Properties of an STR multiplex marker system suitable for paternity and forensic determinations.

J. Neuweiler, L. Perlee, J. Venturini, I. Balazs

Lifecodes Corp., 550 West Ave., Stamford Ct 06902, USA

INTRODUCTION

Simple sequence repeat polymorphisms composed of tandem di-,tri- or tetranucleotide repeats (STR) are highly abundant in the human genome (Williamson et al. 1990) and are particularly suitable for detection by PCR amplification. These type of loci have been used for population genetic studies, determination of paternity or forensic analysis (Edwards et al. 1991, Kimpton et al. 1993). Analysis of these markers has been performed either by individual amplification of single loci or by the simultaneous amplification of several loci (i.e. multiplexing). Alleles can be detected by gel electrophoresis followed by radioactive labeling, silver staining or fluorescence detection.
We developed a combination of 3 polymorphic loci containing GATA repeats that is highly informative for paternity and forensic determinations and can be amplified in a single PCR reaction.

MATERIALS AND METHODS

DNA was isolated from blood samples of unrelated individuals by either phenol/chloroform extraction or by a non-organic procedure. The amount of DNA in each sample was determined with the use of a yield gel.

The amplification reaction was assembled using the components of the Multiplex I Kit (Lifecodes Corp.) consisting of 5 ul Primer Mix , 5 ul of 10 x PCR buffer, 5 ul of dNTPs. The mixture was combined with 1.25 U Taq polymerase and 10 ng of human DNA. Final volume was 50 ul. The Multiplex-I Primer Mix contained primers specific for the amplification of D3S1744, D12S1090 and D18S849 loci. Samples were amplified in a Perkin-Elmer 480 Thermo-cycler with 30 cycles of 1 min at 95°C, 1 min at 65°C and 1 min at 72°C.

The amplified material was denatured and fractionated by electrophoresis in 4% acrylamide/8M urea gel (0.4 mm x 17 cm x 32 cm) for 1 hour at 2000 volts. Following electrophoresis, the gel was fixed in acetic acid, stained with silver nitrate and developed with sodium carbonate/thiosulfate. A photograph of the results was obtained by placing the gel on a light box and exposing it to an X-ray duplicating film (Kodak).

For each sample, the size of alleles was measured by comparison to size standards made by mixing the individual alleles of these loci (i.e. allelic ladder). The allelic ladder was loaded in lanes flanking the sample(s). The exact size of each rung in the ladder was determined by DNA sequence analysis of individual alleles (Perlee et al. 1995)

RESULTS AND DISCUSSION

Analysis of DNA samples from random individuals resulted in the identification of 9 alleles for D3S1744 and D18S849 and more than 25 alleles for D12S1090. Sequence analysis of

the alleles indicate that these polymorphisms are the result of variations in the number of tetranucleotide repeats present in these loci (Perlee et al. 1995).
The primers used to amplify these loci were designed to produce allele sizes that did not overlap between loci. As a result, these loci can be amplified simultaneously in a single amplification reaction and their respective alleles separated by gel electrophoresis. Locus D18S849 has alleles that vary in size from 93 to 133 bases, D3S1744 has alleles from 150 to 182 bases and D12S1090, from 212 to 306. A summary of the general properties of the loci composing the Multiplex are summarized in Table 1.

The analysis of DNA from random individuals from North American Black and Caucasoid populations was used to generate a table of allele frequencies. An analysis of the frequency of the observed genotypes versus those calculated from the frequency of the alleles indicate that the loci are in H-W equilibrium. A summary of the frequency of alleles in these two populations are shown in Table 2. A large number of alleles have been observed in both populations with a sightly higher heterogeneity observed in the Black population.

The ability of this multiplex system to resolve cases of disputed paternity and for human identification was calculated for these loci. The results indicate that for paternity determinations the combined power of exclusion of the 3 loci is about 98% and the combined power of discrimination is 1 in 5600. Therefore, this multiplex , is a useful combination of genetic markers for application in human identification.

Table 1. General properties of the Multiplex loci.

Locus:	D3S1744	D12S1090	D18S849
Chromosome:	3q24	12q12	18q12-q21
Size range of alleles:	150 to 182 bases	212 to 306 bases	93 to 133 bases
Number of alleles:	9	>25	9
Heterozygosity: Black	87%	96%	75%
Cauc.	83%	95%	75%
P. E. for Paternity: Black	63%	87%	51%
Cauc.	63%	84%	51%
Power of Discrimination: Black	1 in 15	1 in 98	1 in 4
Cauc.	1 in 16	1 in 88	1 in 4
Populations analyzed (number of individuals):		North American Blacks (103); North American Caucasoids (110)	

Table 2. Allele frequency in North American Black and Caucasoid populations.

D3S1744			D12S1090			D18S849		
Allele size	Black	Cauc.	Allele size	Black	Cauc.	Allele size	Black	Cauc.
150	0.005	0.005	212	0.010	0.009	93	0.068	0.005
154	0.053	0.109	216	0.005	0.009	97	0.000	0.000
158	0.121	0.095	220	0.034	0.045	101	0.019	0.000
162	0.146	0.109	224	0.112	0.036	105	0.015	0.000
166	0.325	0.286	228	0.049	0.036	109	0.000	0.000
170	0.141	0.227	232	0.029	0.027	113	0.010	0.068
174	0.160	0.141	236	0.019	0.005	117	0.136	0.136
178	0.044	0.023	240	0.010	0.014	121	0.417	0.395
182	0.005	0.005	244	0.005	0.009	125	0.262	0.268
			248	0.039	0.018	129	0.058	0.123
			253	0.053	0.073	133	0.015	0.005
			257	0.121	0.100			
			261	0.063	0.105			
			265	0.068	0.091			
			269	0.053	0.114			
			273	0.049	0.068			
			277	0.044	0.068			
			281	0.053	0.114			
			284	0.063	0.045			
			288	0.034	0.009			
			290	0.005	0.005			
			294	0.039	0.000			
			298	0.024	0.000			
			302	0.015	0.000			
			306	0.005	0.000			

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