In normal individuals several variations are seen in the genomic DNA sequence which are called polymorphisms. In individuals with genetic disorders, variations in the form of substitutions, deletions or insertions are seen at crucial positions in the disease gene, which ultimately affect the final expression of the gene and are called mutations. The most recent revolution in biology has been the development of the recombinant DNA technology. Discovery of restriction endonucleases and techniques like Southern blotting and cloning have helped to study the defects in the disease gene at molecular level. Using these approaches several human genes of medical interest have been isolated and their function in an in vitro system has been studied. This has helped to elucidate molecular mechanisms responsible for complex processes like cell growth and metabolism. These developments have also enabled the pre- or postnatal diagnosis of genetic diseases as well as in determining an individual’s predisposition to multifactorial conditions such as diabetes or coronary artery disease.

The haemoglobinopathies constitute one of the most common autosomal disorders in India. Besides cancer, haemophilia and glucose-6-phosphate dehydrogenase (G 6 PD) deficiency also pose significant public health problems in our country. Characterization of mutations is one of the important aspects in the study of these haematological disorders. Several powerful and sensitive molecular techniques are available to diagnose these disorders. This write-up discusses the application of various techniques to detect the molecular lesions in the hereditary haematological disorders.

**APPROACHES FOR GENE ANALYSIS**

**Specific Detection of the Desired DNA Fragment by Hybridization**

This involves bringing together a labelled single stranded DNA or RNA (probe) with single stranded test DNA and allowing the complementary strands to anneal. A DNA probe is a piece of DNA labelled either with radio-isotopic molecules like $^{32}$P or $^{35}$S or with a non-isotopic molecule like biotin, which is used in hybridization assays to identify other DNA or RNA sequences (target sequences) which are closely related to it in base sequence. DNA probes may be single or double stranded molecules but the working probe must be single stranded. Double stranded DNA probes are denatured by heating before use. If the target sequences are double stranded, they must also be denatured before hybridization.

The DNA probe can be isolated by DNA cloning (length about 0.1-45 kb) or single stranded DNA can be
synthesized chemically (length 15-50 nucleotides). A good probe should form stable duplexes with target sequences from the heterogenous mixture of many sequences in genomic DNA under appropriate conditions. It should not hybridize to other regions within the template and it should not anneal to itself. A special computer programme is available for selecting the sequence of a good probe.

Heteroduplexes formed between the probe and test DNA are very stable due to a high degree of base complementarity. Generally, target DNA is fixed on a solid support like a nitrocellulose or nylon membrane. Excess probe is removed by washing and heteroduplexes between probe and target DNA are detected either by autoradiography using an X ray film or by a colour reaction.

Detection of large deletions

Southern blot hybridization

This protocol was developed by Southern in 1975. In this technique the test DNA is digested with a restriction enzyme, size fractionated by agarose gel electrophoresis, denatured and transferred to a nitrocellulose or nylon membrane for hybridization. The immobilized single stranded target DNA sequences are allowed to associate with labelled single stranded DNA probe. Their position on the membrane can be related back to the original gel in order to estimate their size. A considerable degree of mismatching can be tolerated if the overall region of base complementarity is long (>100 bp). Therefore, it cannot be used to detect point mutations or small insertions or deletions.

Northern blotting uses the same principle but uses RNA as a target sequence. It is used to examine the stability of mRNA and to measure the length of the mRNA transcript.

Detection of point mutations and small insertions or deletions by hybridization

Dot blot hybridization using allele specific oligonucleotide probes

A specific segment of DNA is amplified by PCR. The amplified product is dot blotted and immobilized on a nitrocellulose or nylon membrane. A total of 96 samples can be checked at a time using a special apparatus. Two oligoprobes (18-20 bases long) are synthesized for detection of each mutation, one complementary to the normal sequence and the other to the mutated sequence. They are labelled with either radioactive molecules or are covalently attached to an enzyme like horse radish peroxidase. Specific probes are synthesized for different mutations. Stringent hybridization conditions are employed so that the DNA duplex between probe and target is only stable if there is perfect base complementarity between them. A single mismatch between probe and target sequence is sufficient to render the short heteroduplex unstable. Detection is done either by autoradiography or by using a colour reaction. The same membrane can be stripped and rehybridized with different allele specific oligonucleotides (ASOs) to detect various mutations/alleles in target DNA. This approach has been applied to detect the sickle cell gene and -thalassaemia mutations.

