

# **Bio203 Final term notes**

**Regards By Admins:**

**VU Friends**

## **Q.1 Explain nomenclature?**

- A suitable system was proposed by Smith and Nathans (1973)
- The species name of host organisms is identified by the first letter of genus and first two letters of specific epithet
- E. coli = Eco
- H. influenzae = Hin
- Strain identification is written as EcoK
- In case, host strain has several restriction and modification systems, these are identified by roman numerals, for example, in case of H. influenzae
- HindI, HindII, HindIII etc
- All restriction enzymes have general name endonuclease R and modification-methylase M followed by the system name, for example, in case of H. influenzae
- R. HindIII or M. HindIII

### **TABLE:**

| Enzyme     | Enzyme source                                    | Recognition sequence |
|------------|--|----------------------|
| SmaI       | Serratia marcescens, 1st enzyme                  | CCCGGG               |
| HaeIII     | Haemophilus aegyptius, 3rd enzyme                | GGCC                 |
| HindII H.  | influenzae, strain d, 2nd enzyme                 | GTPyPuAC             |
| HindIII H. | influenzae, strain d, 3rd enzyme                 | AAGCTT               |
| HamHI      | Bacillus amyloliquefaciens, strain H, 1st enzyme | GGATCC               |

### **Target sites:**

Type II endonucleases recognize and cleave DNA within particular sequences of four to eight nucleotides which have a twofold axis of rotational symmetry i.e. referred as palindromes

5'-GAATTC-3'

5'-CTTAAG-3'

## Q.2 Describe number and size of restriction fragments?

- The number and size of fragments generated by restriction enzyme depend on the frequency of occurrence of the target site in the DNA to be cut
- Four base recognition site occurs every 44 (256) bp
- Six base recognition site occurs every 46 (4096) bp
- Eight base recognition site occurs 48 (65,536) be

### **TABLE:**

| Enzyme       | Target | Arabidopsis | E.coli | Human |
|--------------|--------|-------------|--------|-------|
| <i>Apal</i>  | GGGCCC | 25000       | 15000  | 2000  |
| <i>BamHI</i> | GGATCC | 6000        | 5000   | 5000  |
| <i>SpeI</i>  | ACTAGT | 8000        | 60000  | 10000 |

### **Summary of restriction endonucleases:**

Type II restriction enzymes are heavily responsible for the current explosion in the field of gene manipulation in that they are essential in forming recombinant DNA molecules

## Q.3 Describe DNA modifying enzymes?

- Nucleases
- DNA Polymerase
- Reverse transcriptases
- DNA ligases

### **(i)Nucleases:**

- Nucleases or DNases are the enzymes that degrade DNA
- Two broad classes of nucleases
  - Exonucleases
  - Endonucleases

### **(ii)DNA Polymerase:**

An enzyme that catalyzes template-dependent synthesis of DNA

### **(iii)Reverse transcriptases:**

An RNA-directed DNA polymerase in retroviruses; capable of making DNA complementary to an RNA

### **(iv)DNA ligase:**

An enzyme that creates a phosphodiester bond between the 3' end of one DNA segment and the 5' of Another

#### Q.4 Write methods of joining DNA fragments?

Mainly three methods are used for joining DNA in vitro

- (i) Joining covalently annealed cohesive ends by DNA ligase
- (ii) Joining blunt-ended fragments by DNA ligase from phage T4 infected E. coli
- (iii) The third utilizes the enzyme terminal deoxynucleotidyltransferase to synthesize homopolymeric 3' single stranded tails at the ends of fragments

#### Q.5 What are DNA ligase?

E. coli and phage T4 encode enzyme, DNA ligase, which seals single stranded nicks between adjacent nucleotides in a duplex DNA chain

##### ❖ DNA ligase to create covalent recombinant DNA:

DNA ligase can repair the nicks produced after the association of cohesive ends of DNA strands.

