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Systematic review on optimization of STR (D7S820) for forensic studies

Alima khan, Mahnoor Afzal, Zukhruf Asif, Ammara Sameen,Shehreen Sohail, Tayyaba khan, Hamza Rana, Fareeha Sohail

Abstract:

In last twenty years DNA profiling has become an obligatory technology which has brought scientists towards forensic identification. Thousands of STR markers are present in human genome but only core set of loci are selected for forensic DNA and human identification. STRs have become famous in forensic labs because even low amount and degraded form can be easily typed. STRs are found in prokaryotes and eukaryotes, including humans. They appear scattered almost evenly throughout the human genome, resulting in about 3% of the genome. However, their distribution in chromosomes is not quite uniform. Usually, STRs occur in the noncoding regions, only about 8% are located in the coding regions. In humans, chromosome 19 has the highest density of STRs. On an average, one STR occurs per 2,000 bp in the human genome. On the basis of repeat units, STRs can be differentiated into different types. The repeats of STR markers are highly variable in human population. STR marker evaluation very precisely figures out individual humans at the molecular level even from very small quantities. STR locus in the identification selected is D7S820 from NIST (National Institute of Standards and Technology). Conditions for STR with the changes in temperature, magnesium ion concentration, primer and setting up, PCR of the marker used to carry out. PCR product is inspected of agarose gel. The results showed that the STR locus being investigated is detectable by PCR. PCR showed that the detection of primer and temperature conditions measured by using the fixed amount of magnesium, D7S820 locus bands are weaker than expected. Using a buffer and setting magnesium condition towards changes in primer and temperature, addition of Taq polymerase at a temperature of 94°C, bands become visible desirably.

Key words: Taq polymerase, DNA sequence, STR variation, PCR, polymorphism, human genome

Introduction:

Short tandem repeats or microsatellite are path of tandemly replicate short (1-6 bp) DNA sequence motifs. Short tandem repeats have one or another intergenic or intragenic area, as well as genes, which contain 3% of human genome. Most of the microsatellites are more changeable to show sequence or length polymorphism. But the others are maintained or provide many information which is available in the marker for the population genetics, mapping or interconnection studies [1]. Basically, the repeated DNA sequences hold full of human genome by bear on polymerase chain reaction STRs can easily be identified which can be used as a DNA marker it is possible that the STRs and the PCR product are similar to find the result easily. A particular position of trinucleotide replicate is merge of nucleotide are organize duplicative such as (CAG, CGG, that can be replicate) [2]. Numerous STRs loci are used in many countries all over the world. Basically, STRs used as a marker which is also help in laboratories or help to enhance the quality insurance. STRs used at the same time of PCR amplification that can obtained by allowing the 15 STR loci. Before to be relevant on population genetic studies it is necessary to be aware about the STRs in detail. In commercial kits DNA sequence can be originate to the presences of X chromosome or Y chromosome that give a name amelogenin which already present in STRs.

For genotyping approaches microsatellite are used. To increase their genetic essential quality in the field of botany, microsatellite is used. Microsatellite have non-coding or coding region, that are present in prokaryotes as well as euchromatin of the eukaryotes. SSR divided into two categories in cereal the first one is repetitive sequence or the second one is unique sequences. In the oats the repetitive sequence is less polymorphic. SSR basically smaller than 12bp nucleotide which have mutation potential SSR is microsatellite. When the mutation rate increase in SSR it led to change in gene expression [3]. STRs categorically helps to differentiate individuals by their genome. When analyzing the STR variation many of STR shows the consequences of the alleles that can be absent in the genome. Human genome has STR that are polymorphic. STR are polymorphic in nature, they are helpful in forensic or use to classify the genetic disorder. Approximately 700,000 STR loci were assemble from 1000 individuals from the 1000 genome project in phase 1. These simply show that the STR or SNPS are not relate to each other [4].

Electrophoresis and PCR is used to detect the pathogenic STR in a single locus when there is detection of pathogenic STR which means it cause many diseases. To identify the new STR loci short-read sequence of STR is used which bring down the cost and time. Disease is caused by the

new STRs and was not identify because long-read sequence can be detected by many tools. STRetch can be used to detect the whole human genome STR. The variation at STR loci can be analyzed by 97 human genomes with the STRetch.