Covalent reverse dot blot hybridization

The target DNA is amplified with a biotin group attached to the 5-end of one of the primers. Specific probes for different mutations are synthesized with an amino group attached to the 5-end of the probes. Several probes are immobilized onto a solid support like a biodyne C transfer membrane which bears a carboxyl group, to covalently bind to the amino group of the probe. Optimization of conditions for hybridization with several probes in a single step is crucial. Once this is achieved, biotin labelled DNA from the duplex can be detected via a streptavidin-alkaline phosphatase conjugate and chromogenic substrate. A major advantage of this protocol is that several mutations can be detected in a single hybridization. This method has been used for detection of mutations in -thalassaemia.

Oligo cleavage detection using hybridization

The probe may provide a restriction site when hybridized to one of the strands of the amplified DNA. After restriction digestion of the complex, the length of the released fragment of the radiolabelled probe can be visualized after polyacrylamide gel electrophoresis (PAGE) and autoradiography. The size dependent migration of the fragment will demonstrate whether or not a restriction site is present in the sequence of the amplified product. This has been applied to detect the sickle mutation.

Use of oligonucleotide microchips for hybridization

Recently, light directed chemical synthesis has been used to generate miniature high density arrays of oligonucleotide probes. A microchip is defined as an array of such oligonucleotides immobilized into gel elements fixed on a glass plate. Hybridization of this microchip is carried out with fluorescently labelled amplified DNA from an individual. This reaction is monitored.
simultaneously for all microchip elements with a two wavelength fluorescent microscope equipped with a charge coupled device camera. Sequencing by hybridization to oligonucleotide microchips (SHOM) has been used to detect b-thalassaemia mutations in individuals by hybridizing PCR amplified DNA with the microchips. This technique allows the study of specific hybridization using several hundred oligonucleotides simultaneously. It is highly sensitive with a low background, however, the resolving power is low. Short arrays (4-8 mers) are useful for detecting mutations but long arrays (25 mers) may give false positive results.

Restriction fragment length polymorphism

Restriction endonuclease analyses has expanded our knowledge of the molecular structure and arrangement of certain genes. It has revealed variations in DNA sequence using indirect or direct approaches and these variations provide a new class of markers for genetic analysis.

Indirect detection of mutations by linkage analysis

Kan and Dozy first reported an association of the sickle mutation with a 13 kb HpaI fragment containing the b-globin gene. They subsequently found an association between a 9.3 kb BamHI fragment and b-thalassaemia in Sardinia. This is called restriction fragment length polymorphism (RFLP). If two alleles lie on the same chromosome, there is a fair possibility that they will be inherited together. The closer they are on the same chromosome, the greater the chance of staying together at meiosis and co-segregation. If they are very far apart on the chromosome, the chances of recombination are so rare that they may not appear to be linked at all. Of these two alleles, one is the restriction site for a specific enzyme which is called the marker allele while the other is the disease gene. Family studies are required to study the linkage pattern in all members. Orkin and co-workers found an association between specific b-thalassaemia mutations and a particular pattern of seven restriction enzyme cleavage sites in the b-globin gene designated as haplotype. These earlier studies were done using Southern blot hybridization.

More recently, RFLP analysis for b-thalassaemia is being done by amplifying different regions of the b-globin gene cluster containing polymorphic restriction endonuclease sites by PCR, digesting the amplified DNA with the respective enzyme and running on an agarose or acrylamide gel to look for the presence or absence of the particular site. This approach has also been used for haemophilia A, where extragenic markers like St-14 are also useful.

Direct detection of mutations by restriction endonuclease analysis

Certain mutations can create or abolish a restriction enzyme recognition site. This makes direct detection of the mutation possible after digestion of the amplified DNA followed by electrophoresis. Some abnormal haemoglobins and b-thalassaemia mutations can be detected in this way.

HbS b(GAG → GTG) abolishes a site for Mst II
HbE b(GAG → AAG) abolishes a site for Mnl I
IVS II - 745 (C→G) creates a site for Rsa I
IVS I-1 (G→T) abolishes a site for Bsp MI
- 88 (C→T) creates a site for Fok I
Mutations causing G6PD deficiency can be detected in a similar way.

