Allele Frequencies of STRs (F13A01, FESFPS and vWA) in Random Iban Population of Malaysia

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ABSTRACT: DNA fingerprinting has become such an established technology that has been featured frequently in the media and is quite well known to the general public. Many techniques are used to profile the DNA. DNA profiling methods were established throughout the time to develop database for use in individual identification in Forensic Science institution in the world. Tandemly repeated DNA sequences are widespread throughout the human genome and show sufficient variability among individuals in a population that they have become important in several fields including genetic mapping, linkage analysis and human identity testing. In this present study, distribution of allele frequencies for three validated STRs (F13A01, FESFPS and vWA) was compiled for ethnic Iban population of Malaysia.DNA samples from 100 random Iban individuals were processed using multiplex primer kit. The highest percentage of allele distribution is reported as the most frequent alleles with allele 6 for F13A01 (36.5%), allele 12 for FESFPS (37.5%) and allele 17 for vWA (35%).

Keywords: DNA profiling, STR, human genome, allele frequencies

Introduction

Crime occurs everywhere and every time with or without being detected. Criminals have advantages of stealth to commit crime at night or in the isolated area where nobody is there. Locard postulated that, 'every contact leaves traces' [1]. The clues left behind are witness to their crimes. Deoxyribonucleic acid (DNA) is a physical evidence that is likely to be found present in the crime scene. Physical evidence provides clues regarding the crime scene or events indirectly that can lead to the findings and solutions [2].

DNA fingerprinting is used to trace heredity [3]. The human genome contains approximately 3 billion base pairs and the range of variations is from 1 in 100 to 1 in 1000 nucleotides. This variation is located in the non-coding region which contains of tandem repeat sequences. The numbers of repeat sequences vary between individuals so that it produces alleles of different length known as length polymorphism [4].

The most popular technique and always preferred by the analyst is short tandem repeat (STR) analysis. DNA profiling using STR is easier compared to other analysis due to its ability to deal with less quantity of samples [5]. The STR typing protocol amenable for amplifying multiple loci is multiplex primer kits. Multiplexing is very valuable in profiling or typing of biological materials and sample mixtures containing degraded DNA molecules.

In this study, commercial multiplex STR kit for STR loci-F13A01, FESEPS and vWA was used to study the distribution of their alleles in Iban population in Malaysia.

Literature Review

Tandemly repeated regions of DNA are typically classified into several groups depending on the size of repeat region. Minisatellites (variable number of tandem repeats, VNTRs) have core repeats with 9-99 bp, while microsatellites (short tandem repeats, STRs) contain about 2-7 bp repeats. Figure 1 shows the examples of STR polymorphisms with 7, 8 and 9 repetitions. PCR technique is used for VNTR and STR loci [6]. The alleles were characterized by measurement of their molecular weight. Compared to VNTR loci, STR loci are the most preferred by the analyst as STR loci will give more advantages in DNA typing. Some of the reasons are STR loci can be easily amplified by PCR without problems of differential of amplification in heterozygous individual, the technique is more appropriate for degraded DNA; and also single base

resolution of DNA fragments can be obtained easier with sizes below 500 bp using denaturing polyacrylamide gel electrophoresis [7]. In the middle of 1990s, the PCR based STR technique was introduced. STR loci can be multiplexed together using several different STR primer pairs to amplify several loci in one reaction. Early multiplex tests were based on simple STR loci. The four-locus 'quadruplex' have been described as the first to be widely used for court reporting purposes. In 1996, a six-locus STR system has been established in UK and New Zealand known as 'second-generation multiplex' (SGM). The introduction of SGM preceded the launches of the UK and New Zealand national DNA database. Presence of commercial multiplex systems manufactured by related companies nowadays can help the scientists in order to establish the database which can be used as a valuable reference in solving crimes [5]. In the literature, population database for many different populations are available. There are several databases on ethnic population groups in Malaysia on STRs that has been published. Hence in the present study allelic distribution for three validated STRs (F13A01, FESFPS and vWA) for Iban ethnic group from eastern Malaysia is studied.

