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STR MARKER ASSISTED SELECTION FOR THE DETECTION OF GENETIC POLYMORPHISM IN NCR's RANDOM UNRELATED HUMAN MALE POPULATION

KUMAR A[1*], SINGH N[1#], GAURAV SS[2]

1: Department of Biotechnology, Mewar Institute of Management, Ghaziabad, U.P-201012

2: Dept of Biotechnology, Choudhary Charan Singh University, Meerut-U.P

***Corresponding Author: E mail: ajaykmr1986@gmail.com, #neetu.avni@gmail.com**

ABSTRACT

Being the Industrial and residential centre, National Capital Region (NCR) attracts migrant's population from different parts of the country, making the population a heterogeneous one. However any polymorphism study in the random population gives an idea of mixing of population and the polymorphism variation among them, if their antecedents are properly verified. Humans have approximate 3164.7 million nucleotide bases in their genomic DNA. Most human identity testing is performed using markers on the autosomal chromosome and the gender determination is done with markers on the sex chromosomes. The fundamental basis of DNA polymorphism in a population is variation of the nucleotide sequence at homologous location in the genome due to single nucleotide mutations or deletions and inversions of variable number of contiguous nucleotides. The loci chosen for forensic use generally have tandem repeat units of 2 to 6 base pairs and number of alleles present in the population varies from about 5 to 20 depend upon loci. STR loci are detected as discrete alleles and thus can be compared directly to an allelic ladder run on the same gel, simplifying comparison and analysis. The Y-chromosome is becoming a popular method for male identification in forensic situations as it improve the chance of detecting, low levels of committer's DNA in a high background of female victim's DNA in case of sexual assault. Range of Y-STRs used are DYS 19, DYS 385, DYS 388, DYS 434, DYS 435 etc. Study on over 200 unrelated individuals using DYS-19 STR markers, the variation in R_f was found to be between 0.642 and 0.927. The variation in molecular

weight was found to be 58.08 and 78.34. The locus shown sufficiently polymorphic and can be used for forensic applications.

Keywords: DNA, Genome, Mutation, Polymorphism, STR, Y-Chromosome

INTRODUCTION

Indian population, comprising of more than a billion people, consists of more than 4000 communities with several thousands of endogamous groups, over 300 functioning languages and 25 scripts. To address the questions related to racial diversity, founder populations, migrations or pharmaco-genomics, one needs to understand the diversity and relatedness at the level of genes in such a diverse population. Molecular genetic evidence supports the fact that a major population expansion of modern humans took place within India **[1]**. Indian population can be, to a large extent, sub structured on the basis of their cultural origin as well as dialectal lineages. To allow accurate estimation of ancestral sizes, it therefore, becomes domineering to determine the extent of genetic variability and affinity within and between the Indian subpopulations as well as their affinity to other worldwide populations. Thus, the analysis of sequence variation through SNPs and VNTRs in the context of genomic diversity would help in identifying genetic substructures **[2]**.

DNA Polymorphism

The fundamental basis of genetic polymorphism in a population is variation of the nucleotide sequence of DNA at homologous locations in the genome. These differences in sequence can result from mutations involving a single nucleotide or from deletions or insertions of variable numbers of contiguous nucleotides. Allelic variants can occur anywhere in the genome (i.e. Introns or Exons) **[3]**. Different types of Genetic Polymorphisms are-

Single-Nucleotide Polymorphisms (SNPs) are allelic variants generated as the result of conversion of a nucleotide to another at a homologous position. When present within a exons, the expressed product may or may not have a single amino acid difference depending on the resulting codon change.

Deletion or Insertion Mutations

The consequence of a deletion depends upon the precise location of the deletion; whether it produces a nonsense frame shift; and whether

it alters the function of the expressed product **[4]**.

Short Tandem Repeat (STR) Polymorphism

Short sequences of two to four base pairs at a given location can be duplicated back-to-back a specific number of times and inherited as a genetic variant **[5]**.

