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Generation of 15 Hypervariable Short Tandem Repeats Profile from Human Blood Traces

Rakesh Ranjan and Abhijit Dutta

Department of Zoology, Ranchi University, Ranchi – 834008, Jharkhand, India

Abstract: Over the last two decade, individual identity testing has settled on a set of core short tandem repeats (STR) loci that are widely used for DNA profiling. It is mandatory to estimate the amount of human DNA present in blood traces before going into amplification of STRs. This study was performed for STR profiling of four different blood sample of human origin. The absolute amount of DNA in different sample were 1.69 ng/µL, 5.81 ng/µL, 12.80 ng/µL and 7.53 ng/µL for sample A1, A2, A3 and A4 respectively quantified by Real Time PCR (RT-PCR). All the samples suggest high amount of DNA. 1.5 to 2.0 ng/10µL DNA is optimum to get balanced STR profile. Therefore, it is subjected to dilution with appropriate factor with TE buffer. AmpFl STR kit enabled robust amplification of the CODIS (Combined DNA Index System) loci with additional two loci and amelogenin (for gender typing). From the comparison chart of allelic genotype of 15 + 1 STR loci, it is observed that STR profile of an individual is consists of 16 different loci. Each locus is having Bi allelic pattern of inheritance that is one descent from father and the second from mother. A Pair of number in a locus represents number of repeat unit of STRs which is the sole determinant of discrimination. Each locus has multiple alleles in population therefore chances of matching between each locus decreases as we analyze more number of locus.STR profile of 15 hyper variable autosomal loci is strong enough in discriminating power of 7.2×10^{-19}.

Key words: STR · Hypervariable · DNA · Teteranucleotide repeats · Amelogenin

INTRODUCTION

An STR is a region of human DNA containing an array of tandem repeats and a repeat unit can be 2 to 6 base pair (bp) long [1,2]. STR sequences account for approximately 3% of total human genome [3] which are highly polymorphic non coding DNA sequences, each STR locus contains a core repeat region in which the number of tandem repeat units varies among individuals.

The numbers of tandem repeat units determine genotype for human identification. More than 20,000 tetranucleotide STR loci have been characterized in human genome [4]. Several STR loci have been characterized for human identification. The discriminating power of an STR locus used for human identification can be measured by a population match probability (Pm). Lower the Pm value the less likely a match will occur between two randomly chosen individuals.

The present study was performed for STR profiling of four different blood sample of human origin.

MATERIALS AND METHODS

Collection of Blood: About 48µL of blood was collected in 1.5 mL eppendorf tube for analysis.

Extraction of DNA: The process of DNA extraction was organic extraction method followed by Lincoln and Thomson [5]. The process is based on the use of organic solvent Phenol + Chloroform + Isoamyl alcohol and extraction buffer.

Quantitation and Dilution of DNA: The DNA was quantified using Real time PCR (Applied Biosystem 7500 Real-Time PCR system) and quantifiler human DNA kit (Applied Biosystem).

PCR Amplification and Fluorescent Labeling of STRs: After the quantification of DNA, it is diluted with appropriate TE buffer and DNA is subjected to selective amplification of 15STR loci plus amelogenin gender

Corresponding Author: Rakesh Ranjan, Department of Zoology, Ranchi University, Ranchi-834008, Jharkhand, India. Cell: +91 9534217495.

loci simultaneously in Gene Amp® PCR system 9700 using identifiler kit. The identifiler kit have all the necessary reagents to amplify the target loci along with the 5 Fluorescent dye labeled oligonucleotide primers incorporated into 5- end. Each of the fluorescent dye emits maximum fluorescence at different wavelength.

Capillary Electrophoretic Detection of Alleles: The amplified product of PCR reaction was subjected to capillary electrophoresis in 3130 genetic analyzer (Applied Biosystem). This reaction is conducted in denatured state for STR. Separation in capillary is performed in high voltage. During electrophoresis capillary is connected in buffer reservoirs. The injection of sample into capillary is performed by auto sample using an electro kinetic mechanism. Instrument is equipped with detection system having data collection software v3.0 which utilizes excitation source. This excites the labeled DNA fragments. Optical filters are used to detected light emitted at a particular wave length. These signals are recorded in optical filter with the help of mathematical algorithm matrix. The experiment done in this contest with the help of identifiler® kit of Applied Biosystem which enabled identification of human with a matching probability of $7.2x10^{-19}$ [6].