Population variation

To let out the true number of repeats standardized allele ladder can be used which can be performed according to the certain condition that is comparison of STRs typing and their size which is most important in it [5]. To obtain the population variation STR kit provide allelic ladders that contain different allelic variety. When the sample is run according to the condition with STR loci with the passage of time the sample can run the new alleles can be came across where the size cannot be obtained with the ladder loci. These off-ladder repeats are available in common alleles which can be characterized by the commercially available allelic ladder it is present because of the less repetitive units' variants. To adjacent the region of the repeats the variant allele contains such deletion or insertion of the flanking region.

To verify this type of process we took the example of insertion or deletion of the flanking region that create off ladder repeats which is D7S820, that easily carry GATA repeats establish in the ladder alleles or carry 8, 9, and 10 adjacent T nucleotide [6]. Basically, alleles can be categories by the wide-ranging number of alleles rather than the alleles which can be repeated in the ladder which we can run to obtain the specific alleles. To analyze the result, we can used NIST or STR Base website which is used to analyze the diallelic pattern that has been observed for the STR central loci [7].

Combined DNA index system (CODIS)

In November 1997, combined index system by using the genetic markers were selected that can be obtained by the FBI laboratory it takes almost 13 years. Not only this system can be selected it can be used national DNA database of the FBI, UU (NDNAD) and further criminals. In criminal justice 5 million profiles can be collected in the USA UU or the UK alone. From the central loci and subset, they get information of the DNA database [8].

Material and Method:

1. 0.5M EDTA
2. 1M Tris HCL Ph 8
3. 1M Tris HCL Ph 7.5
4. Sodium acetate
5. 10%SDS
6. TE buffer
7. 20X TBE buffer
8. 1X TBE buffer
9. 6X DNA Loading dye
10. Lysis buffer (1M Tris HCL Ph 8+0.5M EDTA+10%SDS)
11. Proteinase K
12. RNase

Sample collection

The STR primer were selected from whole blood sample to identifying the optimization of STR (D7S820) for forensic study. The blood samples were collected from 4 student of University of Central Punjab

Total of four blood samples was collected from university of central Punjab Lahore. We collected 4ml blood sample in EDTA in sterile collection tubes. The particular of individual were recorded in a prescribed consent from dully signed by the participating volunteer. One aliquot of 300mL whole blood sample in micro centrifuge tube was preserved at -70°C for each sample as backup source and remaining whole blood was preserved at -20°C in the sterile tubes and in micro centrifuge tubes.

DNA extraction

1. Firstly,200µl EDTA blood samples were thawed by keeping at 37ºC for 10 minutes.
2. Then vortex it
3. Then wash with washing buffer (20Mm tris HCL with 7.5) for all the removal of material of blood except white blood cells. This removes all red blood cells and serum protein.
4. Wait for 20 minutes mix gently followed by centrifuge at 3,000 rpm for 15 minutes. Then discard the supernatant and save the pallet.

5. Repeat the process for 2,3 times until the pinkish color of blood is removed.
6. Add 100µl lysis buffer, 15µl proteinase K and 10% SDS.
7. Incubate at 37’C overnight
8. Shaking incubator
9. Equal volume of PCI is added in Eppendorf tubes followed by centrifugation at 3000 rpm for 15 minutes and save the pallet.
10. Equal volume of Isopropanol and centrifuge for 15 min at 3000rpm
11. Discard supernatant add 70% chilled ethanol and centrifuge at 5000rpm for 5 minutes.
12. Dry the pellet
13. Add 5micro liter RNASE incubate it and water bath at 60’C for 1:30 hours
14. If the pallet is visible than add 50µl TE Ph 8.
15. For complete suspension place on water bath at 70’C for 10-15 minutes.

PCR Master Mix

PCR Master Mix consists of Nuclease Free Water and PCR Master Mix two2X. PCR Master Mix is a combination of two solution containing Taq DNA polymerase, dNTPs, MgCl2 and reaction buffers at most effective concentrations for environment friendly amplification of DNA templates through PCR.

Composition of Buffers and Solutions

PCR Master Mix	50ml
Taq DNA polymerase pH 8.5	400uM
daTP, dGTP. dCTP, dTTP	3mM

Procedure:

Polymerase Chain Reaction

PCR amplifies the DNA at an exponential rate. It consists of two profiles reaction profile and temperature profile. It is automated cycler which can heat and cool the tubes with the reaction mixture in a very short time.

For the amplification of the TH01 was done by using the primers. Amplification was carried out using the master mix, primers and DNA template PCR is performed using a thermocycler.