METHODOLOGY

Detection of Genetic Polymorphism

SNPs can easily be detected, with a high degree of specificity and sensitivity using DNA-based genotyping methods. The assays depend on amplification of the polymorphic locus in question to produce sensitivity in the setting of a background of sample genomic DNA. Specificity is ensured by using tailored oligonucleotides that are complementary to the DNA sequence of the allele one wants to detect.

Polymerase Chain Reaction (PCR)

One strategy for typing is to use Polymerase Chain Reaction to amplify a segment of DNA that includes the polymorphic position and a moderate amount of flanking DNA on both the 3' and 5' sides of the polymorphic position. This is done with primers that are complementary to conserved sequences in either side of the desired segment to be amplified. This yields an amplicon of known size that contains inherited alleles and is present in an amount that can be tested for the presence of specific alleles without significant interference from genomic DNA. The amplicon can then be probed, using a set of radiolabeled oligonucleotides, each of which is complementary to the DNA sequence of one of the possible alleles **[4] [6]**.

Site Specific Priming (Ssp)

Another strategy for SNP typing is called Site Specific Priming**,** for detection of SNPs; one can craft a set of PCR primers that are complementary to the alleles to be detected, with the terminal 3A base of one of the primers located at the polymorphic position. The second PCR primer is usually complementary to a conserved segment of DNA and positioned to yield a product of a convenient size. If an allele is present, use of the appropriate set of primers will produce an amplicon. The amplicon can be separated from genomic DNA by simple agarose gel electrophoresis and identified by ethidium bromide staining under ultraviolet light, and the expected size can be confirmed **[6] [7]**.

Short Tandem Repeats (STRs)

The loci chosen for forensic use generally have tandem repeat units of 2 to 6 bp and maybe repeated from a few to dozens of times. The number of alleles present in the population varies from about 5 to 20 depending on the locus **[8]**. STR loci are detected as discrete alleles and thus can be compared directly to an allelic ladder run on the same gel, simplifying comparison and analysis. The size of the DNA fragment produced by the amplification of the STR loci is in the range of about 200 to 500 bp. This makes STR's an ideal choice for degraded DNA. STR loci may be detected and analysed manually using a silver stain **[9]**. The analysis of the human Y-chromosome DNA variation is proving to be a useful genetic tool for the reconstruction of recent human population history through paternal lineages. This is due mainly to its high mutation rates and its absence of recombination during meiosis. Among the Y-chromosome molecular-markers, DYS19 and YAP (Y-Alu polymorphism) have been analysed in detail from a population genetics approach. The ability to separate and identify the male component of a mixture is valuable for many forensic situations. Using Y chromosome specific primers can improve the chance of detecting low levels of the committer's DNA in a high background of female victim's DNA **[10-12]**.

RESULTS AND DISCUSSIONS

The complete procedure for the study is given as:

1. The blood samples were collected from random male population of NCR region.
2. The DNA was isolated from these blood samples using Phenol Chloroform genomic DNA isolation method.
3. This DNA was visualized on the Agarose gel **(Figure 1)**.
4. After this, the DNA samples were amplified by PCR using forward and reverse primers **(Figure 2)**

   5' – GTTATATATATATAGTGTTTAG-3' FORWARD

   5' – GTTAAGGAGAGTGTCACTA-3'-REVERSE [13];
5. The quality of the PCR products was checked on PAGE **(Figure 3, 4, 5, 6) (Table 1, 2)**.
6. The results of PAGE were used to determine the R_f values and Molecular

weight of the PCR products **[6, 14]**. **(Table 1-4)**.

Agarose Gel Pictures

All samples contain a fair amount of DNA except samples 1, 9 and 10. The feeble bands are due to random human error in the loading of the samples into the gel. All samples were hence further processed by PCR by applying primers specific to the DYS 19 locus **(Figure 1)**.

All samples showed a fair amount of DNA except sample number 1 and 16. All samples were hence preceded for PCR procedures **(Figure 2)**.