G6PD Mediterranean creates a site for Mbo II
G6PD Orissa abolishes a site for Hae III
G6PD Kerala – Kalyan abolishes a site for Mnl I

Selective Amplification of the Desired DNA Fragment

This can be achieved in vivo by cloning though it is time consuming. With the advent of the polymerase chain reaction (PCR), effective amplification of specific regions of genomic DNA can be done within 2 to 3 hours using a thermal cycler. Primers or synthetic oligonucleotides (15-20 bases in length) flanking the target sequence to be amplified, hybridize to opposite strands of the target sequence. They are oriented with their 3' ends directed towards each other. Repeated cycles of heat denaturation of the template, annealing of the primers to their complementary sequences and extension of the annealed primers with a DNA polymerase results in the amplification of the segment defined by the 5' ends of the PCR primers. Since the extension product of each primer can serve as a template for the other primer, each cycle essentially doubles the amount of the DNA fragment produced in the previous cycle. This results in an exponential accumulation of the specific target fragment. Therefore, currently PCR is the first step in most of the mutation detection techniques.

Detection of deletions by PCR

PCR electrophoresis

A set of specific primers are synthesized to cover the area of the deletion. The presence of the mutation is
indicated by amplification of a smaller fragment. The 619 bp deletion, one of the common Indian \( b \)-thalassaemia mutations can be detected in this way.

**PCR-HPLC**

This strategy has been used to detect heterozygotes with gene deletions involving target sequences from haptoglobin, Factor IX and the Duchenne muscular dystrophy gene\(^{19} \). Target sequences are amplified with specific pairs of primers and amplified products are analysed by high performance liquid chromatography (HPLC).

**Analysis by manipulating PCR conditions**

It has been observed that modification in the sequence at the 5\( \_\)end of a primer does not affect the yield of PCR but the sequence at the 3\( \_\)end is very crucial; modification at that end affects the yield of the reaction considerably. Several approaches for mutation detection are devised based on these properties.

**Amplification refractory mutation system**

Allele specific primers are designed such that the nucleotide at the 3\( \_\)end of each primer is complementary to the change of DNA sequence caused by the mutation that is being looked for\(^{20} \). To enhance their specificity, a deliberate additional mismatch is introduced at position-4 from the 3\( \_\)end. Each of these primers is tested with positive and negative control DNA samples under uniform stringent PCR conditions to ensure that successful amplification occurs only in the presence of the mutation that is being looked for. In some instances, despite the additional deliberate mismatch near the 3\( \_\)end, false amplification may occur. It is then necessary to alter the additional mismatch or even reverse the direction of the primer to obtain specificity. This strategy has been used to characterize \( b \)-thalassaemia mutations\(^{18} \).

**Mismatch PCR**

Mutation specific primers are designed to either create or abolish a restriction enzyme site in the presence of the mutation. The amplified product is treated with the respective enzyme and electrophoresis is run to detect fragments of specific sizes. This has been applied to detect point mutations in the Kirsten ras oncogene\(^{23} \) and \( b \)-thalassaemia\(^{24} \).

**Single nucleotide primer extension**

DNA from the region of interest is amplified with a specific pair of primers and purified by gel electrophoresis to remove excess of dNTPs and primers. One more short primer is synthesized. It is designed in such a way so that it ends at the nucleotide immediately 5\( \_\)to the point mutation, which is to be detected in purified DNA. Using this primer, two separate primer extension reactions are performed in the absence of any free, unlabelled dNTPs. In the first reaction, radiolabelled wild type dNTP is added while in the other, radiolabelled dNTP corresponding to the mutant sequence is added. The primer will be extended by one nucleotide and radiolabelled only if the appropriate template sequence is present. The PAGE and autoradiography detect the radiolabelled extended primer. This is also called mini sequencing\(^{25} \).