RESULTS AND DISCUSSION

DNA isolated from blood samples in the form of precipitate. The quantity of DNA obtained from Real time PCR from four samples are presented in table 1.

The presence of IPC (Internal positive control) indicates that the entire DNA extracted from their raw sample was free from inhibitor of PCR. During the Quantification process R2 value and average slope came out to be 0.996 and -3.1 respectively. All the sample indicates presence of high amount of DNA therefore each sample were diluted in TE buffer with proper dilution factor so as to get balanced STR profile in subsequent step. The dilution factor of each sample is listed in table 2.

All the diluted DNA samples contain 1.5-2.0 ng/10µL of DNA which was multiplex in hot stat PCR system (Applied biosystem). Hot stat PCR system amplify definite target STR DNA. The amplified products were subjected for capillary electrophoresis. The result of electrophoresis was analyzed in gene mapper ID software which are listed in table 3 and a specimen of STR profile of sample A1 is generated in 3130 genetic analyzer (Figure 1).

It has more than two decades since 13 STR loci become the core of the FBI laboratory`s (CODIS) for human identification [7]. Their precise chromosomal information became available with completion of human genome project. Applied Biosystems released their 16 plex Identifiler kit in July 2001 which amplify 13 core loci, amelogenin and two tetranucleotide loci D2S1338 and D19S433 [8]. The extra two loci provide improve power of discrimination. STR allele size are measured relative to an internal size standard during electrophoresis and depending upon the DNA strand that is dye labelled may have a different apparent size [9].

Table 1: Absolute amount of DNA in different sample.

Sample	Quantifiler H (ng/µL)	IPC (ng/µL)	Quantity of DNA (ng/µL)
A1	22.61	37.38	1.69
A2	26.04	25.80	5.81
A3	25.32	26.40	12.80
A4	25.00	25.82	7.53

Table 2: Dilution factor of different sample.

Sample	Amount of DNA (ng/µL)	Dilution factor
A1	1.69	1µL DNA + 8µL TE
A2	5.81	3µL DNA + 24µL TE
A3	12.80	1µL DNA + 63µL TE
A4	7.53	1µL DNA + 37µL TE

Table 3: Comparison chart of allelic distribution (genotype) of 15 different loci + amelogenin (gender typing) locus of DNA tested

Sl. No.	Name of Loci	Sample A1	Sample A2	Sample A3	Sample A4
1	D8S1179	11,13	14,15	14,14	11,16
2	D21S11	28,31.2	28,29	19,32.2	29,50
3	D7S820	8,11	11,11	8,8	8,11
4	CSF1PO	10,12	12,12	10,10	11,12
5	D3S1358	15,18	16,17	17,17	15,16
6	THO1	6,9	8,19	7,8	9,9.3
7	D13S317	8,10	8,14	8,11	8,9
8	D16S539	11,11	9,11	12,12	11,12
9	D2S1338	17,21	17,20	19,19	19,23
10	D19S433	12,13.2	13,14	13,15.2	14,15.2
11	VWA	16,18	18,19	17,19	16,20
12	TPOX	8,11	11,11	9,11	9,11
13	D18S51	13,15	14,15	15,16	13,18
14	D5S818	11,12	12,12	12,13	10,12
15	FGA	24,24	19,25	23,23	22.2,24
16	Amelogenin	X,Y	X,Y	X,Y	X,Y

