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ABSTRACT:

The allele and genotype frequency values of the three tetranucleotide short tandem repeat (STR) loci, D7S820, D13S317 and D16S359, were analysed in blood samples of 25 unrelated randomly selected individuals in the National Capital District, Papua New Guinea.

Gene-Print Silver-STR III Multiplex kit (Promega Corp., Medison, WI, USA) was used for the PCR amplification in GeneAmp®PCR System 9700 thermal cycler (Applied Biosystems). Data analysis was carried out using the PowerStatsV12.xl workbook template obtained from Promega Corporation. The three STR loci were in Hardy-Weinberg equilibrium.

Five alleles (9 – 13) were identified for D16S539, five alleles (8 – 12) for D7S820 and six alleles (8, 9, 11 – 14) for D13S317. No new or microvariant alleles were observed. The most frequent genotypes for D16S539 were 11-11 and 11-12; for D7S820 were 10-11 and 12-12; for D13S317 was 8-12. Observed Heterozygosity was highest in D13S317 (0.880). The combined power of discrimination was 0.99733 and the combined power of exclusion was 0.9363.

The data suggests that the three loci are useful for identity testing, forensics and for solving paternity cases among the population in National Capital District, Papua New Guinea.

Key words: STR loci, DNA typing, Allele, Genotype, Forensic, PNG,

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INTRODUCTION:

The non-protein-coding segments in the DNA consist of tandemly repeated sequences of about 2 – 7 base pairs in length [1-3]. The short tandem repeats (STR) loci are well distributed throughout the human genome, are polymorphic in nature and represents important genetic markers for population studies [1 – 3].

Polymerase chain reaction (PCR) can be used to amplify the STR loci in low amount or degraded DNA in biological samples [2 – 6]. STR data can be highly informative and extremely effective for individualizing a wide range of biological samples, especially when multiplex PCR is used for simultaneous amplification of the STR loci [2 – 6].

Polymorphic loci whose alleles are the result of STR are the most informative PCR-based genetic markers for human identity testing, personal identification, and paternity testing because of their high statistical capability of discrimination and individualization [2, 4, 5].

The many uses of STR loci in forensics and criminal investigations have been highlighted by many researchers [1 – 6]. Selected STR loci are currently used for DNA typing and for setting up of DNA database in various countries [2,3]. Determination of the allele frequencies and distribution of genotype are prerequisites for DNA typing of any population.

In resource limited countries like Papua New Guinea DNA typing can be carried out with the multiplex STR system using silver stain for manual detection [7,8]. The Gene Print Silver STR III multiplex system containing three tetra-nucleotide repeat STR loci, D16S539, D7S820, and D13S317 can be used for DNA typing [7,8]. The characteristics of these STR loci are presented in Table 1.

There are no published data on the allele frequency of STR loci in the DNA of the population in the National Capital District (NCD) in Papua New Guinea (PNG). This project was an attempt to initiate the setting up of a DNA database for forensic testing in NCD.

The aim of this project was to determine the allele frequency of the three tetra-nucleotide STR loci, D7S820, D13S317 and D16S359, in a population sample in NCD, PNG.

MATERIALS AND METHODS:

The study site was the NCD, which is the incorporated area around Port Moresby the Capital of PNG. The study population consisted of 25 randomly selected and consented unrelated individuals resident in the NCD.

Whole blood was collected by finger stick and spotted onto special filter paper, which was then left to dry overnight [9,10]. Each dried blood spot obtained was then stored in a refrigerator until required for analysis [9,10].

DNA extraction was carried out using 5% Chelex®100 (Bio-Rad Laboratories, Hercules, CA) extraction procedure [10]. Gene-Print Silver-STR III Multiplex kit (Promega Corp., Medison, WI, USA) was used for the PCR amplification, of the loci D16S539, D7S820 and D13S317, in GeneAmp®PCR System 9700 thermal cycler (Applied Biosystems), following the standard procedures [9]. DNA amplification was done in 25µl per reaction sample.

The master-mix contained 140µl of primer pair mix (Multiplex 10x) and 8.4µl of Taq DNA polymerase mixed into 140µl of STR buffer (10x) and 971.6µl of sterile water, which gives a total volume of 1260µl.

