Swiss Population Data For 3 STR-Systems (SE33, HUMTHO1, D21S11), HLA DQα And D1S80

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1. Introduction

The application of any new genetic marker system in forensic casework requires a sufficiently large data base of the relevant population. This study presents a data base of the 5 PCR-systems SE33, HUMTHO1, D21S11, HLA DQα and D1S80 for a Swiss population sample. Allele and genotype frequencies were determined of at least 195 unrelated individuals.

2. Methods

a) STR-Systems SE33, HUMTHO1 and D21S11

Primer sequences: SE33 [5]: 5'->3' AAT CTG GGC GAC AAG AGT GA; ACA TCT CCC CTA CCG CTA TA . HUMTHO1 [2]: 5'->3' GTG GGC TGA AAA GCT CCC GAT TAT; GTG ATT CCC ATTGGC CTG TTC CTC. D21S11 [7]: 5'->3' GTG AGT CAA TTC CCC AAG; GTT GTA TTA GTC AAT GTT CTC C.

PCR reaction mixture: 10 ng template DNA, 5 µl 10x PCR buffer (GeneAMP, Perkin Elmer Cetus), 8 µl 1.25 mM dNTP's, 1 µl 12.5 µM of each primer (one primer labeled with a fluorescent dye), 1.5 U AmpliTaq DNA Polymerase (Perkin Elmer Cetus), 4µl 0.2% BSA, sterile H_2O was added to a final volume of 50 µl; the reaction mixture was overlayed with a drop of oil.

Amplification conditions (Thermocycler: Biometra Triothermoblock):
SE33 and HUMTHO1: 94°C - 45 sec., 60°C - 30 sec., 72°C - 30 sec; 30 cycles.
D21S11: 94°C - 45 sec., 55°C - 30 sec., 72°C - 30 sec.; 30 cycles.
SE33 and HUMTHO1 were coamplified.

Analysis and detection: The PCR-products were analyzed and detected on an ABI 373A automated DNA sequencer (ABI GeneScan 672). The electrophoresis was carried out on 6 % denaturing polyacrylamide gels according to the ABI protocol (running conditions: 1600 V, 24mA, 8hs). The PCR-products of the 3 STR-systems are labeled with different fluorescent dyes (blue, green and yellow), therefore they were analyzed together on the same gel. An internal lane standard coelectrophoreses with the PCR-products in each lane using a color (red) different from the STR-samples. The standard is used to build up a calibration curve for each gel lane. The lengths of the STR-alleles are automatically calculated from the calibration curve.

Nomenclature: The alleles of SE33 and HUMTHO1 were designated as suggested in the collaborative STR-exercise of EDNAP [2]. For D21S11 we chose the following designation: we named the most common allele as allele 10 and numbered the next shorter or longer alleles according to repeat sizes of 4 bp. In all 3 STR-systems we designated intermediate alleles with decimals, e.g. allel 10.2 is two bp longer than allele 10.

Allelic ladder: For each STR-system we constructed our own allelic ladder from alleles observed in the examined population sample. DNA from different individuals were amplified and their PCR-products were mixed to an allelic cocktail. 0.5 - 1 µl of the cocktail were loaded in a gel track.

b) HLA DQ α: Amplification and typing reactions were performed according to the Cetus Protocol (HLA DQα Forensic DNA Amplification & Typing Kit).

c) D1S80: Amplification was carried out by following the recommended protocol of the D1S80 Forensic DNA Amplification Reagent Set (Perkin Elmer Cetus). The PCR-products were separated on 6% polyacrylamide gels with piperazine diacrylamide as cross-linker [1]. The bands were visualized by silver staining. The alleles were determined by side-to-side comparison with the allelic ladder provided in the reagent kit from Cetus.

3. Results

SE33: For SE33 a total of 33 different alleles could be observed in a sample of 195 individuals; their frequencies are shown in table 1. The allele frequencies range between 0.3% and 10%. Allele 6 and allele 16 are the most common alleles with frequencies of 10% and 9.5% respectively. We could distinguish between 130

genotypes; 90 genotypes were represented only once. The power of discrimination for SE33 is 0.989.