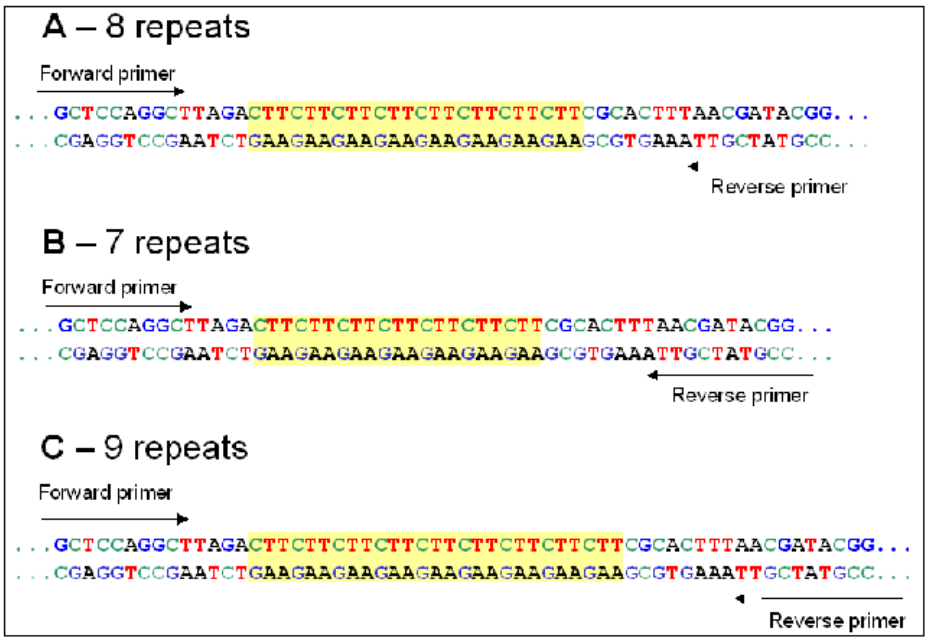

Figure 1: Examples of STR Polymorphisms [6]

Methodology

Materials and reagents used in this study were made sterile by autoclaving. The STR typing was done according to the guidelines given by the manufacturer of STR kits (GenePrint STR System Technical Manual D004, Promega, USA). Saliva samples (buccal swabs) were used and taken from 100 unrelated random Iban populations in Sarawak, Figure 2. Sterile cotton buds were used. Each individual was asked to streak the cotton buds from inside their mouth towards their cheek for at least 10 seconds. Then, all the cotton buds were air dried at room temperature away from direct sunlight and kept in envelopes separately which were labeled with necessary brief information such as subject's name, father's name, age, sex, and the place of domicile. Then all the samples were extracted using organic method. The extracted samples later were amplified using FFv multiplex primer kits and went through electrophoresis, along with reference allelic ladder. Then the products of amplified DNA were detected by developing the allele bands with silver staining technique. The identified alleles later were analyzed through statistical analysis.

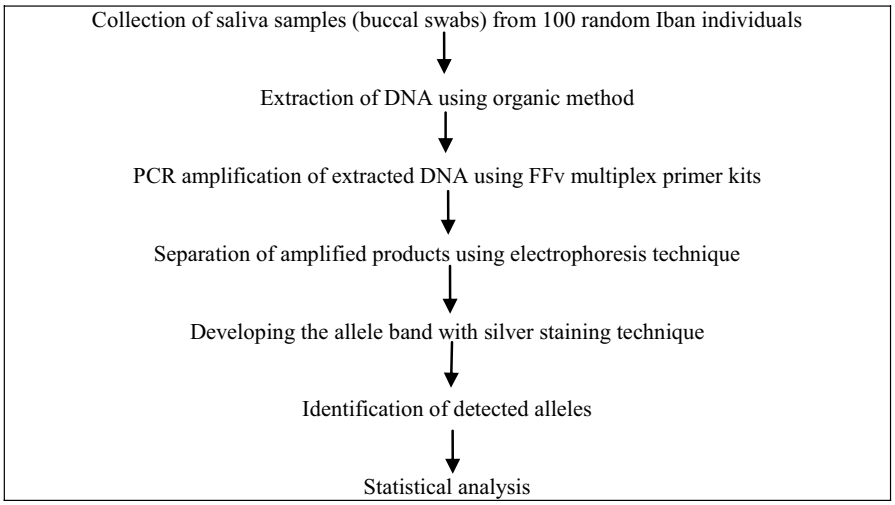

Figure 2: Schematic representation of analytical protocol

Results and Discussions

In this study, seven alleles were found in the Iban population (Table 1). The highest percentage of allele distribution is allele 6 with 36.5%.The other 6 alleles are 3.2,4, 5, 7,14 and 15 with the distribution of 24.5%, 17.5%, 17%, 2.5%,0.5% and 1.5% respectively. Allele distribution in the present study shows similar pattern with the allele distribution in other Asians population such as Filipino population, Korean population, Thai population, Chinese population in China and Chinese population in Malaysia [8-12]. Similarity in distribution of allele was also found between Italian populations [13-14] (Cossutta et al., 2000 and Domenico et al., 2001), Tamil and Badaga population in India [15].