Figure 1: Agarose Gel Pictures

Figure 2: PCR Results

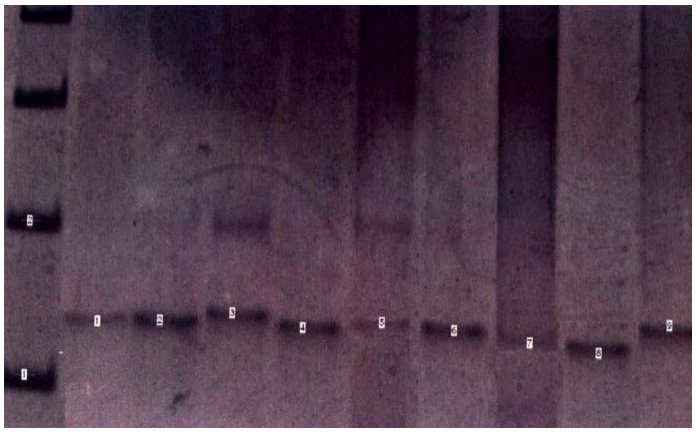

Figure 3: Poly Acrylamide Gel Electrophoresis

Table 1: PAGE data

MARKERS			
BAND	**POSITION**	**MOLECULAR WEIGHT**	**R_f-VALUE**
1	**753**	**50**	**0.782**
2	**612**	**100**	**0.643**
QURIES			
BAND	**POSITION**	**MOLECULAR WEIGHT**	**R_f-VALUE**
1	**700**	**66.08**	**0.684**
2	**690**	**64.24**	**0.694**
3	**678**	**69.73**	**0.682**
4	**697**	**65.22**	**0.668**
5	**694**	**67.35**	**0.686**
6	**702**	**64.70**	**0.684**
7	**708**	**62.35**	**0.698**
8	**714**	**60.50**	**0.702**
9	**696**	**65.80**	**0.679**

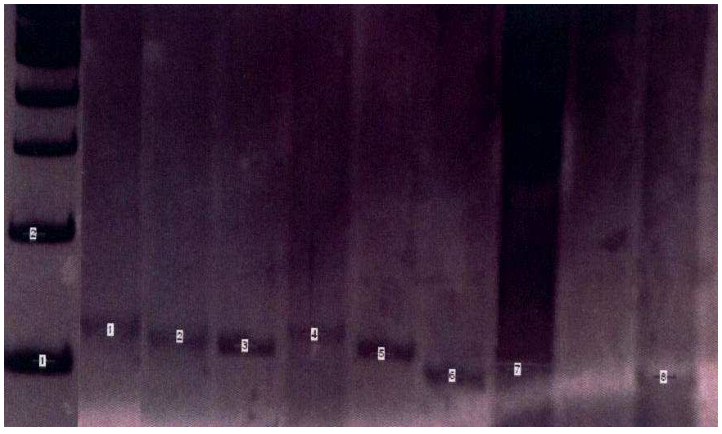

Figure 4: Poly Acrylamide Gel Electrophoresis

Table 2: PAGE data

MARKERS			
BAND	**POSITION**	**MOLECULAR WEIGHT**	**R_f-VALUE**
1	**872**	**50**	**0.938**
2	**746**	**100**	**0.718**
QURIES			
BAND	**POSITION**	**MOLECULAR WEIGHT**	**R_f-VALUE**
1	**798**	**68.20**	**0.764**
2	**790**	**69.32**	**0.782**
3	**814**	**64.66**	**0.781**
4	**790**	**71.68**	**0.766**
5	**812**	**60.30**	**0.788**
6	**822**	**60.80**	**0.790**
7	**820**	**59.91**	**0.770**
8	**830**	**59.88**	**0.798**

