APPLICATIONS OF MOLECULAR BIOLOGY TECHNOLOGY

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Introduction

The development of Molecular Biology technology (or Recombinant DNA technology or Genetic Engineering) and the advances made in the various techniques have had a major impact on almost all aspects of biological sciences. In plant and animal breeding and in human medical sciences, the impact has been of tremendous value. Some of the medical application are listed in Table 1. Figure 1 summarises these applications in a diagramatic presentation. With further advancements applications in new avenue will come to light in the near future.

I Diagnosis of disease state

Both single gene and chromosomal disorders can be diagnosed by recombinant DNA technology. The approaches can be divided into 'direct' and indirect methods.

A. Direct Methods

(i) Direct detection of the mutation by mutation specific RFLPs.

In some single gene disorders, the mutation either eliminates an RE restriction site or creates a new site. This effects the size of the fragments generated on RE treatment e.g. the sickle cell mutation abolishes the restriction site of Mst II, and hence in presence of the mutation a 1.35 kb fragment is produced carrying ß-globin gene compared to a 1.15 Kb fragment produced normally. Other examples include mutations producing and abnormal haemoglobin (e.g. Hb O Arab, Hb Lepore), some α and ß-thal., α1-AT deficiency, and G-6-PD deficiency. However, there is limited use of this approach as the occurrence of a mutation in the recognition site of an RE is relatively rare.

(ii) Detection of deletion in the gene

Deletion of a whole or major part of gene can be detected in various ways:

- Fragment generated on treatment with RE. If the the gene/s are deleted the fragment will not be produced e.g. in case of deletion type of α-thalassaemnia, Bam HI produces a 14.5 kb fragment as it cuts on both sides of the α-globin genes. If one α gene is deleted, the fragment is smaller (10.5 kb). Heterozygotes to this deletion have 14.5 and 10.5 kb fragments while homozygotes have only 10.5 kb fragment.
- A cDNA probe for ß-globin gene does not hybridize to the DNA fragments in persons who have a ß-globin deletion - δß-thalassaemia, due to the absence of the ß-gene.

(iii) Direct detection using allele specific oligonucleotides

Allele specific oligonucleotides are short synthetic oligonucleotides with sequence corresponding to the allele and another to the normal allele. After DNA amplification, treatment with the ASO can reveal the presence or absence of the mutation depending on whether the hybridization occurs with the mutant or normal ASO. This principle is used in several techniques, with the most widely used one is dot blot analysis and reverse dot blot.

B. Indirect methods for diagnosis of a genetic disease

These method makes use of sequence variations in the non-coding sequences on the DNA. The types of variations which are considered useful for diagnosis are those known as restriction fragment length polymorphisms (RFLPs) and the hypervariable DNA length polymorphism.

(i) RFLPs

Mutations frequently occur in the intergenic regions and are inherited in a Mendelian codominant manner. It is suggested that these mutations occur once in every 200 bp. A RE restriction site may be lost or new one created by presence or absenece of these mutations. This results in production of different fragment sizes that can be detected following electrophoresis. This is termed as RFLP. Some RFLPs are linked to a specific mutation in the gene and hence some disease genes can be detected not by the mutation in the gene itself, but by the associated RFLP. This also forms the basis of linkage analysis and several diseases including cystic fibrosis, Huntington's disease, adult onset polycystic kidney disease, Duchennes muscular dystrophy, homocystiuria and myotonic dystrophy were diagnosed by linkage analysis.

(ii) Hypervariable DNA length polymorphism

Variable number of tandem repeats (VNTR) DNA sequence polymorphism occur in human DNA which are hypervariable. It occurs due to presence of variable number of tandem repeats of short DNA sequences. These are inherited in Mendelian codominant fashion and are believed to have arisen by unequal crossing over during chromosomal recombinations. This can be demonstrated using RE, as different length fragments are produced e.g. hypervariable region 5' to the insulin gene, hypervariable region near α-globin gene on chromosome 16 which is linked to the adult onset polycystic kidney disease. Other such regions are mini satellites which are short - 10-15 bp "core" sequences and microsatellites (variable no. of tandem repeats dinucleotide).

CA: These are called CA repeats or microsatelline. Individuals differ in the number of these CA repeat sequences. These indirect detection of mutations have been of extensive use for prenatal diagnosis, carrier detection and preclinical diagnosis with-in high risk families.