FESFPS (Human c-fes/fps proto-oncogene) STR is located on chromosome 15 at 15q25-qter. Eight alleles i.e. allele 7, 8, 9, 10, 11, 12, 13 and 14 (Table 1) are reported in the literature. The repeat sequence of the locus is AAAT tetrametric. There is presence of rare allele 15 in the population database. Allele 7 and 15 are the least frequent alleles in many populations. The most common allele with the highest distribution in most of the populations is allele 11.

Third STR genetic marker used in this study is vWA. vWA (Human von Willebrand factor gene) STR is located on chromosome 12. Eight alleles namely (13, 14, 15, 16, 17, 18, 19 and 20) were reported in the literature. The repeat sequence of the locus is AGAT tetrametric. Alleles 10, 11, 12, 21 and 22 were found to be rare alleles in most of the population [16]. Allele 17 was the most frequent allele in several populations [17]. The other frequent alleles are allele 14, 15, 16 or 18. Allele 22 in the Garo population [18] was found to be 1% in the distribution. The Bhutia population [19] exhibits absence of allele 19 and Chinese Han population in the middle China exhibits absence of allele 18 and allele 19. Allele 17 in the Lepcha population [19]was reported to have the maximum distribution of 42%.

In the Iban population studied, seven alleles (allele 14, 15, 16, 17, 18, 19 and 20) were recorded. Allele 17 with a distribution of 35% is the most frequent allele (Table 1). The other alleles 14, 15, 16, 18, 19 and 20 were counted with frequency of 7%, 2.5%, 15%, 35%, 27.5%, 12.5% and 0.5%, respectively in the population. The pattern of allele distribution in the present study is similar to the pattern of allele distribution in the population of Sumatera/Sulawesi in Indonesia [20], Tamil and Badaga population in South India [15], Naga population in Eastern India and Indian population [18], Chinese population in China [11], Asian population, Caucasian population and aboriginal population in West Australia [21], Turkish population [22], Yadav and Kurmi Community in India [19], Dhaka population in Bangladesh, population in Surabaya , Indonesia and Chinese population in Malaysia [12].

Table 1: Allele frequency of STR loci (F13A01, FESFPS, vWA) in Iban population of Malaysia

Allele	Frequency (x/200)		
	F13A01	FESFPS	vWA
3.2	0.2450	-	-
4	0.1750	-	-
5	0.1700	-	-
6	0.3650	-	-
7	0.0250	-	-
8	-	0.0150	-
9	-	0.0250	-
9.3	-	-	-
10	-	0.0600	-
11	-	0.3150	-
12	-	0.3750	-
13	-	0.1650	-
14	0.0050	0.0450	0.0700
15	0.0150	-	0.0250
16	-	-	0.1500
17	-	-	0.3500
18	-	-	0.2750
19	-	-	0.1250
20	-	-	0.0050
Chi	15.510	12.590	14.070
H	0.8709	0.8489	0.8847
PE	0.5169	0.4938	0.5473
PD	0.9025	0.8528	0.8984

(Note: H-heterozygosity, PE- Power of exclusion, PD- Power of discrimination, Chi- Chi square)

Conclusion

The foregoing discussions explicitly show least similarities among various populations which are separated geographically. This indicates that the distributions of the alleles for various STRs in various population are not determined by geography. There are several racially different populations that had the same pattern of allele distribution. From these considerations, it is important to compile database in allele distribution of all subpopulations in a country for personal identification. The purpose of the present study is to compile a database on three validated STRs – F13A01, FESFPS and vWA in Iban population. Due to the uniqueness and individualization of allele distribution, differences occurred in the distribution cannot be assigned to racial or geographical difference.

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