##### ❖ Alkaline Phosphatase:

- An enzyme responsible for removing phosphate groups from many types of molecules including DNA
- Treatment of linearized plasmid vector DNA with alkaline phosphatase to remove 5'-terminal phosphate groups will prevent recircularization and plasmid dimer formation

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##### ❖ Blunt end ligation via linker molecules:

- Joining DNA fragments with cohesive ends by DNA ligase is a relatively efficient process
- T4 DNA ligase has been used to join blunt-ended DNA molecules

##### ❖ Adaptors:

- Chemically synthesize adaptor molecules which have a preformed cohesive end can be used to insert blunt ended foreign DNA into vector
- The main difference between linker and adaptor is that former having blunt ends while later have one cohesive end

#### Q.6 Explain Southern blotting?

- The Southern blot is used to detect the presence of a particular piece of DNA

- in a sample by a molecular probe.
  - Southern Blotting is named after its inventor, the British Biologist Edwin Southern (1975).
  - Identify DNA sequence (gene) of interest.
  - Identified DNA may be a small piece of DNA or a mutation.
- Other blotting method:
- Other blotting methods with similar principles, but using protein or RNA, have been named in reference to Edwin Southern's name.

### Q.7 Write a note on procedure of Southern blotting?

- (i) DNA is extracted from cells, leukocytes.
- (ii) DNA is cleaved into many fragments by restriction enzyme (e.g, BamH1, EcoR1 etc)
- (iii) The resulting fragments are separated on the basis of size by electrophoresis.
- (iv) The DNA fragments are denatured and transferred to nitrocellulose membrane for analysis.
- (v) The labeled probe is added to the blocked membrane in buffer and incubated for several hours to allow the probe molecules to find their targets.
- (vi) Blot is incubated with wash buffers containing NaCl and detergent to wash away excess probe.
- (vii) Radioactive probes enable autoradiographic detection.

#### **Conclusion:**

Southern blot is used to detect the presence of a particular piece of DNA in a sample by a molecular probe.

### Q.8 Explain Southern blotting probes?

- Labeled material to detect a target.
  - For DNA: 20–30 nucleotides, complementary to a region in the gene or DNA.
- ❖ **Radioactive probe- P32:**
    - Sensitive
    - Relatively cheap
    - Hazardous
    - Radioactive waste disposal regulations should be followed
  - ❖ **Non radioactive probe biotin:**
    - Sensitive
    - Relatively expensive
  - ❖ **Hybridization of probes:**
    - The binding between single stranded labeled probe to a complementary nucleotide sequence on the target DNA.

#### **PROBES**

Labeled material to detect complementary region in the gene or DNA.

### Q.9 Write transfer method in southern blotting?

#### Transfer method of DNA to membrane:

- Upward capillary transfer
- Downward capillary transfer
- Simultaneous transfer to two membranes
- Electrophoretic transfer
- Vacuum transfer

### Q.10 Identify mutation by southern blotting?

#### Mutation identification:

- The presence of a mutation affecting a restriction site causes the pattern of bands to differ from those seen in a normal gene.
- A change in one nucleotide may alter the nucleotide sequence so that the restriction endonuclease fails to recognize and cleave at that site.
- Digestion of genomic DNA to DNA fragments.
- Size-separation of the fragments.
- In situ denaturation of the DNA fragments.
- Transfer of denatured DNA fragments into a solid support (nylon or nitrocellulose).
- Hybridization of the immobilized DNA to a labeled probe (DNA, RNA).
- Detection of the bands complementary to the probe (e.g. by autoradiography).
- Estimation of the size & number of the bands generated after digestion of the genomic DNA will be different.
- Mutations can be identified by Southern blotting.

### Q.11 Write Northern blotting and its procedure?

- Northern blot is a method used for detection of a specific RNA sequence in RNA samples.

#### Procedure of northern blotting:

- Isolation of intact mRNA.
- Separation of RNA according to size (through a denaturing agarose gel).
- Transfer of the RNA to a solid support.
- Fixation of the RNA.
- Hybridization of the immobilized RNA to probes complementary to the sequences of interest.
- Removal of probe molecules that are nonspecifically bound to the solid matrix.
- Detection, capture and analysis of an image of the specifically bound probe molecules.