HUMTHO1: DNA samples of 197 individuals were amplified and typed for the system HUMTHO1. We found five common alleles with frequencies between 11.4% and 30.9% and one rare allele (allele 5) with a frequency of 0.2%. There also exist two variants designated as 8.3 and 10.1, which differ by just one base from the main alleles 9 and 10, respectively (see table 1).We obtained 20 different genotypes; the most frequent genotype was 6/10 with 13.8%. The power of discrimination is 0.92.

D21S11: Among 200 examined individuals 15 different alleles were detected. In the Swiss population there are 9 common alleles (3.3% and 24.6%) and 6 rare alleles with frequencies between 0.3 and 1%. The allele frequencies are listed in table 1. We found 42 genotypes; the most frequent genotype is the homozygote type 10/10 with 8.2%. The power of discrimination for this system is 0.956.

HLA DQα: A sample of 227 individuals were analyzed for the HLA DQα locus. All 6 described alleles and 21 genotypes were found. We obtained allele frequencies between 8.6% and 32.4% (see table 1). The most common allele in the Swiss population is HLA DQα 4. The observed heterozygosity rate is 80.2 % and the power of discrimination 0.93.

D1S80: DNA samples of 201 Swiss individuals were typed for the D1S80 locus. We found 20 different alleles; their length ranges between 371 and 739 bp (17 - 37 repeat units of 16 bp). The allele frequencies are shown in table 1. The most common alleles are the allele 24 with a frequency of about 33% and the allele 18 with a frequency of 26%. 61 different genotypes could be observed. The most frequent genotypes are 18/24 (19%), 24/24 (10%) and 18/18 (8%). The power of discrimination is 0.95. For all 5 PCR-systems the Hardy-Weinberg equilibrium was tested; the genotype frequencies did not deviate from a Hardy Weinberg equilibrium.

4. Discussion

For the PCR-Systems HLA DQα, D1S80 and HUMTHO1 the distributions of allele frequencies in our Swiss population sample are similiar to those observed in other Caucasians, e.g. in Germany, in England and in the Netherlands % [3, 6, 8, 9]. The more complex STR-systems SE33 and D21S11 are difficult to compare with other population studies because there is no standardisation of allelic ladder markers and nomenclature, but this does not exclude the application of this powerful marker systems in individual laboratories.

The automated analysis of the STR-systems by use of fluorescence detection and internal lane standards demonstrates many advantages. There are no typing problems because of electrophoretic migration variability and bandshifting. One can simultaneously analyze at least 3 STR-systems . The precision of measurements is one base for allele lengths of less than 300 bp. The automated analysis by the Genescanner provides higher accuracy, reproducibilty and sensitivity than traditional techniques. The 5 investigated polymorphic systems are reliable and sensitive marker systems that are well suited for use in forensic casework.

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Table 1: Allele frequencies for SE33, D21S11, HUMTHO1, D1S80 and DQα.

Allele	SE33 Frequency	Allele	D21S11 Frequency	Allele	HUMTHO1 Frequency	Allele	D1S80 Frequency
-2	0.003	6	0.003	5	0.002	17	0.007
-1	0.003	6.2	0.003	6	0.243	18	0.261
1	0.01	7	0.033	7	0.166	19	0.002
2	0.044	8	0.16	8	0.114	20	0.03
2.2	0.003	8.2	0.003	8.3	0.005	21	0.017
3	0.033	9	0.211	9	0.146	22	0.05
3.2	0.003	10	0.246	10	0.309	23	0.025
4	0.041	10.2	0.048	10.1	0.015	24	0.328
5	0.067	11	0.046			25	0.07
6	0.1	11.2	0.089			26	0.017
6.2	0.003	11.3	0.01			27	0.022
7	0.069	12.2	0.109			28	0.032
7.2	0.005	13	0.003			29	0.047
8	0.039	13.2	0.033			30	0.017
8.2	0.013	14.2	0.005			31	0.05
9	0.018					32	0.007
9.2	0.018					33	0.002
10	0.003					34	0.007
11	0.044					36	0.002
11.2	0.003					37	0.002
12	0.054						
13	0.023						
14	0.028						
15	0.065						
16	0.095						
17	0.072						
18	0.056						
19	0.046						
20	0.026						
20.2	0.003						
21	0.005						
23	0008						
24	0.003						

Allele	DQ α Frequency
1.1	0.134
1.2	0.17
1.3	0.086
2	0.167
3	0.119
4	0.324

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