A master-mix of 22.5µl was placed into each reaction well and the reaction plate was placed on ice, after which 2.5µl of template DNA was added to each reaction well. A total of 31 cycles of PCR were performed [9].

The amplified PCR products were separated in 6% Polyacrylamide denaturing gel on vertical Sequi Gen GT gel apparatus (Bio-Rad Laboratories, CA) using standard protocol [9, 11]. After electrophoresis the gel was stained according to the Promega Multiplex STR Silver Staining protocol [9]. The allele bands were identified by comparison with the allelic ladders in the Gene-Print Silver-STR III Multiplex kit (Promega Corp., Medison, WI, USA) Allelic Ladder Mix.

Ethical clearance and permission for this study was obtained from the ethics and research grant committee of the School of Medicine and Health Sciences, University of Papua New Guinea. Signed informed consent was obtained from all selected participants.

Data analysis was carried out using the PowerStatsV12.xl workbook template obtained from Promega Corporation [12].

The alleles were assigned their numerical values according to the reference information in the Promega technical manual [9].

These figures were used in the PowerStatsV12.xl software to calculate the allele frequencies and forensic efficiency parameters, such as Power of Discrimination (PD), Probability of Match (PM), Polymorphic Information Content (PIC), Power of Exclusion (PE), Heterozygosity (H), and Typical Paternity Index (PI).

Table 1: Characteristics of the STR loci D16S539, D7S820 and D13S317 [9]

STR Locus	Chromosome Location	Repeat Sequence	Allele Size Range (Bases)	Known Alleles
D16S539	16q24-qter	AGAT*	264 – 304	5, 8 – 15
D7S820	7q11.21-22	AGAT*	215 – 247	6 – 14
D13S317	13q22-q31	AGAT*	165 – 197	7 – 15

* Repeat sequences represent all four possible permutations (e.g. AGAT is used for AGAT, GATA, ATAG or TAGA) [9]

RESULTS:

The results indicated that the three loci were in Hardy-Weinberg Equilibrium [13]. Table 2 shows the observed allele frequencies for the three STR loci of the population sample in NCD. Five alleles (allele 9 – 13) were identified for D16S539, five alleles (allele 8 – 12) for D7S820 and six alleles (allele 8, 9, 11 – 14) for D13S317. New alleles or Microvariant alleles were not observed in any of the STR loci.

The most frequent allele for D16S539 was allele 11 (observed 22, frequency 0.478), for D7S820 the most frequent was allele 12 (observed 13, frequency: 0.271), and for D13S317 most frequent was allele 8 (observed 18, frequency 0.360).

The most common frequency ranged from 0.360 (D13S317) to 0.478 (D16S539).

Table 3 shows the observed genotype frequencies of the 3 STR loci (D16S539, D7S820 and D13S317) as well as the homozygosity and heterozygosity of the genotypes of the population sample in NCD. The most frequent genotypes for the loci were, for D16S539 genotypes 11-11 (frequency 0.261) and 11-12 (frequency 0.261), for D7S820 genotypes 10-11 (frequency 0.167) and 12-12 (frequency 0.167), and for D13S317 genotype 8-12 (frequency 0.280). The observed Heterozygosity was highest in D13S317 (0.880).

The statistical parameters and forensic data for the three STR loci calculated for the population sample in NCD are presented in Table 4. The matching probability ranged from 0.104 (D7S820) to 0.176 (D16S539), the power of discrimination ranged from 0.824 (D16S539) to 0.896 (D7S820), polymorphic information

content ranged from 0.62 (D16S539) to 0.73 (D7S820 and D13S317), and the power of exclusion range from 0.322 (D7S820) to 0.755 (D13S317).