### **Q.12 Write application of northern blotting?**

- Study of gene expression in eukaryotic cells.
- To measure the amount & size of RNAs transcribed from eukaryotic genes.
- To estimate the abundance of RNAs.
- To equalize the amounts of RNA loaded into lanes of gels.
- Use of housekeeping gene (endogenous constitutively-expressed gene).
- Normalizing samples according to their content of mRNAs of the housekeeping gene.
- Northern blot is used in many ways while studying RNAs.

### **Q.13 Explain Western blotting?**

- A technique used to detect the presence of a specific protein in a complex protein mixture.
- To determine the molecular weight of a protein.
- To measure relative amounts (quantitation) of the protein present in complex mixtures of proteins that are not radiolabeled.
- Western blots have become one of the most common analytical tools for the detection of viral proteins.
- Characterization of monoclonal and polyclonal antibody preparations and in determining the specificity of the immune response to viral antigens.

### **Q.14 Write western blotting procedure?**

- 1 Sample preparation
- 2 Gel Electrophoresis
- 3 Blotting (or transfer)
- 4- Blocking
- 5 Antibody probing
- 6 Detection

1. The choice of extraction method depends primarily on the sample and whether the analysis is targeting all the proteins in a cell or only a component from a particular subcellular fraction.
2. Samples are loaded into separate wells. A protein marker is also loaded. The separated protein mixtures are transferred to a solid support for further analysis.
3. Transfer can be done in wet or semi-dry conditions. Semi-dry transfer is generally faster. Wet transfer is recommended for large proteins, >100 kD.
4. Blocking is a very important step in the immunodetection phase of Western blotting because it prevents non-specific binding of antibody to the blotting membrane. Protein of interest is detected and localized using a specific antibody. Western blotting protocols utilize a non-labeled primary antibody directed against the target protein. A species-specific, labeled secondary antibody directed against the constant region

of the primary antibody is then used. The most common antibody label used in Western blots is HRP.

The signal is detected when HRP is exposed to a substrate solution in the final step of the immunodetection procedure

### Q.15 Write applications of western blotting?

- Analysis Of IgG Fractions purified from human plasma.
- Diagnosis of HIV by ELISA, involves the western blotting technique.
- Western blotting technique is also used to Detect Some Forms Of Lyme Disease.
- Western blotting technique is used in Definitive Test For BSE, which is commonly known as Mad cow disease.
- Confirmatory Test For Hepatitis-B involves western blotting technique.
- Western blotting test is used in the Analysis Of Biomarkers such as hormones, growth factors & cytokines.
- This technique is also employed in The Gene Expression Studies

### Q.16 Write limitation and conclusion of western blotting?

#### Limitations in western blotting :

Very delicate and time consuming process. A minute imbalance at any level of the procedure can skew the results of the entire process.  
Incorrect labeling of the protein can happen due to the reaction of secondary antibody.

Cause erroneous bands or no bands due to insufficient transfer.

Well trained technicians are required for this technique.

Primary antibody availability is crucial.

It is just a semi-quantitative at best. Only an approx. estimation & not a precise measurement of molecular weight of the protein is possible

#### Conclusion:

Western blotting technique is simply a way to identify unknown proteins on a polyacrylamide gel. It is sometimes called as protein blotting or immunoblotting. It is a widely used analytical technique in the fields of molecular biology, immunogenetics, and other biochemistry disciplines. Western blotting technique is also used in the field of medical diagnostics. i.e., in the analysis of various kinds of diseases. Apart from the limitations of western blotting, it is more helpful now a days. Hence, The discovery of Western blotting technique has become a boon in the field of science & technology

### Q.17 Write difference between different blotting technique?

#### Type of molecule detected:

| Northern Blotting | Southern Blotting | Western Blotting |
|-------------------|-------------------|------------------|
|-------------------|-------------------|------------------|

|   |  |  |
|---|--|--|
| Northern blotting detects a specific RNA sequence from an RNA sample. | Southern blotting detects a specific DNA sequence from a DNA sample. | Western blotting detects a specific protein from a protein sample. |
|---|--|--|

