg equilibrium for the D21S11-locus in our database (p = 0.03), but the differences between observed and expected D21S11 allele pair frequencies were of negligible practical significance in forensic calculations.

**Key words** Forensic · DNA · STR · D21S11 · HUMFIBRA · Duplex PCR · Population study

## Introduction

Typing of short tandem repeat (STR) loci by the polymerase chain reaction (PCR) has become the method of choice for the forensic identification of human DNA (Lygo et al. 1994). The use of multiplex-PCR and automated detection of the fluorescently labelled products provides a precise, rapid and sensitive test system with high sample throughput and high discrimination power (Kimpton et al. 1994). After the introduction of the fluorescence-based quadruplex PCR of the four STR-loci HUMTHO1, HUMFES/FPS, HUMVWA31/1, and HUMF13A1 (Sjerps et al. 1995) in the Dutch Forensic Science Laboratory there was a need to further enhance the power of discrimination (DP) in routine forensic DNA typing. In this paper we report on a population genetic study of the two STR loci D21S11 (Sharma and Litt 1992) and HUMFIBRA (Mills et al. 1992). The amplification of these loci is based on a duplex-PCR and the fragments are analysed using an ABI 377 DNA sequencer. We present data on the genotype and allele frequencies in a sample of 205 unrelated Dutch Caucasian individuals. Furthermore, the observed heterozygote frequency and the expected frequency assuming Hardy-Weinberg equilibrium (HWE) were calculated, as well as the precision of fragment length estimates and the DP. To check if the frequency of a profile consisting of these loci can be estimated by multiplying within and across loci, we tested for both HWE and linkage equilibrium (LE). Furthermore we tested for LE against the STR systems presently in use at the Dutch Forensic Science Laboratory.

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## Materials and methods

### Population sample

The sample consisted of 205 unrelated Caucasian donors (employees and students of the Dutch Forensic Science Laboratory). DNA was isolated from air-dried bloodstains using Chelex extraction and quantified by using the Quantiblot kit (Perkin Elmer).

### Amplification conditions

The primer sequences for the STR-loci employed are given in Table 1. The primers were synthesised and HPLC purified by ABI division, Perkin Elmer (NL).

PCR amplification was performed using 2 ng of genomic DNA in a 25 µl reaction volume containing 1 × PCR-buffer (GeneAMP, Perkin Elmer), 1.25U Amplitaq DNA polymerase (Perkin Elmer) and 200 mM of each dNTP. Primer concentrations were 0.30 mM. Samples were amplified for 28 cycles of 60 s at 94°C, 60 s at 60°C and 60 s at 72°C followed by a 10 min extension period at 72°C on a Perkin-Elmer 9600 thermal cycler. Typing of the amplified DNA samples was according to the method of Kimpton et al. (1994), using the automated fluoresecent detection system on an ABI 377 DNA sequencer. Electrophoresis was carried out on 4% polyacrylamide denaturing sequencing gels (36 cm well-to-read). Allelic ladders for D21S11 and HUMFIBRA were provided by Prof. Brinkmann (Institut für Rechtsmedizin, Münster, Germany)

**Table 1** Chromosomal location, primer sequences and allelic ladders of the D21S11 and the HUMFIBRA STR loci

| | D21S11 | HUMFIBRA |
|---|---|---|
| Chromosomal location | 21 | 4q28 |
| Forward primer (label) | ATATgTgAgTCAATTCCCCAAg (none) | gCCCCATAggTTTTgAACTCA (none) |
| Reverse primer (label) | TgTATTAgTCAATgTTCTCCAg (6-FAM) | TgAATTTgTCTgTAATTgCCAgC (HEX) |
| Alleles in allelic ladder | 56, 59, 61, 63, 65, 66, 67, 68, 70, 72, 73, 74, 75, 77 | 17, 18, 18.2, 19, 19.2, 20, 20.2, 21, 22, 22.2, 23, 23.2, 24, 24.2, 25, 26, 27, 46.2 |
| References | Sharma and Litt 1992, Urquhart et al. 1995, Möller et al. 1994 | Mills et al. 1992, Urquhart et al. 1995, Barber et al. 1996 |

**Table 2** Genotype and allele distributions for D21S11 and HUMFIBRA in a sample of 205 Dutch Caucasians.
Below each part of the table are shown:

– the DP-value with its standard error (SE)

