CHAPTER 3

AN INTRODUCTION TO GENOMICS

Genomics involves the study of the genome of an organism including the information relating to both organization and function of the genome. The study of genomics has facilitated in answers for various questions in life pertaining to evolution, reproduction, cellular functions, and diseases and so on. In this chapter a brief introduction to various aspects in genomics is presented.

A genome is defined as a complete set of DNA of an organism. DNA or deoxyribo nucleic acid is the genetic material present in the nucleus of a cell, which is responsible for propagation of life. DNA is a double helical molecule. The monomer units of DNA are called as nucleotides, and the polymer is known as a polynucleotide. Each nucleotide consists of a 5-carbon sugar (deoxyribose), a nitrogen containing base attached to the sugar, and a phosphate group. There are four different types of nucleotides found in DNA, differing only in the nitrogenous base. The four nucleotides are given one letter abbreviation as shorthand for the four bases, namely A is for adenine, G is for guanine, C is for cytosine, and T is for thymine.

Genomes vary widely in size. The smallest known genome for a free-living organism (a bacterium) contains about 600,000 DNA base pairs, while human and mouse genomes have about 3 billion base pairs. Except for mature red blood cells, all human cells contain a complete genome. DNA in the human genome is arranged into 23 distinct chromosomes, physically separate molecules that range in length from about 50 million to 250 million base pairs.

Each chromosome bears many genes. In fact chromosomes are effectively the carrier for genes. Genes can be defined as the basic physical and functional unit of heredity. In molecular terms, a gene is defined as the entire nucleic acid sequence that is necessary for the synthesis of a functional polypeptide. The discovery of genes gave an answer to the question that is central to all of biology: how genetic information is replicated and transmitted from cell to cell and organism to organism.

The classical principles of genetics were deduced from Gregor Mendel's theory of inheritance in 1865 derived on the basis of breeding experiments with peas. Mendel studied the inheritance of a number of well-defined traits, such as seed color, and was able to deduce general rules for their transmission. He correctly interpreted the observed patterns of inheritance by assuming that each trait is determined by a pair of inherited factors or alleles, which are now called as genes.

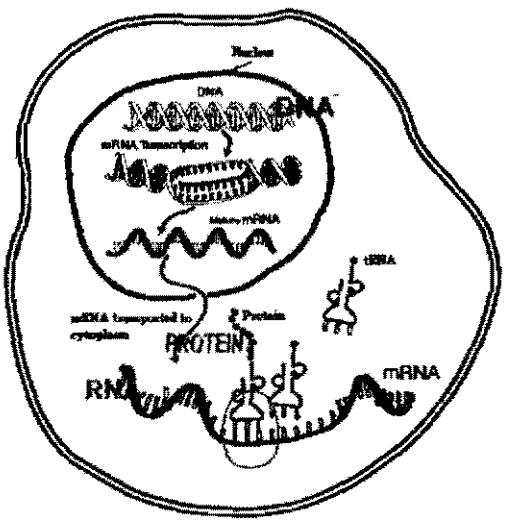
The study of genetics helps in determining the genetic basis of phenotypes. Knowing how many genes determine a phenotype, and where the genes are located, is a first step in understanding the genetic basis of a phenotype. The second step involves studying the sequence of the gene, or genes, determining the phenotype and understanding how the expression of the genes is regulated at the transcriptional level. Subsequent steps involve analysis of post-transcriptional events, understanding how the genes fit into metabolic pathways and how these pathways interact with the environment.

Genes are specific sequences of bases that encode for amino acids, which form the basic units of a protein. Genes are fragments of DNA. However the reverse is not true. Genes constitute only the coding DNA. The human genome is estimated to contain 20,000-25,000 genes, which constitute only 2% of the human genome. The remainder consists of non coding regions, whose functions may include providing chromosomal structural integrity and regulating where, when, and in what quantity proteins are made. Although genes determine the formation of proteins, it is the proteins that performs most life functions and even make up the majority of cellular structures. Proteins are large, complex molecules made up of smaller subunits called amino acids. Chemical properties that distinguish the 20 different amino acids cause the protein chains to fold up into specific three-dimensional structures that define their particular functions in the cell.

The constellation of all proteins in a cell is called its proteome. Unlike the relatively unchanging genome, the dynamic proteome changes from minute to minute in response to tens of thousands of intra- and extracellular environmental signals. A protein's chemistry and behavior are specified by the gene sequence and by the number and identity of other proteins made in the same cell at the same time with which it associates and reacts. The study of protein structure and activities is known as proteomics. Proteomics is now the focus of research to reveal the molecular basis of health and disease[81].

The central dogma of molecular biology

The central dogma of molecular biology is based on the principle that the flow of genetic information travels unidirectional from DNA to RNA and finally to the translation of proteins.



CENTRAL DOGMA IN MOLECULAR BIOLOGY

(Source - National human genome research institute)

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[81] Genomics and Its Impact on Science and Society: The Human Genome Project and Beyond, http://www.ornl.gov/sci/techresources/Human_Genome/publicat/primer2001 Visited on 15th January 2006.

