Evaluation of New STR Loci for Forensic DNA Typing

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Introduction

STR DNA typing has become the method of choice for the identification of the source of human biological material. The main reasons are its sensitivity and its ability to analyse degraded material. The main drawback of most of the STR loci presently in use in forensic science is their very limited polymorphism which contrasts with the extraordinary polymorphism of the VNTR loci used in RFLP typing. A few STRs are very polymorphic, but they are also rich in microheterogeneities (for example locus HUMACTBP2) which makes the identification of alleles difficult and the comparison of results between laboratories uncertain.

Table 1: Polymorphism of STR loci commonly used in forensic science

Locus		Heterozygosity (caucasian)	Ref.	Matching probability	Ref.
THO1	① ②	0.74	[6]	0.09	[4]
VWA	① ②	0.75	[6]	0.07	[6]
D21S11	①	0.83	[6]	0.05	[6]
FIBRA (FGA)	①		[6]	0.04	[6]
D19S253	①	0.76	[6]	0.08	[6]
D18S51	①	0.88	[6]	0.03	[6]
TPOX	②	0.66	[1]		
HPRTB	②	0.77	[3]	0.08	[3]
CSF1PO	②	0.74	[3]	0.11	[3]
FES/FPS	②	0.67	[4]	0.19	[4]
ACTBP2		0.94	[4]	0.02	[4]
F13A1		0.72	[4]	0.13	[4]
F13B		0.66	[5]		
LPL		0.68	[3]	0.16	[3]
CD4		0.68	[3]	0.16	[4]

① belongs to the heptaplex of the British DNA database
② belongs to the Promega multiplex STR systems

Recent advances in genetics have allowed the discovery of hundreds or even thousands of STR loci each year. All these new loci are stored in large databases which are then a potential rich source of STR loci. Although most of the stored STR loci display a limited polymorphism, it is certain that STR loci should be found which would bring substantial improvements compared to the current set of loci.

A set of 6 loci have been chosen for this study. The goal was to do a crude evaluation to sort out the best loci deserving a future more thorough examination. This first step was essentially an allele frequency determination.

Material/Methods

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Database : The Human Genome Data Base (GDB), at Johns Hopkins University in Baltimore, has been searched (http://gdbwww.gdb.org/.). GDB is a database created for gene mapping purposes. In early 1995, it contained more than 7000 STR loci, among which more than 2000 tetranucleotide repeats. When questionned for tetranucleotide repeat STRs, with a heterozygosity greater than 0.85, the database brought about 200 hits. Most of these loci are, in fact, poorly described with only an estimate of the true polymorphism in the form of a "maximum heterozygosity" based on a very limited sample. A few of these loci have been chosen for more detailed examination.

Samples : The DNA samples were extracted from blood given by anonymous donors from the local blood transfusion center.

PCR : The amplification conditions were essentially those described in the files from the Human Genome Data Base (GDB). The amplifications were done on either a Thermocycler TC480 (Perkin-Elmer) or Crocodile (Appligene), in 25µl of a solution containing 10mM Tris-HCl pH 9, 50mM KCl, 0.2mM dNTP, 1.5mM $MgCl_2$, 0.25µM primers, 0.01% gelatine and 0.25U Taq polymerase. After 3 min incubation at 94°C, the samples went through 30 cycles (45"/94°C ; 45"/annealing temperature ; 1'/72°C), followed by a final 5' incubation at 72°C. The annealing temperature was adjusted to the requirements of each locus, as described in the files from the database.

Electrophoresis [2]: The samples were separated on discontinuous horizontal polyacrylamide gel electrophoresis (acrylamide / piperazine diacrylamide : 6 - 8.5% T, 5% C ; 60mM formate-Tris), on 12 - 28 cm long gels. The electrode buffer (0.75M borate-Tris pH 9) was absorbed in 6 sheets of 1.5 cm wide blotting paper. The PCR products were revealed by silver staining.

