FLUORESCENT BASED TYPING OF TWO SHORT TANDEM REPEAT LOCI IN A SWEDISH POPULATION SAMPLE AND REPRODUCIBILITY OF FRAGMENT SIZE ESTIMATES

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SUMMARY

The objective of this study was to compile a population database for two tetrameric short tandem repeat loci (STR) and investigate the reproducibility of fragment size estimates. An Applied Biosystems 373A Sequencer with Genescan 672 collection and analysis software was used for typing of HUMTH01 (intron 1 in human tyrosine hydrolase gene) and HUMACTBP2 (also named SE33, human cytoplasmic beta-actin related pseudogene). PCR products from both loci were sized in one lane using an internal size standard.

The amount of genetic variation was investigated in the Swedish population (n=301). Seven alleles were found at HUMTH01 and the observed heterozygosity was 0.78. At HUMACTBP2 locus more than thirty alleles were found and the observed heterozygosity was 0.96. The observed genotype frequencies at HUMTH01 and HUMACTBP2 did not deviate significantly from Hardy-Weinberg expectation.

In order to compare within and between gel reproduciblity of fragment size estimates a control sample was typed three times on each of 20 gels. Within gel, the standard deviation (S.D.) was less than 0.1 bp for four fragment sizes between 158-291 bp. Standard deviations between gels were slightly higher for the two shorter fragment sizes (HUMTHO1), while the larger fragments gave a 0.3 and 0.4 bp S.D (HUMACTBP2).

We conclude that both HUMTHO1 and HUMACTBP2 can be reliably typed. However the latter require an allelic ladder to be run on each gel. The combined chance of a random match between two unrelated individuals from the Swedish population is approximately 1 in a 1000.

INTRODUCTION

PCR-based typing of VNTR/STR loci has become a technique of growing importance in paternity and crime investigations. It is likely that compilation of population databases will be generated faster than for the VNTR markers used in DNA-profiling with RFLP-technique. Also, the simplicity of many STRs make the standardisation of nomenclature and comparison of allele frequencies in different populations much easier. The result of this investigation will influence the choice of further STRs to be introduced in forensic casework.

MATERIALS AND METHODS

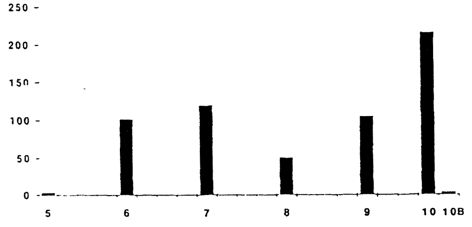
Blood samples were collected from 301 blood donors with Swedish names from three cities in Sweden. One individual, with one short and one long allele at both loci, was selected for repeated analysis in order to test the reproducibility of the estimates of fragment sizes. A total of 20 gels were run, each with three replicas randomly distributed among a total of 24 samples.

Primer sequences for HUMTH01 were 5'-L GTG GGC TGA AAA GCT CCC GAT TAT-3' (L, aminolink) and 5'-GTG ATT CCC ATT GGC CTG TTC CTC-3'. Primer sequences for HUMACTBP2 were 5'-L AAT CTG GGC GAC AAG AGT GA-3' and 5'-ACA TCT CCC CTA CCG CTA TA-3'. The left primer in each pair was prepared for labelling with a fluorescent dye by an aminolink. Amplification was performed with a Perkin Elmer 9600 in 0.2 ml tubes. The same PCR cycle was used for both loci and consisted of a 15 seconds predenaturation at 94°C, thirty cycles with 15 seconds denaturation at 94°C, 15 seconds annealing at 60°C and extension for n seconds, where n equals cycle number, at 72°C. The amplification reaction mixture was the same for the two genes and consisted of 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$, 0.01 % gelatine, 200 mM of each dNTP, 250 nM of each primer, 1 unit AmpliTaq and 10 ng template DNA per 50 µl reaction.

RESULTS AND DISCUSSION

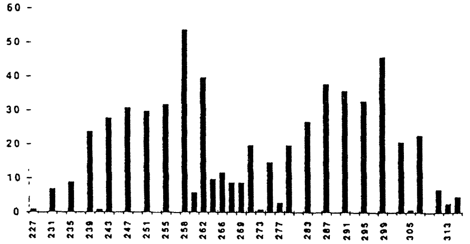
The nomenclature for alleles at the tetrameric HUMTHO1 is based on the number of repeats of $(TCAT)_{5-10}$. HUMACTBP2 is a complex tetrameric locus and it is not possible to base the nomenclature on number of repeats of the sequence $(AAAG)_n$. We choose to base the nomenclature on the estimated fragment size. The vast majority of fragments fell into discrete groupes of fragments and we use the mean fragment size in each group as allele name. An allelic ladder for HUMTHO1 and HUMACTBP2 respectively was constructed, using amplified samples from the database.