1. PCR tubes were label with permanent marker
2. Then in PCR tube added PCR water (double distilled water) 3.5 ul
3. Added chilled 12.5ul master mix that consist of dnTPs, Taq polymerase, and buffer
4. After that we added 2ul forward primer and 2ul reverse primer
5. At last, we added 5ul of extracted DNA sample
6. Placed PCR tubes into the PCR machine
7. Adjusted the temperature profile

PCR condition optimization

The DNA concentration in a working solution of about 10 ng/µl in ddH20 was confirmed by spectrophotometric evaluation at 260 nm. For optimization, the concentration of genomic DNA, 1X buffer plus MgCl2, STR primers and Taq DNA polymerase were optimized for the STR. The primers were synthesized from genetic research. Taq polymerase, collectively with 5X PCR buffer, MgCl2 and dNTP were synthesized locally. The amplification was performed once in the thermal cycler PTC-100 for 30 cycles. The constituents with their exact composition that were used for PCR reaction profile are given and listed in table 1.During the amplification in thermal cycler some conditions were applied in order to get better result especially at first time which were observed at different amplification temperature and time listed all in table 2 below.

Sr. No	CONSTITUENTS	AMOUNT
1	Primer forward	2µl
2	Primer backward	2µl
3	Double distillation water	3.5µl
4	Master mix	12.5µl
5	DNA sample	5µl
6	Total	25µl

Table 1: Composition of PCR reaction profile

Sr. No	Phases	Temperature	Time
1	Initial temperature	95°C	3 minutes
2	Denaturation	93°C	45 seconds
3	Annealing	58°C	45 seconds
4	Extension	72°C	45 seconds
5	Final extension	72°C	10 minutes
6	Hold	4°C	10 minutes

Table 2:

Conditions for first PCR amplification

Result:

Sample was collected from a student from university. This study was performed with three other research students' samples. In order to avoid any confusion our sample was named A1, Z1, M1, K.DNA extraction was carried out of the sample. Extraction of DNA was confirmed using gel electrophoresis method. This was performed to get the conformation. After the extraction of DNA, the samples were amplified in thermo cycler PCR machine by using PCR machine by using STR locus the results for D7S820 were collected.

Discussion:

The short tandem repeat is developing rapidly as a useful method for human identification for forensic and other purposes. A short tandem repeat is actually a microsatellite which consist of a unit of 2-13 nucleotides that are repeated several times to tens of times in the DNA. In the STR analysis we measure the exact number of repeating units. Short tandem repeats primers bind to the desired regions of DNA and in order to determine the length of STR we then perform Polymerase chain reaction. For the purpose of human identification, it is very important to have DNA markers with the best version to distinguish different samples. The amplification products

of PCR are often challenging due to the fact that the DNA of these samples is often degraded or mixed as in the case of asexual reproduction. The small size of the alleles makes STR markers better candidate for use in forensic applications where degraded DNA is common. PCR amplification of degraded DNA samples can be performed higher with smaller sized products. We can easily separate STR alleles from different chromosomal locations to ensure that linked loci are no longer selected, all this is possible because of small size of STR alleles predictable pattern of random distribution in the population is not followed by the closely related loci which eventually makes the statistical analysis difficult. Because of these problems, STR with higher discriminatory force is chosen for the purpose of human identification in forensic and criminal cases. It is used to select and confirm the victim, the perpetrator, the missing person and others. At the start of 1996, the FBI laboratory launched a national forensic effort to configure key STR loci for inclusion in the National database known as CODIS (Combined DNA index system). The thirteen CODIS loci are: CSF1P0, FGA, TH01, TPOX, VWA, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51 and D21S11. These loci are used nationally and internationally are recognized for human identification.

The development of molecular system in order to study STR loci and the optimization of PCR method for the analyzation of STR loci provide a way for the forensic investigation and study of these loci in different population. In this study optimization of STR loci (D7S820) the blood sample was extracted from an individual and the DNA extraction was done and the optimization of STR loci was performed using PCR. The D7S820 loci had the lowest allelic diversity, polymorphism and heterozygosity. At the level of D7S820 locus, the uniform distribution of alleles in the samples examined makes it informative for the purpose of paternity and forensic testing. As indicated previously, this locus has been highly polymorphic with a high degree of variability in different populations. Reports from other population have indicated a high degree of variation in allele number of this STR loci, making it particularly informative for forensic and paternity testing. The analysis of short tandem repeat (STR) DNA sequences is of elementary significance in forensic science because they have turn into the recognized standard in establish public database. The STR loci used in this study are D7S820 extraction is done on different samples and the allele variation for these STRs is done in this study using PCR.

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