**Analysis based on heteroduplex formation**

**Chemical mismatch cleavage**

This protocol detects mismatched bases in hybrid duplexes formed between wild type and mutant DNAs\(^{26} \). The PCR product derived from the mutant gene is denatured and reannealed in a 10:1 ratio to uniformly radiolabelled homologous sequences called probes. Mutations in the target strand give rise to mispaired C or T residues in the heterodimers formed. Mispaired residues are specifically modified by hydroxylamine (for C mismatches) and osmium tetroxide (OsO\(_4\); for C and T mismatches). The sites of these mismatches are then cleaved by treatment with piperidine and reaction products are detected by autoradiography after denaturing PAGE. This method is very sensitive, detecting more than 95\% of mismatches when the probe is prepared from the wild type of DNA only and 100\% when the probe is prepared from both, wild type and mutant DNA. Using this technique one can scan up to a 1700 bp fragment of DNA. The precise localization and nature of the change is also indicated by the size of the cleavage band and the cleaving agent. The limitations are that deletions and insertions are detected indirectly to some extent. The method is labour intensive,
time consuming and uses toxic chemicals like OsO₄. Recently, potassium permanganate and tetraethyl ammonium chloride have also been tried as efficient substitutes for OsO₄. This method has been applied to identify molecular lesions in β-thalassaemia²⁷, Factor IX gene²⁸ and Factor VIII gene²⁹. One of the disadvantages of chemical mismatch cleavage (CMC) is that mismatched G and A bases cannot be detected directly but only indirectly via C and T mismatches; hence both sense and antisense probes must be used. Recently this has been applied in its most precise format on the automated sequencers with sense and antisense strands labelled with a fluorescent dye in both heteroduplexes. It is called fluorescent assisted mismatch analysis (FAMA)³⁰.

Heteroduplex generator molecules

Synthetic DNA heteroduplex generator (DHG) molecules mimic genomic DNA sequences but contain controlled nucleotide substitutions, deletions or insertions (collectively called identifiers) at nucleotide positions opposite to and contiguous with known mutation sites within the genomic DNA. Depending on the number, type and position of the mismatches, unique heteroduplexes are generated between different allelic PCR products and the DHG which migrate at different rates on 15% PAGE. This gives rise to specific banding patterns for the particular mutations. This approach can detect sickle cell disease³¹. Savage and co-workers³² constituted three DHGs to detect five common and six rare β-thalassaemia mutations in the Singapore population.

RNase A cleavage

The SP6 system is used to prepare a radioactive colinear RNA probe (ie probe transcribed from cloned genomic DNA) in vitro. This is mixed with double stranded DNA from the patient. RNA: DNA heteroduplexes are generated either by heating and reannealing or by PCR. When they are treated with RNase A, it removes 5’ and 3’ overhangs of the probe and cleaves the probe strand at the position of the mismatch due to the RNA probe appearing to be single stranded at these positions. The reaction is analysed by electrophoresis and autoradiography. The presence and location of a mutation is indicated by a cleavage band of a given size³³. It has been used to detect the sickle mutation³⁴. This method has limited value as it requires radiolabelling and it can detect only about 50% of mismatches. At the same time it requires many contiguous RNA probes from cloned genomic DNAs to span the length of a suspect gene.

Exon scanning technique

This strategy uses RNA probes derived from cDNA templates (cRNAs) to detect lesions in suspect genes. The cRNA probes form heteroduplexes with the exons of the target gene, while the introns which may constitute most of the gene, loop out as single stranded DNA. Therefore, RNase A can be used to scan an entire coding region composed of exons scattered over many kilobases for point mutations³⁵. This technique is similar to the RNase cleavage method. It can detect single base substitutions and deletions but not single base insertions. It has been employed to detect mutations in β-thalassaemia.

4 Ligase chain reaction

The enzyme DNA ligase covalently joins two synthetic oligonucleotide sequences selected so that they can base pair with a target sequence in exact head to tail juxtaposition. Ligation of the two oligomers is prevented by the presence of mismatched nucleotides at the junction region. This procedure allows for the distinction between known sequence variants in samples of cells without the need for DNA purification. The joining of the two oligonucleotides may be monitored by immobilizing one of the two oligonucleotides and observing whether the second labelled oligonucleotide is also captured.

Using this principle, a technique was developed to detect the sickle mutation³⁶. Two pairs of oligonucleotides complementary to each target strand and which can bind to target DNA immediately flanking the sickle mutation are synthesized. When these four oligonucleotides and target DNA are incubated with Taq ligase for several cycles, matched oligonucleotides will join at their ligation site and will be amplified. Two oligonucleotides flanking the sickle mutation will show negligible ligation. The oligonucleotides can be labelled with a fluorescent dye. The signal from correctly paired ligation products is 50-500 fold higher than that from mismatched products.

