Acta Biologica Hungarica 53 (4), pp. 495–498 (2002)

DNA PROFILING BY DETECTION OF REPETITIVE NUCLEOTIDE SEQUENCES ON HUMAN CHROMOSOME 6*

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(Received: June 5, 2002; accepted: July 1, 2002)

Genetic/genomic polymorphism, i.e. variations in DNA sequences are ideally assayed by direct nucleotide sequencing of a gene region or other homologous segment of the genome. An easier and cheaper approach, however, if the variants are analyzed by hybridization technology using restriction fragment length polymorphisms (RFLPs) or by *detection of the number of tandem repeats* (VNTR) of small DNA segments, the "minisatellites". In this study we describe results of the DNA analysis of repetitive sequences of human 6th chromosome by the application of a chemiluminescent labeled probes. The allele frequency distribution of polymorphic DNA sequences has been determined in unrelated individuals. The isolated genomic DNA was cut with Pst I restriction enzyme, size fractionated on agarose gel and hybridized with a chemiluminescent labeled D6 S132 probe. At this locus the Pst I cleaved DNA fragments are ranging from 1841 to 6098 base pairs (bp).

Specific genetic pattern was characterized by *more frequent fragments* (3313 and 3884 bp), *and the rarely occurring ones* (clustered between 1841–2595 and 5227–6098 bp). Our study provides a further possibility for characterization of individual genomic patterns.

Keywords: Gene technology – forensic medicine – RFLP – VNTR – chemiluminescent probe

INTRODUCTION

Human genome map and gene sequences became recently widely available [8]. The total DNA content of a single human somatic cell is approximately 3×10^6 kb, weighs some 6 picograms, has a contour length of about 2 m. The human genome is estimated to contain about 25–40,000 genes [7] with an approximate average length of 5–10 kb including non-coding introns. Spacer DNA, signal sequences, protein coding DNA, unique sequences account for approximately 70–80% of the total human genome. The remaining 20–30% of the human genome consists of repetitive, primarily non-coding sequences of uncertain function. Repetitive DNA can be divided into two classes: tandemly repeated sequences [6] and interspersed repeats [2]. One form of repetitive DNA is called as *satellite* DNA. The repeated units range in length

* Dedicated to Professor György Ádám on the occasion of his 80th birthday.

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from 5 to 250 base pairs and they are repeated many thousand times in tandem arrays. In humans there are several classes or families of satellite DNAs. A minor fraction of tandemly repeated DNA occurs as short tandem repeats. One of these regions termed *minisatellites* or *VNTRs* (variable number of tandem repeats) consists of variable length (between 9–64 base pairs) so-called "core sequences" in tandem arrays at unique chromosomal loci. The length of the region depends how many times the core sequence is repeated.

The largest class of repetitive DNA consists not of tandem arrays, but individual units interspersed with longer tracts of single copy DNA: these elements account for as much as 20% of the total DNA. This class has two fractions: one of two classes is "short interspersed elements" *(SINES),* and the another is "long interspersed elements" *(LINES)* [2, 4, 6].

There are two distinct approaches to the analysis of genetic variation, at the DNA level: the detection of restriction fragment length polymorphism *(RFLP)* and the use of the polymerase chain reaction *(PCR)* to amplify specifically the gene sequence.

The DNA fragment patterns (RFLP or VNTR) are used to solve many problems of human genetics [8], including disputed paternity, individual identification of blood or other forensic biological stains [3, 4, 7], linkage analysis of inherited diseases, zygosity testing and bone marrow transplantation [9].

In this study RFLP analysis was performed using chemiluminescent labeled single locus probe from human 6th chromosome.

MATERIALS AND METHODS

Isolation and purification of DNA

DNA was prepared from peripheral blood samples collected from random, unrelated blood donors. High molecular weight DNA was isolated by salting out procedures using lysis buffer (0.155 M NH_4Cl, 0.01 M $KHCO_3$, 0.001 M EDTA, pH 7.4) followed by proteinase K digestion and isopropanol/ethanol precipitation. The dried DNA was dissolved in distilled water at 4 °C. The ratio between the readings at 260 nm and 280 nm (OD_{260}/OD_{280}) provided an estimate of the purity of the nucleic acid [7, 9].

Digestion of genomic DNA with restriction endonuclease and blotting

DNA was digested with Pst I restriction endonuclease (Sigma), using at least 2 U Pst I/μg of DNA (according to the manufacturer's instruction). Samples were digested overnight at 37 °C, and was stopped with cooling to –20 °C. DNA fragments were transferred into nylon membranes, then immobilized by drying, vacuum-baking and cross-linking by UV.

Hybridization of labeled probe to specific DNA sequences

The membranes were treated by labeled D6 S132 probe (0.5–1.0 µl per ml hybridization solution). The signal intensity was tested in the presence of LumiPhos® 480 substrate (Lifecodes Corporation). Subsequent exposure of the nylon membrane applying LumiPhos® to an X-ray (Polaroid) film resulted in a pattern of bands.

The size of the alleles, detected with probe, was calculated by measuring their mobility relative to that of DNA fragments of known molecular weight.

The allele frequency was calculated by regular way without further statistic analysis.

RESULTS

Using DNA digests of 19 independent persons typical RFLP patterns were obtained with the chemiluminescent D6 S132 probe. Table 1 illustrates results of our survey. The table shows bands in the size-range from 1841 to 6098 kb. The highest frequencies were found at 3884, 3313 and 2549 kb, the lowest were between 1481–2195, 5227–6098 and at 2918 and 3620 kb.

In this study the distribution of Pst I-fragments detected by chemiluminescent labeled D6 S132 probe was shown. Our studies provided evidences for the advantageous usage of non-isotope labeled single locus probe for VNTR analysis. This type

Table 1
Size distribution and frequency
of DNA fragments binding D6S132 probe

Size (kb)	Frequency (D6S132 probe)
6.098	0.026
5.707	0.026
5.227	0.026
4.830	0.052
4.514	0.052
4.205	0.08
3.884	0.34
3.620	0.026
3.313	0.16
3.084	–
2.918	0.026
2.739	–
2.549	0.1
2.386	–
2.195	0.026
2.019	0.026
1.841	0.026

of detection of DNA polymorphisms represents a powerful technique for the identification of individuals. This approach is highly useful many hereditary diseases and also in forensic medicine since even the minor individual differences could be detected.

DISCUSSION

Variable number of tandem repeat (VNTR DNA) markers are contributing as a new potential to human genetic studies because their hypervariable nature allows individualization at the DNA level. In the course of RFLP analysis the size of the alleles was calculated by measuring their mobility relative to that of DNA fragments of known size. It is reasonable to size each band accurately by including a standard molwt ladder on each test, and to cross-compare the size of each band between profiles.

Using the database, the frequency of occurrence of each band within the population is established. It is necessary to generate a database for each population, since distribution of the alleles detected by any given probe may vary significantly between members of different ethnic groups [1, 5].

Because of the capacity of DNA profiling in identifying different origins of human tissues, it is of enormous utility both to crime investigators and to legal counsel. Moreover DNA profiling has application to a number of civil disputes involving paternity [5, 10]. Due to ethnic differences before application for criminal or paternity assays each probe should be tested for population distribution.

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