**Type of gel:**

| Northern Blotting                   | Southern Blotting         | Western Blotting              |
|-------------------------------------|---------------------------|-------------------------------|
| This uses Agarose/formaldehyde gel. | This uses an Agarose gel. | This uses Polyacrylamide gel. |

**Blotting method:**

| Northern Blotting             | Southern Blotting             | Western Blotting              |
|-------------------------------|-------------------------------|-------------------------------|
| This is a capillary transfer. | This is a capillary transfer. | This is an electric transfer. |

**Probes used:**

| Northern Blotting  | Southern Blotting   | Western Blotting                       |
|--|---|--|
| cDNA or RNA probes labeled radioactively or nonradioactively | DNA probes are labeled radioactively or nonradioactively. | Primary antibodies are used as probes. |

**Detection method:**

| Northern Blotting  | Southern Blotting   | Western Blotting   |
|--|---|--|
| This is done using an autoradiograph, or detection of light or color change. | This is done using an autoradiograph, detection of light or color change. | This is done using the detection of light or color change. |



### Q.18 What is Single nucleotide Polymorphism (SNP) and write its some fact also?

A SNP is defined as a single base change in a DNA sequence that occurs in a significant proportion (more than 1 percent) of a large population.

#### Some Facts of SNP:

In human beings, 99.9 percent bases are same.

Remaining 0.1 percent makes a person unique.

Different attributes / characteristics / traits

how a person looks,

diseases he or she develops.

#### These variations can be:

Harmless (change in phenotype)

Harmful (diabetes, cancer, heart disease, Huntington's disease, and hemophilia )

Latent (variations found in coding and regulatory regions, are not harmful on their own, and the change in each gene only becomes apparent under certain conditions

#### e.g:

susceptibility to lung cancer.

### Q.19 Where SNP are found and write its occurrence and abundance also?

#### ❖ SNPs are found:

Coding and (mostly) noncoding regions.

#### ❖ Occur with a very high frequency:

About 1 in 1000 bases to 1 in 100 to 300 bases.

#### ❖ Abundance:

The abundance of SNPs and the ease with Which they can be measured make these genetic variations significant.

❖ SNPs close to particular gene acts as a marker for that gene.

❖ SNPs in coding regions may alter the protein structure made by that coding region.

### Q.20 Write Single nucleotide polymorphism-2 and SNP profiles?

#### SNP maps:

- Sequence genomes of a large number of people
- Compare the base sequences to discover SNPs.
- Generate a single map of the human genome containing all possible SNPs => SNP maps.

#### SNP profiles:

- Genome of each individual contains distinct SNP pattern.
- People can be grouped based on the SNP profile.
- SNPs Profiles important for identifying response to Drug Therapy.
- Correlations might emerge between certain SNP profiles and specific responses to treatment.

### Q.21 How to detect SNPs?

**There are following technique to detect SNP:**

#### **Hybridization technique:**

Micro arrays

Real time PCR

#### **Enzyme based Techniques**

Nucleotide extension

Cleavage

Ligation

Reaction product detection and display

Direct Sequencingn Poly

Microarray

Cleavage / Ligation

Electrophoretic mobility assays

### Q.22 Write direct sequencing?

- Sanger dideoxysequencing can detect any type of unknown polymorphism and its position, when the majority of DNA contains that polymorphism.
- Misses polymorphisms and mutations when the DNA is heterozygous
- limited utility for analysis of solid tumors or pooled samples of DNA due to low sensitivity
- Once a sample is known to contain a polymorphism in a specific region, direct sequencing is particularly useful for identifying a polymorphism and its specific position.
- Even if the identity of the polymorphism cannot be discerned in the first pass, multiple sequencing attempts have proven quite successful in elucidating sequence and position information.