Methods based on physical characters of DNA

Single strand conformational polymorphism

Wild type and mutant DNA are amplified. During PCR they are radiolabelled. Both the samples are denatured before loading on the gel and then electrophoresed side by side through a non-denaturing PAGE. The bands are visualized by autoradiography. Single stranded DNA in a non-denaturing condition, has a folded structure that is
determined by intramolecular interactions. These sequence
based secondary structures affect the mobility of the DNA
during electrophoresis in non-denaturing PAGE. A DNA
molecule containing a mutation will have a different
secondary structure than the wild type resulting in a
mobility shift during electrophoresis when compared to
the wild type37. PCR products with altered migration can
be analyzed by DNA sequencing to determine the exact
nature of the mutation. Visualizing the bands with silver
staining, automation by using fluorescent labelled primers,
multiplexing, restriction enzyme digestion of a longer
amplification product prior to electrophoresis38 and
asymmetric PCR-single strand conformational polymorphism (SSCP)39 are some of the improvements
of this technique. The sensitivity of this technique is 70-
95% if the DNA fragment is <200 bp long; it decreases
with the size of the PCR product. It is about 50% if the
DNA fragment is about 400 bp. The sensitivity depends
on gel matrix, gel additives like glycerol, the dimensions
of the gel, electrophoretic conditions like temperature,
ionic strength, acrylamide concentration, type and position
of the mutation, sequence composition, surrounding
mutations and concentration of primers during PCR.
Sometimes the mutations are missed due to formation of
loops within single stranded DNA. Although this is a
simple technique, many variables make it difficult to apply.

It has been applied to detect mutations in N-ras, K-ras, H-ras oncogenes37, Factor IX gene40, b-thalassaemia41,
a-thalassaemia42 and G6PD deficiency43.

RNA SSCP analysis

RNA is used instead of DNA and is generated by T7
RNA polymerase transcription from a PCR amplified
DNA fragment. Sense and antisense RNA strands yield
different conformational patterns. This method is more
efficient than SSCP. Scanning nine overlapping frag-
ments of the Factor IX gene, ranging from 180-497 bp
in a prospective manner has helped to detect 35% of mu-
tations by SSCP. When the same fragments were scanned
by rSSCP, 70% of mutations were detected.

Denaturing gradient gel electrophoresis (DGGE)

Stretches of DNA ranging from 30-300 bp are called
domains. The change in the structure of DNA from an
orderly helix to a disordered, unstacked structure without
base pairs is called melting. The temperature at which
50% of the DNA of a particular domain melts is called
the melting temperature of the domain. When a DNA
molecule is allowed to travel in an acrylamide gel, if the
temperature of electrophoresis or concentration of
denaturant (like formamide or urea) is raised, DNA starts
melting. This melting is not continuous but stepwise.
Discrete fragments of DNA which have different melting
temperatures undergo melting in a predictable manner.
The mobility of a particular fragment is retarded considerably once it reaches the melting temperature of
that fragment44.

Pre-requisites for DGGE:

Before analysing target DNA, one should know its
complete sequence. Using this, two computer programmes
viz. SQHTX and MELT-87, must be run to obtain the
following information which is required to carry out
electrophoresis.

(i) The melting profile of target DNA.
(ii) Separation of two strands of target DNA at various
times after the run is initiated.
(iii) The relation between melting temperature of different
domains and concentration of denaturant in the
solvent.
(iv) Temperature at which electrophoresis should be
carried out.
(v) Expected mobility of different bands of amplified
DNA from the point of origin in a gradient gel.
(vi) Time required for electrophoresis of different domains
of target DNA.

When DNA which is heterozygous for a particular
mutation is amplified for more number of PCR cycles,
both, homoduplexes and heteroduplexes are formed. When
this sample is run by DGGE using the conditions obtained
from the computer programmes, a particular pattern of
bands is obtained. Initially one has to sequence each pattern
to characterise the mutation. Once a comprehensive
catalogue with different patterns corresponding to various
mutations is prepared, subsequently a mutation can be
identified by comparing the pattern with that from the
catalogue.

This is a very powerful and versatile technique which
localizes the mutation in a DNA fragment of 200-300 bp.
Initially this technique was not very sensitive but the
attachment of a ‘GC clamp’ to one of the PCR primers46
altered the melting profile of a domain and increased the
sensitivity considerably.