Table 2: Observed allele frequencies of three STR loci of the population sample in NCD (frequency calculated as "Observed" out of "Total alleles")

Alleles	**D16S539**		**D7S820**		**D13S317**	
	Observed	**Frequency**	**Observed**	**Frequency**	**Observed**	**Frequency**
5		0.000				
6				0.000		
7				0.000		0.000
8		0.000	11	0.229	18	0.360
9	3	0.065	2	0.042	6	0.120
10	1	0.022	11	0.229		
11	22	0.478	11	0.229	11	0.220
12	11	0.239	13	0.271	11	0.220
13	9	0.196		0.000	3	0.060
14		0.000		0.000	1	0.020
15		0.000				0.000
Total	46	1.000	48	1.000	50	1.000

Table 3: Observed genotype frequencies for the STR loci of the population sample in NCD

	Genotype Frequencies (observed in bracket)		
Genotypes	**D16S539**	**D7S820**	**D13S317**
8-8		0.083 (2)	0.080 (2)
8-9		0.042 (1)	0.080 (2)
8-10		0.083 (2)	
8-11		0.083 (2)	0.160 (4)
8-12		0.082 (2)	0.280 (7)
8-13			0.040 (1)
9-11	0.087 (2)	0.042 (1)	0.120 (3)
9-12			0.040 (1)
9-13	0.043 (1)		
10-10		0.083 (2)	
10-11		0.167 (4)	
10-12		0.042 (1)	
10-13	0.043 (1)		
11-11	0.261 (6)	0.042 (1)	0.040 (1)
11-12	0.261 (6)	0.083 (2)	0.040 (1)
11-13	0.087 (2)		
11-14			0.040 (1)
12-12	0.043 (1)	0.167 (4)	
12-13	0.130 (3)		0.080 (2)
13-13	0.043 (1)		
Total samples	1.000 (23)	1.000 (24)	1.000 (25)
Homozygotes	0.348 (8)	0.375 (9)	0.120 (3)
Heterozygotes	0.652 (15)	0.625 (15)	0.880 (22)

Table 4: Statistical parameters and forensic data for the three STR loci D16S539, D7S820 and D13S317 for the population sample in NCD

Statistical Parameters	D16S539	D7S820	D13S317
Probability of Match (PM)	0.176	0.104	0.146
Power of Exclusion (PE)	0.358	0.322	0.755
Power of Discrimination (PD)	0.824	0.896	0.854
Polymorphic Information Content (PIC)	0.62	0.73	0.73
Heterozygosity observed (Ho)	0.652	0.625	0.880
Typical Paternity Index (TPI)	1.44	1.33	4.17
Combined PD	0.9973		
Combined PE	0.9663		

DISCUSSION:

The combined allele distribution for the three STR loci in the present study ranged from 8 to 14, which is within the range (7 – 14) reported in other populations [4 – 6]. Allele 11 was the most common in D16S539, which support similar findings for other populations [4 – 6].

The observed allele range for locus D16S539 was from 9 to 13 (5 alleles), compared to allele range of 8 to 14 (7 alleles) reported for population in Banladeshi [4], Marmara region of Turkey [5] and Southern Italy [6].

The observed allele range for locus D7S820 was from 8 to 12 (5 alleles), compared to allele range of 7 to 13 (7 alleles) reported for population in Banladeshi [4], 7 to 14 (8 alleles) reported for Marmara region of Turkey [5] and 7 to 14 (8 alleles) for Southern Italy [6].

For locus D13S317 the observed allele range was 8, 9, 11 – 14 (6 alleles), compared to allele range of 7 to 14 (8 alleles) reported for population in Banladeshi [4], 8 to 14 (7 alleles) for Marmara region of Turkey [5] and 8 – 15 (8 alleles) for Southern Italy [6].

PIC values for the three loci were highly informative (PIC > 0.5). For forensic analysis the higher the PD of a locus, the more efficient it is in discriminating between members of the population [2 – 6]. The PD obtained for each locus can distinguish samples from different individuals with a probability or efficiency of 82.4% (D16S539), 89.6% (D7S820) and 85.4% (D13S317). The calculated combined PM for the three loci was 2.67 x 10-3, thus the combined PD was 0.99733. This implies that when used in combination, these loci can distinguish between samples from different individuals with an increased probability of 99.73 %.

Although the individual PE value for each of the locus is low, the calculated combined PE value (0.9363) is high, which indicates high degree of exclusionary power.

In conclusion, the data indicate that the three STR loci show high discriminating power, which suggests that they are useful for identity testing, forensics and for solving paternity cases among the population in NCD.

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