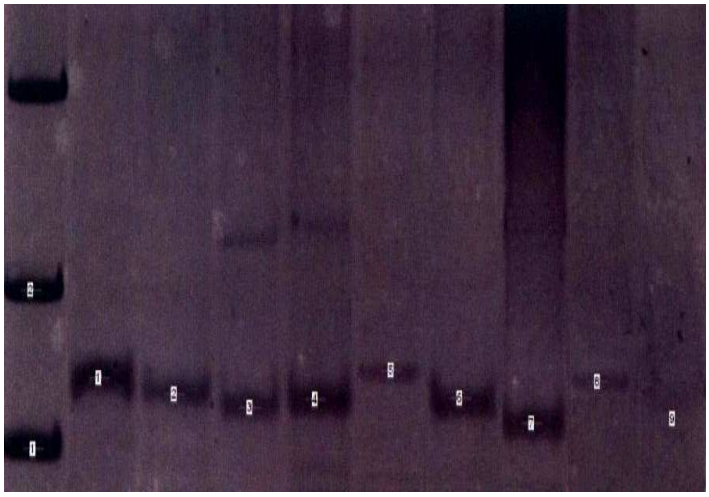

Figure 5: Poly Acrylamide Gel Electrophoresis

Table 3: Shows PAGE data represented in Figure 5

MARKERS			
BAND	**POSITION**	**MOLECULAR WEIGHT**	**R_f-VALUE**
1	**742**	**50**	**0.722**
2	**626**	**100**	**0.618**
QURIES			
BAND	**POSITION**	**MOLECULAR WEIGHT**	**R_f-VALUE**
1	**684**	**69.64**	**0.656**
2	**692**	**65.21**	**0.672**
3	**710**	**61.56**	**0.683**
4	**696**	**64.29**	**0.679**
5	**686**	**71.32**	**0.664**
6	**688**	**65.24**	**0.675**

7	**714**	**56.04**	**0.691**
8	**690**	**70.04**	**0.670**
9	**722**	**58.77**	**0.689**

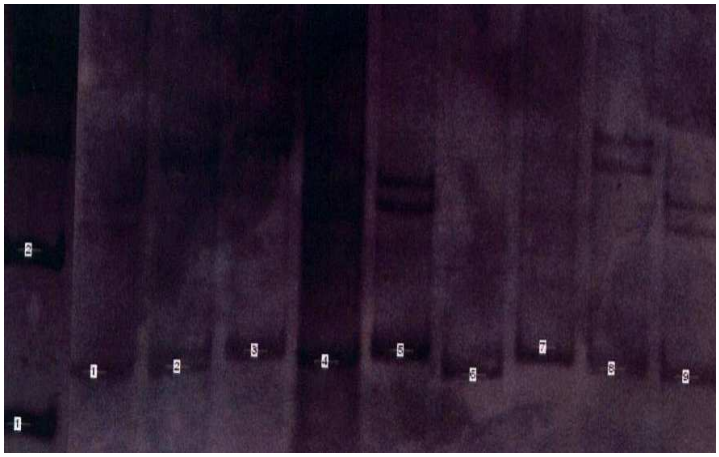

Figure 6: Poly Acrylamide Gel Electrophoresis

Table 4: Shows PAGE data represented in Figure 6

MARKERS			
BAND	**POSITION**	**MOLECULAR WEIGHT**	**R_f-VALUE**
1	**752**	**50**	**0.780**
2	**630**	**100**	**0.628**
QURIES			
BAND	**POSITION**	**MOLECULAR WEIGHT**	**R_f-VALUE**
1	**698**	**68.62**	**0.686**
2	**628**	**67.32**	**0.684**
3	**668**	**75.71**	**0.652**
4	**680**	**71.92**	**0.682**
5	**682**	**80.17**	**0.660**
6	**688**	**72.77**	**0.662**
7	**664**	**81.17**	**0.640**
8	**688**	**77.64**	**0.664**
9	**680**	**74.78**	**0.660**

CONCLUSION

- NCR attracts migrant population from all parts of the country making the population a heterogeneous one. Hence, any polymorphism study in the random population gives an idea of the mixing of populations and the polymorphism variation among them if their antecedents are properly verified.
- The variation in R_f was found to be between 0.638 and 0.938.

- The variation in molecular weight was found to be 56.04 and 81.17.
- The locus shows sufficiently polymorphic and can be used for forensic applications like DNA fingerprinting for individual identification and paternity testing.

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