(iii) DNA haplotypes i.e. the pattern of DNA sequence polymorphism on the chromosome generated by using 2 or more RE. The presence of a restriction site is indicated by (+) and absence by a (-). Patterns are generated which are referred to as haplotypes. Specific haplotype as may be linked to a specific disease and may be used for prenatal, presymptomatic diagnosis and carrier detection.

II. Study of molecular pathology of diseases

It is possible since the advent of molecular biology techniques to identify the precise molecular defect which has caused a specific genetic diseases.
Of the 6000 Mendelian characteristics in humans, many of which are associated with a disease, over 2000 disease genes have been cloned and the exact mutation leading to these diseases has been identified. In those cases where there is no identifiable protein or enzyme abnormality, the finding of a linked DNA sequence polymorphism has led to the isolation and cloning of the structural gene and hence recognition of the protein product. This process is called "reverse genetic". Table 2 lists the molecular basis of some single gene disorders.

III. Gene mapping/chromosome mapping

Various techniques are utilized to map genes on chromosome:

(a) Chromosome mapping: assigning a gene or DNA sequence to a specific chromosome or a particular region of a chromosome. The techniques used for chromosome mapping includes:

- gene dosage stimulation
- somatic cell hybridization
- insitu hybridization

(b) DNA mapping involves detailed mapping at DNA level which includes physical relationship to flanking DNA sequence polymorphisms and detailed structure of genes. The techniques used include:
- Pulse field electrophoresis
- Chromosome jumping/linking
- cloning in YACs (Contigs) followed by chromosome walking.
- Positional cloning.

By applying these techniques, an exponential growth has been observed in the mapping of genes. By 1993, over 3000 genes had been mapped and a map is out of date almost soon after it is collected, due to the rapid growth in knowledge.

IV. Biosynthesis of human proteins

It has been possible to introduce human genes in bacterial vectors and by allowing the bacteria to grow and multiply, the human protein is produced by the bacterial cell, purified and used for humans e.g. insulin, growth hormones, erythropoietin, interleukin, and a variety of other human proteins are produced in this way.

V. Treatment of genetic diseases

One of the major aims of the recombinant DNA technology is to provide ways and means by which a defective gene may be corrected. This forms the basis of gene therapy. Several disease are under investigation and some have shown very encouraging results. (Discussed in a separate lecture)

VI. In forensic medicine

DNA fingeprints are specific in each individual. This knowledge has been utilized in forensic medicine to confirm or otherwise the involvement of a suspect in a crime.

VII. Diagnosis of bacterial and viral diseases

In interesting application of reconbinant DNA technology has been in the diagnosis of bacterial and viral diseases by using probes specifically directed towards their DNA.

VIII. In agriculture and animal husbandry

Outside of the medical fields DNA technology has shown tremendous potentials in the field of agriculture and animal husbandry where it is utilized to improve verieties of crops and increase thier production. Simultaneously animal husbandry has been tremendous advancement utilising these technologies.

Table 1: Application of mulecular biology technology

- Diagnosis of diseases
- Study of molecular pathogenesis
- Clinical genetics
  - Prenatal diagnosis
  - Presymptomatic diagnosis
  - Carrier detection
- Gene structure/mapping/function
- Biosynthesis of human proteins e.g. insulin, growth hormone, interferon
- Treatment of gene diseses e.g. gene therapy
- Agriculture e.g. nitrogen fixation
- Animal and plant breeding

Table 2: Molecular basis of some single gene disorders

Disorder	Molecular defect
Autosomal Recessive	
α1-AT deficiency	Point mutation, deletion Insertion, duplication
CF	Deletion, point mutations, insertions
Phenylketonuria	Deletion, point mutations, insertions
Congenital adrenal hyperplasia	Point mutations deletions

Tay Sachs	Deletions, point mutations
α-thalassaemia	Deletions, point mutations
ß-thalassaemia	Deletions, point mutations
Haemoglobinopathies	Point mutations
Autosomal Dominant	
Antithrombin III deficiency	Deletions, point mutations, insertions
Familial hypercholesterolaemia	Deletions, point mutations, insertions Deletions, duplications
Huntington's disease	CAG triplet repeat expansion
Myotonic dystrophy	CTG triplet repeat expansion
Osteogenesis imperfecta	Deletions, point mutations
X-linked Disorders	
Duchenon muscular dystrophy	Deletions, duplication, point mutations
Fragile X-mental retardartion	CGG triplet repeat expansion
Hemophilia A	Point mutation, deletion, insertion, duplication, inversions
Hemophil'a B	Point mutations, deletions, inversions
Lesch-Nyhan Syndrome	Deletion, point mutations, insertions, duplications