– the observed fraction of heterozygotes and the expected fraction assuming HWE

– the observed genotypes of the K562 cell line

The mean fragment length and the standard deviation of the alleles were obtained from multiple runs of the allelic ladders. The indicated sizes of the ladder alleles have been derived automatically by the use of an internal lane standard and may not correspond exactly to the true fragment length

| D21S11 Alleles | 61 | 63 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 75.2 | Allele freq | Fragment length (bp) | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 59 | 2 | 2 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0.017 | 216.28 | 0.15 |
| 61 | 9 | 17 | 15 | 1 | 12 | 5 | 0 | 4 | 0 | 1 | 0 | 0.183 | 220.27 | 0.13 |
| 63 | | 12 | 27 | 1 | 3 | 6 | 1 | 4 | 0 | 3 | 0 | 0.215 | 224.36 | 0.13 |
| 65 | | | 17 | 3 | 14 | 4 | 1 | 10 | 1 | 2 | 0 | 0.273 | 228.46 | 0.15 |
| 66 | | | | 0 | 0 | 3 | 2 | 3 | 0 | 0 | 0 | 0.032 | 230.46 | 0.14 |
| 67 | | | | | 0 | 2 | 0 | 2 | 0 | 2 | 0 | 0.085 | 232.53 | 0.15 |
| 68 | | | | | | 1 | 1 | 1 | 0 | 2 | 0 | 0.063 | 234.54 | 0.16 |
| 69 | | | | | | | 0 | 0 | 0 | 0 | 0 | 0.015 | | |
| 70 | | | | | | | | 3 | 0 | 2 | 0 | 0.080 | 238.61 | 0.17 |
| 71 | | | | | | | | | 0 | 0 | 0 | 0.002 | | |
| 72 | | | | | | | | | | 0 | 1 | 0.032 | 242.67 | 0.18 |
| 75.2 | | | | | | | | | | | 0 | 0.002 | | |

| | | | |
|---|---|---|---|
| Heterozygosity | 0.80 | DP (SE) | 0.943 (0.006) |
| Exp heterozygosity (SE) | 0.83 (0.03) | K562 genotype | 63/65 |

| HUMFIBRA Alleles | 19 | 20 | 21 | 22 | 22.2 | 23 | 23.2 | 24 | 25 | 26 | 27 | Allele freq | Fragment length (bp) | SD |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 18 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0.015 | 178.82 | 0.07 |
| 19 | 0 | 0 | 10 | 3 | 0 | 1 | 0 | 5 | 3 | 1 | 0 | 0.059 | 182.69 | 0.07 |
| 20 | | 3 | 11 | 6 | 0 | 16 | 1 | 8 | 4 | 1 | 3 | 0.137 | 186.58 | 0.08 |
| 21 | | | 6 | 11 | 2 | 13 | 1 | 8 | 3 | 3 | 0 | 0.180 | 190.47 | 0.08 |
| 22 | | | | 7 | 2 | 9 | 0 | 10 | 7 | 3 | 0 | 0.166 | 194.38 | 0.07 |
| 22.2 | | | | | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0.017 | | |
| 23 | | | | | | 3 | 0 | 7 | 4 | 1 | 1 | 0.144 | 198.29 | 0.07 |
| 23.2 | | | | | | | 0 | 0 | 0 | 1 | 0 | 0.007 | | |
| 24 | | | | | | | | 4 | 12 | 2 | 0 | 0.154 | 202.24 | 0.09 |
| 25 | | | | | | | | | 0 | 0 | 0 | 0.083 | 206.20 | 0.07 |
| 26 | | | | | | | | | | 0 | 0 | 0.029 | 210.16 | 0.08 |
| 27 | | | | | | | | | | | 0 | 0.010 | 214.11 | 0.08 |

| | | | |
|---|---|---|---|
| Heterozygosity | 0.89 | DP (SE) | 0.961 (0.003) |
| Exp heterozygosity (SE) | 0.87 (0.02) | K562 genotype | 21/24 |

**Table 3** p-Values** for LE test between two loci for a Dutch Caucasian population sample

| | HUMFIBRA | HUMTHO1 | HUMFES/FPS | HUMVWA31/1 | HUMF13A1 |
|---|---|---|---|---|---|
| D21S11 | 0.91 | 0.05* | 0.70 | 0.40 | 0.40 |
| HUMFIBRA | | 0.29 | 0.89 | 0.23 | 0.67 |

