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Allele Frequency of Three Autosomal STR Loci D16S539, D7S820 and D13S317 in a Bangladeshi Population Sample

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Abstract: Allele frequency of three Short Tandem Repeats (STR) loci, D16S539, D7S820 and D13S317 were analyzed by PCR amplification and polyacrylamide gel electrophoresis in 52 unrelated Bangladeshi individuals. A GenePrint™ SilverSTR™ Triplex kit (Promega Corporation, USA) was used in this study. All STR loci were in Hardy-Weinberg equilibrium. Statistical parameters for forensic importance, the Power of Discrimination (PD), observed and expected Heterozygosity values (H), Polymorphism Information Content (PIC), Probability of Match (PM), Power of Exclusion (PE) and typical Paternity Index (PI) were calculated for the loci. These parameters indicated the usefulness of the loci in paternity testing and personal identification in Bangladeshi population. The combined Probability of Match (PM) and using the three loci was 3.88×10^{-4} and hence the PD (1-PM) was 0.99961. The combined power of exclusion for paternity for the three loci was also calculated at greater than 0.9396.

Key words: Allele distribution, STR loci, forensic efficiency parameters

INTRODUCTION

Microsatellites or Short Tandem Repeats (STRs) are loci with alleles composed of tandemly repeated short DNA sequences of 2-7 base pair in length (Weber and May, 1989). These sequences are widespread throughout the human genome and show sufficient variability among individuals of a population. Autosomal STRs now have become an indispensable tools for personal identification and paternity testing in forensics and criminal investigation (Edwards *et al*., 1992; Hammond *et al*., 1994; Santos *et al*., 1996; Sparkes *et al*., 1996). The use of polymerase chain reaction (PCR) for amplifying polymorphic DNA helps determining phenotypes of STR loci series in vary small and old biological evidences (Schumm, 1996). Their importance rises from the fact that they are the most informative genetic markers providing high statistical capability of discrimination and individualization (Edwards *et al*., 1991; Hochmeister *et al*., 1995; Linn *et al*., 1998).

Prior to the introduction of a new DNA profiling method a study of the allele frequencies and genotype distribution of any population needs to be undertaken. In this study, we present the allele distribution and forensic efficiency at three tetranucleotide STR loci, D16S539, D7S820 and D13S317 in a population sample of Bangladesh.

MATERIALS AND METHODS

Whole blood obtained by venipuncture from 52 healthy unrelated individuals residing in Dhaka city, was collected in EDTA vacutainer tubes. Genomic DNA was extracted from the whole blood using standard published protocol (Gustincich *et al*., 1991). The quantity of recovered DNA was determined using the QuantiBlot® Human DNA Quantification Kit (Applied Biosystems, Foster City, USA). Amplification by polymerase chain reaction (PCR) was performed using GenePrint® SilverSTR III System (Promega Corporation, Madison, WI, USA) using 2.0-5.0 ng of template DNA in 9600 thermal cycler (Applied Biosystems) according to the manufacturer's recommendations. PCR products were separated by electrophoresis on a 6% denaturing polyacrylamide gel. The bands were visualized by silver staining. STR alleles at each locus was determined after comparing the band with appropriate allelic ladder provided with the kit. The study was conducted in the Department of Biochemistry and Molecular Biology, University of Dhaka. Written informed consent was obtained from the study participants and local ethical review committee approved the design of the study.

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STATISTICAL ANALYSIS

Allele frequencies and forensic efficiency parameters such as Heterozygosity (H), Polymorphism Information Content (PIC), Power of Discrimination (PD), Probability of Match (PM), Power of Exclusion (PE) and typical Paternity Index (PI) were calculated using the PowerStats Microsoft Excel workbook template provided by Promega Corporation (http://www.promega.com/geneticidtools/). Possible departures from hardy-Weinberg expectations at each locus was evaluated by Fisher's exact test (Guo and Thompson, 1992).

