

ANALYSIS OF THE SHORT TANDEM REPEAT POLYMORPHISM D18S51: ALLELE FREQUENCIES AND SEQUENCE STUDIES

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INTRODUCTION

The analysis of Short Tandem Repeat polymorphisms is widely used as a method for human identification. There is a demand to combine several STR loci with high discrimination power for multiplex analysis. STR polymorphisms with few alleles (e.g.: TC11, F13A) do not have the discrimination power compared to STR-systems like SE33 (ACTBP2). SE33 has more than 30 alleles and thus the space for the combination with other STR loci in multiplex analysis is reduced. Therefore STR polymorphisms like FGA (HUMFIBRA) and D18S51 with about 15-20 alleles seem to be a good compromise.

The aim of the study was to get information about allele frequencies of the STR loci D18S51 in the German population. For typing these alleles correctly in size we started to sequence some alleles on the GATC Direct Blotting System (MWG-Biotech, Germany).

MATERIAL AND METHODS

DNA was extracted from whole blood according to standard procedures. Amplification of the STR locus D18S51 was carried out with the primers:

D18S51/1: 5'-CAAACCCGACTACCAGCAAC-3'

D18S51/2: 5'-Dig-GAGCCATGTTCATGCCACTG-3'

Amplification conditions:

10 mM Tris/HCl, 50 mM KCl (pH 8,3), 1.0 mM MgCl₂, 0.2 mM each dNTP, 0.5 µM each primer, 0.1 U Taq DNA polymerase (Boehringer) and 2.5 ng DNA. The reaction volume was 12.5 µl. Samples were amplified after an initial step of 1 min at 93°C for 30 cycles of 1 min at 93°C, 1 min at 60°C and 1 min at 72°C. PCR products were diluted 1:20 in water and an aliquot was further diluted 1:5 in formamide dye mix. 1.5 µl of this dilution was loaded on a 30 cm long, 0.2 mm thick, 4% DBE sequencing gel (Direct Blotting Electrophoresis GATC 1500 sequencer, MWG, Germany) and run at constant 30 W (about 1700 V, 17 mA). As described previously (Berschick et al., 1994) the samples run through the gel and were blotted onto a nylon membrane (Macherey-Nagel, Germany). DNA was fixed onto the membrane by exposure to UV light for 2 min and the products visualized with the DIG Nucleic Acid detection kit. Some of the samples were analysed with an ABI sequencer.

Sequencing of the PCR products

Homozygous DNA samples, purified after amplification with Nucleotrap kit (Macherey-Nagel), were used as a template for sequencing. About 1/5th of the purified

PCR product was used for sequencing with the Sequitherm Cycle Sequencing kit (Biozym, Germany). We add 3% DMSO to the sequencing reaction to prevent unspecific stops. After 45 cycles of 30 sec at 93°C and 5 min at 55°C the reaction was stopped by adding 3 µl of formamide dye mix. 1.5 µl of the sample was loaded on a sequencing gel and run at constant 30W. After 50 min prerun the blotting membrane was started with a speed of 20 cm/h for 1h 30 min. Fixation of DNA and colorimetric detection was done as described above.

RESULTS AND DISCUSSION

To identify the alleles of D18S51 and for sequencing, DIG labelled samples were separated with direct blotting electrophoresis. With this quick and easy method it is possible to analyse a one basepair difference and therefore it is easy to type alleles of STR loci.

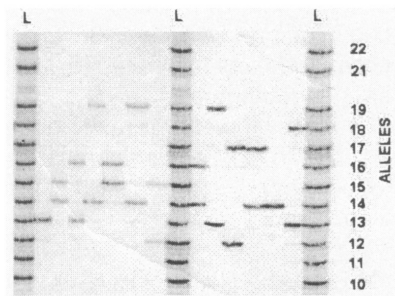
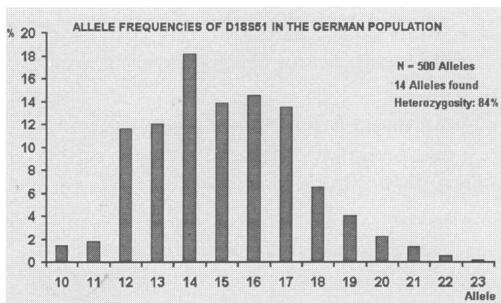


Fig. 1: Allele frequencies at the STR-locus D18S51 in the German population. Allele typing is based on the number of repeats.

Fig. 2: Examples of blotting D18S51 samples of unrelated individuals. L = allelic ladder.

Fig.1 shows the allelic distribution for D18S51 calculated from analysing 250 unrelated German individuals. For this STR loci we could not detect any interalleles so the repeat units were always 4 bp in length. On the blot (Fig. 2) D18S51 shows one distinct band for each allele. The heterozygosity index is 0.84. The distribution found is according to Hardy-Weinberg equilibrium. Analysing 130 meioses we could not detect any de novo mutation.

For typing the alleles we sequenced 3 different alleles. With the use of DMSO we could reduce background of unspecific stops and could read through the sequence. The whole sequence for allele 16 is shown in table 1. The differences between the alleles was the number of GAAA repeats as shown in Table 2. The length of the products differ by 2 basepairs compared to the published data (Straub et al., 1993; Urquhart et al., 1995) but the published allele lengths are not based on sequencing.

Tab. 1: Sequence of allele 16 locus D18S51 amplified from DNA. The length variable site is shown in bold letters. Primers are underlined.

| | |
|--|-----|
| <u>GAGCCATGTT</u> <u>CATGCCACTG</u> CACTTCACTC TGAGTGACAA ATTGAGACCT | 50 |
| GTCTCAGAAA GAAAGAAAGA AAGAAAGAAA GAAAGAAAGA AAGAAAGAAA | 100 |
| GAAAGAAAGA AAGAAAGAAA AAGAGAGAGG AAAGAAAGAG AAAAAAGAAA | 150 |
| GAAATAGTAG CAACTGTTAT TGTAAGACAT CTCCACACAC CAGAGAAGTT | 200 |
| AATTTTAATT TTAACATGTT AAGAACAGAG AGAAGCCAAC ATGTCCACCT | 250 |
| TAGGCTGACG GTTTGTATTAT TTGTGTTGTT <u>GCTGGTAGTC</u> <u>GGGTTTG</u> | 297 |

Tab 2: DNA sequences of three D18S51 alleles. Only the varying repeat units are shown.

| Allele | Length (bp) | Sequence |
|--------|-------------|--|
| 12 | 281 | ...GTCTCA (GAAA) ₁₂ A (AG) ₄ ... |
| 13 | 284 | ...GTCTCA (GAAA) ₁₃ A (AG) ₄ ... |
| 16 | 297 | ...GTCTCA (GAAA) ₁₆ A (AG) ₄ ... |

The sequences determined in this study are perhaps 1 bp shorter than the putative allele length of the allele typing because of the addition of one nucleotide to the end of the PCR product. This is due to the tranferase activity of the Taq polymerase (Clark et al., 1988; Kimpton et al, 1993).

References

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