205 individuals were compared for LE between the D21S11 and the HUMFIBRA loci
190 individuals were compared for LE between the D21S11/HUMFIBRA loci and the quadruplex STR loci (HUMTHO1 alleles 9.3 and 10 were pooled)

*The rejection level at an overall significance level of 0.05 is 0.05/8 = 0.006
(improved Bonferroni, Hochberg 1988)
**p-values were estimated by a bootstrap method using 1000 resamples

and Dr. Gill (Forensic Science Service, Brimingham, UK) respectively. The length of the amplified DNA fragments was determined from the internal lane standard Genescan-350 ROX (Perkin Elmer). Fragment sizes were automatically estimated using Genescan PCR Analysis software (GeneScan Analysis 2.0.1) by the Southern local method. Allele designations were automatically performed using Genotyper 1.1. DNA Fragment Analysis Software (ABI). In order to adjust the window (size range) for each allele we estimated the fragment sizes of the allelic ladders (Table 2) which were run 4 times on 10 different gels. The window for each allele was set to the average size ± 3 standard deviations.

### Ladder re-amplification

An infinite supply of the D21S11 and the HUMFIBRA ladders was obtained by re-amplification. We found that suitable re-amplification conditions (monoplex PCR) for both ladders were as follows: 10 µl of a 600 × dilution of the allelic ladder was re-amplified in a total volume of 50 µl containing 1 × PCR buffer (GeneAMP, Perkin Elmer) and 400 mM of each dNTP. Primer concentrations were 0.60 mM. Further PCR conditions included a 6 min hot start denaturation after which 5 U Amplitaq DNA polymerase was added. Allelic ladders were re-amplified for 14 cycles of 60 s at 94° C, 60 s at 60° C and 60 s at 72° C followed by a 10 min extension period at 72° C.

### Nomenclature

The nomenclature of D21S11 is based on the number of dimeric repeats, thus alleles 59 and 60 differ by 2 bp (Barber et al. 1996). The nomenclature of HUMFIBRA follows the recommendations of the DNA commission (1994) and is based on complete tetrameric repeats, the alleles that possess an incomplete repeat unit are designated with a suffix (.2), thus alleles 22 and 22.2 differ by 2 bp and alleles 23 and 24 differ by 4 bp.

### Statistical methods

Allele frequencies, expected frequency of heterozygotes assuming HWE, the DP and standard errors were calculated as previously described (Sjerps et al. 1995). We performed the exact test for HWE by the Markov Chain method (Guo and Thompson 1992) and the match-matrix test (Risch and Devlin 1992) for LE.

## Results

The sample frequency of genotypes and alleles are presented in Table 2. Standard deviations of the fragment size estimates are also indicated. The longer fragments of the D21S11 locus had the largest standard deviation (the maximum observed SD was 0.18 bp at allele 72). The combined DP for this duplex PCR system was 0.9978.

### Hardy-Weinberg and linkage equilibrium

The exact test did not indicate any deviations from HWE for the HUMFIBRA locus (p = 0.09). However, departure from HWE was noticed for the D21S11 locus (p = 0.03) in the exact test. The match-matrix test did not indicate deviation from LE between the D21S11 and the HUMFIBRA locus or between these loci and the loci of the quadruplex PCR-system (Table 3).

## Discussion

This study shows that the automated detection of STR loci on the ABI 377 sequencer provides an accurate and precise method of detecting and sizing STR alleles. The largest standard deviation of 0.18 bp was found at the D21S11 locus for allele 72. This precision allows the separation of adjacent alleles for both loci and distinction between the alleles with full tetrameric repeats and alleles with the incomplete 2 bp repeats.

The combination of the two STR loci is highly discriminating and in the Dutch Caucasian population the combined DP was 0.9978. Our data showed no deviations from LE. However for the D21S11 locus we observed departure from HWE (p = 0.03). The largest difference (excluding those with frequencies of less than 1%) was observed for the D21S11 61/67 allele pair frequency (observed f = 0.059 and expected f = 0.031) with an underestimation of 2.8% of the actual observed frequency. Conservative calculation procedures for estimating multi-locus profile frequencies (Evett et al. 1996; Budowle et al. 1996; Balding 1995) should sufficiently correct for such small differences.

In conclusion, we consider the duplex PCR of the D21S11 and the HUMFIBRA locus as a further development in the individualisation of DNA-evidence.

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