Results

Table 2 : Description of the STR loci tested and their polymorphism

STR locus	max. het.①	primer sequence	anneal. temp.	chrom. nb.②	allele nb.	obs. het.③	Pm④
D20S85	0.86	5'-ATTACAGTGTGAGACCCTG-3' 5'-GAGTATCCAGAGAGCTATTA-3'	55°C	108	8	0.74	0.06
D7S1517	1.00	5'-AGCCTGATCATTACCAGGT-3' 5'-CTATTGGGGCCATCTTGC-3'	50°C	94	12	0.87	0.03
D7S1520	0.94	5'-AGATGACATACGGATGAATGG-3' 5'-GTCTCCTCTATCATCTTTCGA-3'	50°C	120	6	0.75	0.08
D6S965	1.00	5'-GTCACCTGCGTGAAGGAAA-3' 5'-GGTTGTGGGTTTTGTAGGC-3'	54°C	94	15	0.87	0.02
D1S1171	0.94	5'-GGGCAACAAAGTAAGACCC-3' 5'-TTTCCCATAGCCCCTGTGC-3'	65°C	118	9	0.78	0.07
D2S1242	0.94	5'-TGACATAGCGAGACCCTGTC-3' 5'-CCATTCTCATCCAGCAGGA-3'	55°C	92	10	0.83	0.06

① Heterozygosity displayed in the corresponding file in the Human Genome Data Base
② Number of chromosome tested
③ Percentage of heterozygotes in the sample tested
④ Matching probability ($\sum p_i^2$)

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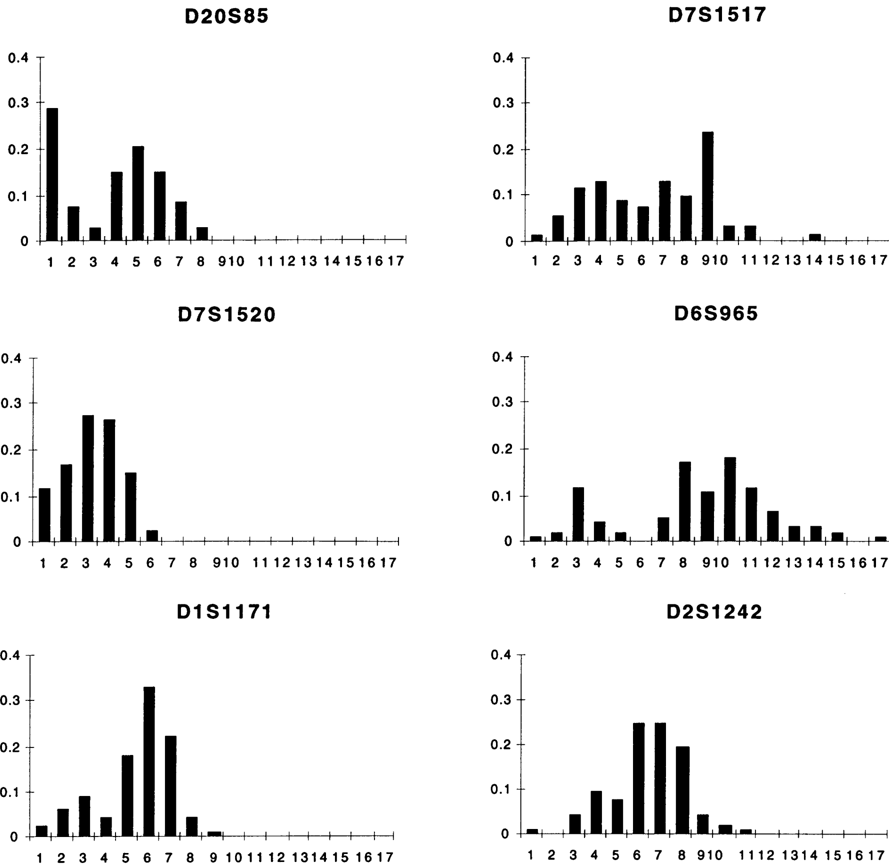

Conclusion

From this small-scale screening of new STR loci, it is evident that sets of STR loci can be found which are more powerful than those already used in forensics. We intend to screen many other loci until we find some which have the capacity to improve substantially the present power of identification. It is worth remembering that a set of 4 loci with a matching probability of 0.05 (discrimination power : 0.95) are more powerful than a set of 6 loci with a matching probability of 0.15 (discrimination power : 0.85).

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