### Q.23 Describe SNP screening?

Two different screening strategies

- Many SNPs in a few individuals

- A few SNPs in many individuals

- Different strategies will require different tools
- Important in determining markers for complex genetic states

SNP genotyping methods for detecting genes contributing to susceptibility or

resistance to multifactorial diseases, adverse drug reactions:  
=> case-control association analysis

Case

GCCGTTGAC...  
...GCCATTGAC...

Control

...GCCATTGAC...  
...GCCATTGAC...

## Q.24 What are significance of SNPs?

There are following significance of SNPs:

- In disease diagnosis.
- In finding predisposition to diseases.
- In drug discover and development.
- In drug responses
- Investigation of migration patterns
- All these aspect will help to look for medication and diagnosis at individual level.
- 

## Q.25 What is SNP-Haplotype?

A set of closely linked genetic markers present on one chromosome which tend to be inherited together (not easily separable by recombination).

Haplotypes:

AG- 2/6(Black eye)  
GTA 3/6(Brown eye)  
AGA 1/6 (Blue eye)

## Q.26 Write Haplotype correlation?

- The “Haplotype centric” approach combines the information of adjacent SNPs into composite multilocus haplotypes
- Haplotypes are not only more informative but also capture the regional LD information, which is assumed to be robust and powerful
- Association of haplotype frequencies with the presence of desired phenotypic frequencies in the population will help in utilizing the maximum potential of SNP as a marker.
- 

## Q.27 Write some important SNP database?

1. dbSNP (<http://www.ncbi.nlm.nih.gov/SNP/>)  
LocusLink  
(<http://www.ncbi.nlm.nih.gov/LocusLink/list.cgi>)

2. TSC (<http://snp.cshl.org/>)
3. SNPper (<http://snpper.chip.org/bio/>)
4. JSNP (<http://snp.ims.u-tokyo.ac.jp/search.html>)
5. GeneSNPs (<http://www.genome.utah.edu/genesnps/>)
6. HGVbase (<http://hgibase.cgb.ki.se/>)
7. PolyPhen (<http://dove.embl-heidelberg.de/PolyPhen/>)
- OMIM (<http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM>)
8. Human SNP database

## Q.28 Explain DNA Fingerprinting?

### Synonyms...

- DNA profiling.
- DNA testing.
- DNA typing.
- Genetic fingerprinting

### Introduction:

The process of DNA fingerprinting was developed by Professor Alec Jeffreys at Leicester University in 1984 as a form of genetic analysis

- It was first used in the law courts of England in 1987 to convict a man in a rape case.
- It has now been used successfully in many crime and paternity cases in worldwide. Although 99.9% of human DNA sequences are the same in every person, enough of the DNA is different to distinguish one individual from another, unless they are monozygotic twins.
- DNA profiling uses repetitive sequences that are highly variable, called variable number tandem repeats (VNTRs), particularly short tandem repeats (STRs).

VNTR loci are very similar between closely related humans.

- The analysis of variable number of tandem repeats (VNTRs), to detect the degree of relatedness to another sequence of oligonucleotides, making them ideal for DNA fingerprinting.

## Q.29 Define Variable number tandem and its principal families ?

A Variable Number Tandem Repeat (or VNTR) is a location in a genome where a short nucleotide sequence is organized as a tandem repeat. These can be found on many chromosomes, and often show variations in length between individuals. Each variant acts as an inherited allele, allowing them to be used for personal or parental identification.

### Its principal families:

There are two principal families of VNTRs:

1. Microsatellites.
2. Minisatellites.

### Microsatellites:

Microsatellites, also known as Simple Sequence Repeats (SSRs) or short tandem repeats (STRs), are repeating sequences of 2-6 base pairs of DNA.

### **Minisatellites:**

A minisatellites (also referred as VNTR) is a section of DNA that consists of a short series of bases 10–60 base pairs. Their analysis is useful in genetics and biology research, forensics, and DNA fingerprinting.