DGGE has been applied to detect mutations in
b-thalassaemia47, Factor VIII gene48, Factor IX gene49,
G6PD gene50 and a-thalassaemia42.
**DGGE using a chemical clamp**

DGGE is expensive because of the high cost of the GC clamp. Also, the yield of the GC clamped primer is reduced drastically during synthesis. Use of a chemical clamp allows these difficulties to be overcome. The end of the DNA fragment can be stabilized by covalently linking both strands with a chemical agent like psoralen (furo-coumarin), a photoactivatable intercalating agent, which is attached to the 5’ end. Using these primers, once a DNA fragment is amplified, it is irradiated with a 365 nm UV lamp for 15 minutes and then analyzed by DGGE. It is applied to screen for mutations in β-thalassaemia.

**Constant denaturant capillary electrophoresis**

The DNA fragment of interest is selected so that it is either a naturally high melting domain or the high melting domain is incorporated during amplification using primers with GC rich sequences. At an appropriate temperature, a single base pair change in the low melting domain of a sequence alters the stability. The analysis is done using a special instrument. Each sample is loaded on a thin, long (30 cm) capillary and the separation of the band is detected by the laser beam. This allows more precision and sensitivity. It has been applied to detect mutations in N-ras gene and β-thalassaemia.

**Detection of Target Sequences in Solution**

Probe/target hybrids may be selectively isolated on a solid matrix like hydroxylapatite which preferentially binds double stranded nucleic acids or the probe may be immobilized on a solid support and used to capture target sequences from a solution.

**DNA Sequencing**

Sequencing of the gene is an essential final step for identification of uncharacterized mutations. Unfortunately, DNA sequencing is time consuming, requires expensive apparatus and chemicals and expertise to interpret the results. With the use of automation and non-radioactive fluorescence technology, this approach is very efficient and may become a primary method of mutation detection.

The most widely used protocol is Sanger’s dideoxy chain termination reaction where DNA synthesis in vitro is carried out in the presence of chemically modified (dideoxy) nucleotides, the incorporation of which in the growing chain leads to termination. Four reactions are set up. Each reaction contains small amounts of only one of the four possible dideoxy nucleotides and all four deoxy nucleotides, one of which carries a radioactive isotope. Consequently a specific ddNTP chain terminator competes with its corresponding dNTP for inclusion in the growing chain. This yields a population of molecules with common 5’ ends but different 3’ ends depending on the site at which a ddNTP is incorporated. All four reactions are then subjected to electrophoresis using a polyacrylamide gel system that separates the fragments on the basis of length of the newly synthesized strand. This is followed by autoradiography.

**Fluorescence in situ Hybridization**

As mentioned earlier, deletion, insertion or substitution of one or more nucleotides is responsible for creating a mutation which can be detected by several techniques mentioned above. Apart from this, chromosome abnormalities is another important cause of some haematological malignancies. This may be in the form of gross deletion or rearrangement of chromosomes and can be detected by studying chromosome morphology. Giemsa banding or G-banding is the most widely used technique to study chromosome morphology. This technique requires dividing cells and chromosomes arrested in either metaphase or prometaphase. An important progress in this is the development of fluorescence in situ hybridization (FISH) technology where chromosome analysis can be done in interphase nuclei of non dividing cells. This involves preparation of specific DNA probes labelled by incorporation of chemically modified nucleotides that fluoresce directly. These probes may be hybridized to metaphase chromosomes or to interphase nuclei of non-dividing cells. This technique is applied to study chromosomal abnormalities associated with haematological malignancies.

**Conclusions**

Thus several techniques for mutation detection have been described. Most of them depend on the various characteristics of DNA. Broadly they can be divided into two categories viz. (i) diagnostic methods which are devised to detect defined mutations; and (ii) scanning methods which can generally scan hundreds of bases of DNA that might harbour a mutation. A single genetic disorder may be caused by a large number of mutations (e.g. thalassaemia). A particular ethnic group may harbour only 5-6 common mutations which could account for 80-85% of the molecular defects. A simple kit can be prepared using one of the diagnostic methods to detect these mutations. On the other hand, scanning methods are generally cumbersome but can detect almost all the nucleic acid alterations.
Today, attempts have been made to refine the existing mutation detection techniques to develop economical as well as effective methods. Any scanning technique should fulfill the following criteria: It should be able to screen large fragments of DNA, detection rate should be 100%, no complex equipment or harmful reagents should be required and finally there should not be any interference due to false positive or false negative results. This would be an ideal scanning technique.

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