RESULTS AND DISCUSSION

The main biological characteristics of three STR loci are reported in Table 1. The overall distribution of the allele frequencies for each system was found to be in fair accordance with Hardy-Weinberg rule by compliance with the exact test (Gou and Thompson, 1992). The allele at all the three loci varied from 7 to 14, falling within the range observed for other local and world population. The most common allele at D16539 locus was 11, on the other hand at D7820 and D13S317 locus the most common alleles were 8, 10 and 8, 12, respectively. This data partly support an earlier study (Dobasi *et al*., 2005) where they found allele 11 as most common for all the three loci (Table 2).

Forensic efficiency parameters such as, the Power of Discrimination (PD), observed and expected Heterozygosity values (H), Polymorphism Information Content (PIC), Probability of Match (PM), Power of Exclusion (PE) and typical Paternity Index (PIt) are shown in Table 3. The observed heterozygosity was highest in D16S539 (0.846). The PIC values for all STR loci were highly informative (PIC≥0.7). The probability that two randomly chosen person have the same unspecified genotype at a locus is the sum squares of the frequencies of all genotypes at that locus (NRC, 1996). This probability is designated as PM or individualization potential of a locus (Hammond *et al*., 1994) and is used to calculate the power of discrimination of a locus, 1-PM. The higher the discrimination power of a locus, the more efficient it is in discriminating between members of the population. The combined PM using these three loci was calculated to be 3.88×10^{-4} and therefore, the combined power of discrimination was 0.99961. This means when used together these loci can distinguish samples from different individuals with a probability of 99.96%.

Another parameters used to evaluate the strength of a locus to exclude falsely accused individuals is the power of exclusion or PE. It represents the percentage of individuals in the relevant population who would not share the same DNA profile presented in a paternity case (Brenner and Morris, 1990). The higher the PE value, the more non-fathers are excluded. Single locus PE values range from 0.543 to 0.687 (Table 3), which indicate low degree of exclusionary power of the loci when used individually. However, combined value using the three loci has increased the forensic utility to 0.9396. In conclusion, analysis of three STR loci by present multiplex PCR method was shown to be highly discriminating and suggesting it would be very useful in forensic practice.

Table 1: Characteristics of three STR loci used in this study

STR locus	Chromosome location	Repeat motif	GenBank accession	Allele range	No. of allele seen	Allele size range (bases)
D16S539	16q24-qter	GATA	G07925	5-15	10	264-304
D7S820	7q11.21-22	GATA	G08616	6-15	22	215-247
D13s317	13q22-31	TATC	G09017	5-15	14	165-197

Table 2: Observed allele frequency of three STR loci in Bangladeshi population

Allele	D16S539	D7S820	D13S317
7	0.000(0)	0.029(3)	0.019(2)
8	0.067(7)	0.250(26)	0.250(26)
9	0.144(15)	0.087(9)	0.106(11)
10	0.087(9)	0.250(26)	0.135(14)
11	0.308(32)	0.163(17)	0.192(20)
12	0.221(23)	0.173(18)	0.240(25)
13	0.144(15)	0.048(5)	0.038(4)
14	0.029(3)	0.000(0)	0.019(2)
All	1.000(104)	1.000(104)	1.000(104)

Table 3: Forensic efficiency parameters for the three STR loci

	STR loci		
Statistical parameters	D16S539	D7S820	D13S317
Hobs	0.846	0.769	0.788
Hex	0.801	0.807	0.811
PIC	0.788	0.794	0.798
PM	0.077	0.073	0.069
PD	0.923	0.927	0.931
PE	0.687	0.543	0.578
PIT	3.25	2.17	2.36
P(exact)	0.784	0.868	0.433

Hobs: Observed heterozygosity; Hexp: Expected heterozygosity; PIC: Polymorphism Information Content; PD: Power of Discrimination; PM: Probability of Match; PE: Power of exclusion; PIt: Typical paternity index; P (Hardy-Weinberg equilibrium, exact test based on Monte Carlo Simulation)

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