### **Q.30 Describe DNA profiling process?**

The process begins with a sample of an individual's DNA (typically called a "reference sample")

- The most desirable method of collecting a reference sample is the use of a buccal swab, as this reduces the possibility of contamination.

- When this is not available may need to be used to collect a sample of blood, saliva, semen, or other appropriate fluid or tissue from personal items (e.g. toothbrush, razor, etc.) or from stored samples (eg: banked sperm or biopsy tissue).

### **Q.31 Write Procedure of DNA profiling?**

There are following procedure:

#### **1) Isolation of DNA :**

- DNA must be recovered from the cells or tissues of the body.
- Only a small amount of tissue, like blood, hair, or skin, is needed.
- For example, the amount of DNA found at the root of one hair is usually sufficient.

#### **2) Cutting, sizing, and sorting :**

- Special enzymes called restriction enzymes are used to cut the DNA at specific sites.
- For example, an enzyme called EcoR1, found in bacteria, will cut DNA only when the sequence 5'..GAATTC..3' occurs.
- The DNA pieces are sorted according to size by a sieving technique called electrophoresis.
- The DNA pieces are passed through a gel agarose. This technique is the DNA equivalent of screening sand through progressively finer meshscreens to determine particle sizes.

#### **3) Transfer of DNA to nylon :**

The distribution of DNA pieces is transferred to a nylon sheet by placing the sheet on the gel and soaking them overnight.

#### **4) Probing :**

- Adding radioactive or colored probes to the nylon sheet produces a pattern called the DNA fingerprint.
- Each probe typically sticks in only one or two specific places on the nylon sheet.

### **5) DNA fingerprint :**

The final DNA fingerprint is built by using several probes (5-10 or more) simultaneously. It resembles the bar codes used by grocery store scanners.

### **Q.32 Write name of type of DNA fingerprinting methods?**

Types of DNA fingerprinting methods are...

- (i) Electrophoresis.
- (ii) Polymerase chain reaction (PCR).
- (iii) Restriction fragment length polymorphism (RFLP).
- (iv) Random Amplified Polymorphic DNA(RAPD).
- (v) Amplified fragment length polymorphism (AFLP).

### **Q.33 Explain type of DNA fingerprinting methods?**

#### **(i) Electrophoresis:**

Electrophoresis is a separations technique that is based on the mobility of ions in an electric field. Positively charged ions migrate towards a negative electrode and negatively-charged ions migrate toward a positive electrode. Ions have different migration rates depending on their total charge, size, and shape, and can therefore be separated.

#### **(ii) Polymerase chain reaction (PCR):**

Polymerase chain reaction (PCR) was developed

- The polymerase by Kary Mullis of the Cetus Corporation in 1983.
- In this process, the DNA sample is denatured into the separate individual strands.
- Specific DNA primers are used to hybridize to two corresponding nearby sites on opposite DNA strands in such a fashion that the normal enzymatic extension of the active terminal of each primer (that is, the 3' end) leads toward the other primer.
- In this fashion, two new copies of the sequence of interest are generated. Repeated denaturation, hybridization, and extension in this fashion produce an exponentially growing number of copies of the DNA of interest.
- The PCR analysis amplified isolated regions on the strands of the DNA under examination.

### **Q.34**

#### **(iii) Restriction fragment length polymorphism (RFLP):**

- RFLP analyzes the length of the strands of the DNA molecules with repeating base pair patterns.
- The basic technique for detecting RFLPs involves fragmenting a sample of DNA by a restriction enzyme, which can recognize and cut DNA wherever a specific short sequence occurs, in a process known as a restriction

- digest.
- The resulting DNA fragments are then separated by length through a process known as agarose gel electrophoresis, and transferred to a membrane via the Southern blot procedure.
- Hybridization of the membrane to a labeled DNA probe then determines the length of the fragments which are complementary to the probe.
- An RFLP occurs when the length of a detected fragment varies between individuals. Each fragment length is considered an allele, and can be used in genetic analysis.