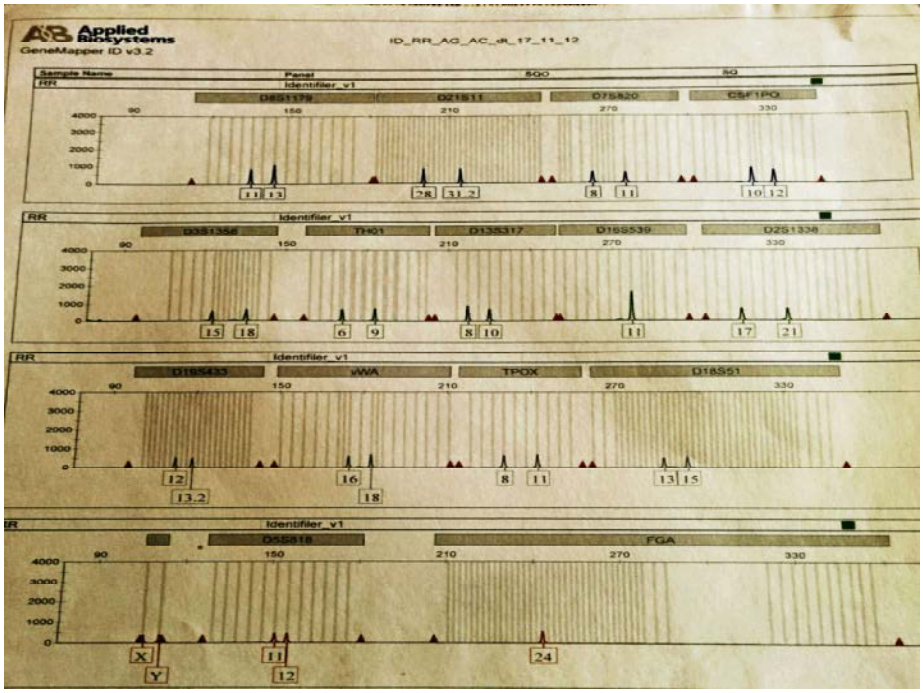

Fig. 1: Specimen of STR profile generated in 3130 genetic analyzer.

STR profiling is performed by using size comparison to standard allelic ladder. Different STR kit manufacturer may supply allelic ladder with slight variation [6]. Since more samples are run with STR loci new alleles can be discovered that do not size exactly with allelic ladder. These are termed as off ladder allele. These off ladder allele can be variants of common allele [6]. A new allele can be discovered which occurs outside the range of commercially available allelic ladder.

Many of the STR loci can be studied for the tri allelic pattern for example TPOX has a highest amount of tri allelic pattern [6]. Medical genetic researchers claim to have shown linkage between a particular disease gene and a core STR marker, these types of findings should not prevent the continued use of the STR locus. HumARA is a X-linked STRs has CAG repeats located in coding region of androgen receptor gene had shown several genetic disease, but none of the core loci used in human identity testing are located in a gene coding region (i.e., exon) or are trinucleotide repeats, which can be prone to expansions that cause genetic defects [10]. In fact, many times these linkage findings can later be proven false with further studies [11].

Million of DNA sample have now been examined across the world for core STR till date. It is perhaps the worth taking that which loci are best suitable. It is desirable to have loci with better allele frequency distribution. Less polymorphic loci have lower mutation rates and more useful in parentage testing. Thus due to different need, not human identity testing desire the same STR marker set.

Thus study of 15 autosomal STR loci for human identity testing will continue to be used for many years due to high degree of variability and implementation to create national data base. Utilization of a uniform set of STR loci provides the capability for national and international sharing of DNA profile.

From the comparison chart of allelic genotype of 15 + 1 STR loci, it is observed that STR profile of an individual is consists of 16 different loci. Each locus is having Bi allelic pattern of inheritance that is one descent from father and the second from mother. A Pair of number in a locus represents number of repeat unit of STRs which is the sole determinant of discrimination. Each locus has multiple alleles in population therefore chances of matching between each locus decreases as we analyze more number of locus.STR profile of 15 hyper variable autosomal loci is strong enough in discriminating power of 7.2×10^{-19}.

In the studied samples as shown in the comparison chart of table 3, it is clear to state that, all the 4 STR profile are unrelated among each other and all 4 sample of blood is of human male origin.

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