The genetic variation we found in the Swedish population sample at HUMTHO1 and HUMACTBP2 are summarised in Figure 1 and Figure 2.



Allele	Size (bp)	Frequency
5	154	0.007 (4)
6	158	0.0169 (102)
7	162	0.199 (120)
8	166	0.085 (51)
9	170	0.174 (105)
10	173	0.359 (216)
10B	174	0.007 (4)
		602 chromosomes

Fig1. Allele frequencies at HUMTHO1



Allele	Size interval	Allele frequency	Allele	Size interval	Allele frequency
227	225.86-227.46	0.002 (1)	271	270.67-272.27	0.033 (20)
231	230.05-231.65	0.012 (7)	273	272.50-274.10	0.002 (1)
235	234.11-235.71	0.015 (9)	275	274.56-276.16	0.025 (15)
239	238.30-239.90	0.040 (24)	277	276.17-277.77	0.005 (3)
241	240.10-241.70	0.002 (1)	279	278.49-280.09	0.033 (20)
243	242.19-243.79	0.046 (28)	283	282.25-283.85	0.045 (27)
247	246.16-247.76	0.052 (31)	287	286.18-287.78	0.063 (38)
251	249.92-251.51	0.050 (30)	291	290.06-291.66	0.060 (36)
255	253.80-255.40	0.053 (32)	295	293.99-295.59	0.055 (33)
258	257.58-259.18	0.090 (54)	299	297.87-299.47	0.076 (46)
260	259.35-260.95	0.010 (6)	303	301.95-303.55	0.035 (21)
262	261.28-262.88	0.066 (40)	305	303.87-305.47	0.002 (1)
264	263.14-264.74	0.017 (10)	307	305.84-307.44	0.038 (23)
266	265.19-266.79	0.020 (12)	311	310.00-311.60	0.012 (7)
268	266.79-268.39	0.015 (9)	313	311.82-313.42	0.005 (3)
269	268.55-270.15	0.015 (9)	315	314.80-316.40	0.008 (5)

Fig 2. Allele frequencies at HUMACTBP2

The observed frequencies of heterozygotes was 0.78 at HUMTHO1 and 0.96 at HUMACTBP2. The heterozygosity and allele frequencies at HUMTHO1 and the fragment size distribution at HUMACTBP2 appears to be in good agreement with what Kimpton *et al* and Wiegand *et al* reports from Caucasian populations in England and Germany. The observed and expected genotype frequencies did not deviate significantly in any of the two loci. At HUMACTBP2 the alleles were pooled in nine groupes (227-243, 247-251, 255-258, 260-264, 266-273, 275-283, 287-291, 295-299 and 303-315).

In this study we have showed that the standard deviation when fragments are estimated on the same gel is less than 0.1 bp for both HUMTHO1 and HUMACTBP2, see Table 1. The variation in size estimates at HUMTHO1 is also lower than 0.1 bp when fragments are estimated on different gels. Typing can therefore rely solely on fragment size estimates. The use of an allelic ladder is therefore only recommended but appears not to be necessary.

Typing of HUMACTBP2 is more complicated and the number of alleles is most likely larger than the number of allelic groups that can be discerned by a size estimate. Kimpton *et al* has recently reported the same level of variation at HUMTHO1 and HUMACTBP2 both within and between gels as in our study. An allelic ladder run in at least one lane on every gel is necessary to confirm the allele designation derived from the size estimation using the internal size standard.

	HUM THO1		HUM ACTBP2	
	Allele 1	Allele 2	Allele 1	Allele 2
n	58	57	59	59
Mean	157.8 bp	173.5 bp	258.8 bp	291.8 bp
Within std.dev. (bp)	0.06 bp	0.04 bp	0.07 bp	0.07 bp
Within std.dev. (%)	0.04 %	0.02 %	0.03 %	0.02 %
Between std.dev (bp)	0.07 bp	0.03 bp	0.31 bp	0.41 bp
Between std.dev. (%)	0.04 %	0.02 %	0.12 %	0.14 %

Table 1. Reproducibility of size estimates for fragments run on the same gel and between gels.

In conclusion, further genetic loci to be introduced as genetic marker in forensic casework should have a low inter-gel S.D., in order to determine alleles automatically without comparison against an allelic ladder. In addition, the nomenclature should be easy to standardise, which means that they should be moderatly polymorphic and alleles should differ be full length repeats. Eventually, a DNA profile will be generated in a few days using PCR, fluorescent tagged primers and an automated sequencer.

REFERENCES

C.P. Kimpton, P. Gill, A. Walton, A. Urquhart, E.S. Millican and M. Adams. Automated DNA profiling employing "multiplex" amplification of short tandem repeat loci. PCR Methods and Applications 3 (1993) 13-22.

P. Wiegand, B. Budowle, S. Rand and B. Brinkmann. Forensic validation of the STR system SE 33 and TC11. Int. J. Leg. Med. (1993) 105:315-320.