### Q.35

#### (iv) Random Amplified Polymorphic DNA(RAPD):

Random Amplified Polymorphic DNA...of PCR reaction, but the segments of DNA

- It is a type that are amplified at random.
- RAPD creates several arbitrary, short primers (8–12 nucleotides), then proceeds with the PCR using a large template of genomic DNA, hoping that fragments will amplify.
- By resolving the resulting patterns, a semi-unique profile can be gleaned from a RAPD reaction.
- RAPD does not require any specific knowledge of the DNA sequence of the target organism: the identical 10–mer primers will or will not amplify a segment of DNA, depending on positions that are complementary to the primers' sequence.

### Q.36

#### Write Advantages and disadvantage of RAPD?

##### Advantages of RAPD:

It requires no DNA probes and sequence information for the design of specific primers.

- It involves no blotting or hybridization steps, hence, it is quick, simple and efficient.
- It requires only small amounts of DNA (about 10 ng per reaction) and the procedure can be automated.
- High number of fragments.
- Arbitrary primers are easily purchased.
- Unit costs per assay are low compared to other marker technologies.

##### Disadvantages of RAPD:

- Nearly all RAPD markers are dominant, i.e. it is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies).
- Co-dominant RAPD markers, observed as differentsized DNA segments amplified from the same locus, are detected only rarely.
- PCR is an enzymatic reaction, therefore, the quality and concentration of template DNA, concentrations of PCR compo-nents, and the PCR cycling

conditions may greatly influence the outcome. Thus, the RAPD technique is notoriously laboratory-dependent and needs carefully developed laboratory protocols to be reproducible.

- Mismatches between the primer and the template may result in the total absence of PCR product as well as in a merely decreased amount of the product. Thus, the RAPD results can be difficult to interpret.
- Lack of a prior knowledge on the identity of the amplification products.
- Problems with reproducibility (sensitive to changes in the quality of DNA, PCR components and PCR conditions).
- Problems of co-migration. Gel electrophoresis can separate DNA quantitatively, cannot separate equal-sized fragments qualitatively (i.e. according to base sequence).

### **Q.37**

#### **(v) Amplified fragment length polymorphism (AFLP):**

- This technique was also faster than analysis and used PCR to amplify DNA samples. It relied on variable number tandem repeat (VNTR) polymorphisms to distinguish various alleles, which were separated on a polyacrylamide gel.
- By using the PCR analysis to amplify the minisatellite loci of the human cell, this method proved quicker in recovery than the RFLP.
- However, due to the use of gel in its analysis phase, there are issues of bunching of the VTRN's, causing misidentifications in the process.

### **Q.38 Write Applications of Fingerprinting?**

#### **1) Diagnosis and Developing cures for inherited disorders :**

- DNA fingerprinting is used to diagnose inherited disorders in both prenatal and newborn babies in hospitals around the world.
- These disorders may include cystic fibrosis, hemophilia, Huntington's disease, familial Alzheimer's, sickle cell anemia, thalassemia, and many others.
- Early detection of such disorders enables the medical staff to prepare themselves and the parents for proper treatment of the child.
- In some programs, genetic counselors use DNA fingerprint information to help prospective parents. In other programs, prospective parents use DNA fingerprint information in their decisions concerning affected pregnancies.

#### **2) Biological Evidence to Identify Criminals:**

- Where fingerprints are not available but biological specimens are available like blood or semen stains, hair, or items of clothing at the scene of the crime then these items may prove to be valuable sources of DNA of the criminal.
- Since the year 1987, innumerable cases have been solved with the help of DNA fingerprint evidence.



### 3) Paternity disputes :

Another important use of DNA fingerprints in the court system is to establish paternity in custody and child support litigation. In these applications, DNA fingerprints bring an unprecedented, nearly perfect accuracy to the determination.

### 4) Personal Identification :

DNA maybe the best way to identify a person as all body tissues and organs contain the same DNA type. The specimen required also is very small. In fact the US army has been doing DNA fingerprinting of all its soldiers and has a huge databank.

REMEMBER US IN  
YOUR PRAYERS

REGARDS =

VU FRIENDS