The DNA self replicates in the nucleus of a cell by using one strand of the double helix as a template. The DNA also codes for mRNA (messenger RNA) in a process called transcription. The structure of RNA is similar to that of DNA except that RNA exists as a single stranded unit with Uracil replacing the DNA counterpart Thymine. The mRNA is then transported out of the nucleus into the cytoplasm of the cell where proteins are formed through a process called translation. A series of 3 nucleotides called a codon is responsible for coding one amino acid. The amino acids are carried to the site of translation by transfer RNA (tRNA). Long chains of 20 different amino acids form proteins[82].

Recombinant DNA Technology

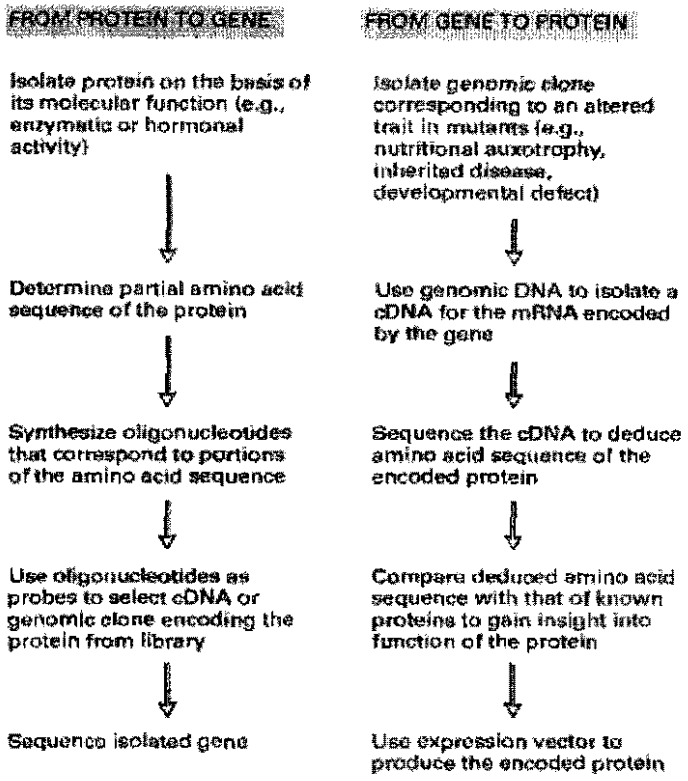
The study of structure of DNA and the genetic code revealed that many deep biological secrets were locked up in the sequence of bases in DNA. But identifying the sequences of long regions of DNA and altering them at will, seemed a distant dream. However, since 1970 there have been dramatic advances in the field of molecular biology, which has enabled us to analyze and manipulate macromolecules, particularly DNA[83].

The science of manipulating DNA molecules is known as recombinant DNA technology and has been one of the approaches taken with the complexity and size of the genome.

Recombinant DNA technology includes a set of techniques for combining genes from different sources and transferring the resulting recombinant DNA into cells, where it can be replicated, and may be transcribed and translated into protein (as per the central dogma). Similarly it also includes techniques, which will enable conversion of protein to genes (by a process called reverse transcription)

[82] http://crystal.uah.edu/~carter/protein/dogma.htm visited on 14th January 2006
[83] Recombinant DNA and Genomics. Chapter 7, Molecular cell Biology, Lodish, Berk et al.

Given below is a flow chart to illustrate the same[84].



Recombinant DNA technology consists o a few basic steps. The first step is to isolate the DNA. The second step is to cut the DNA into smaller pieces which is accomplished with restriction enzymes. The pieces are inserted and propagated in vectors; and collections of pieces are maintained in libraries. DNA targets can be selected via hybridization or by amplification. The nucleotide sequence of the targeted DNA can be determined by sequencing.

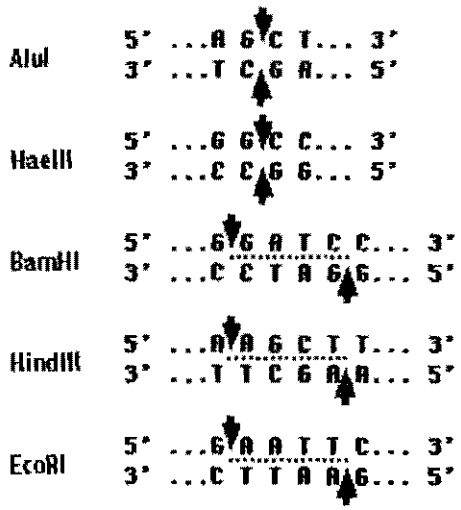
The tools of recombinant DNA technology

1. Restriction enzymes:

Restriction enzymes cut the DNA sequence at defined recognition sites. In nature, these enzymes act as a defense system for bacteria, where they attack and degrade the DNA of attacking bacteriophages. They have been harnessed

[84] Figure at Recombinant DNA and Genomics, Chapter 7, Molecular Cell Biology, Lodish, Berk et al

for the task of systematically breaking up DNA into fragments of small sizes. Each enzyme recognizes a particular DNA sequence and cuts the sequence in a specified fashion, leaving blunt or sticky ends (refer to fig below). An enzyme that has a four-base recognition site will cut approximately every 256 base pairs (4^4), and more frequently than one with a six base recognition site, which in turn will cut more often than one with an eight base recognition site. The restriction enzymes are named for the organism from which they were isolated. The figure below gives a few examples of restriction enzymes and their recognition sites[85].



AluI and HaeIII produce blunt ends

BamHI HindIII and EcoRI produce "sticky" ends

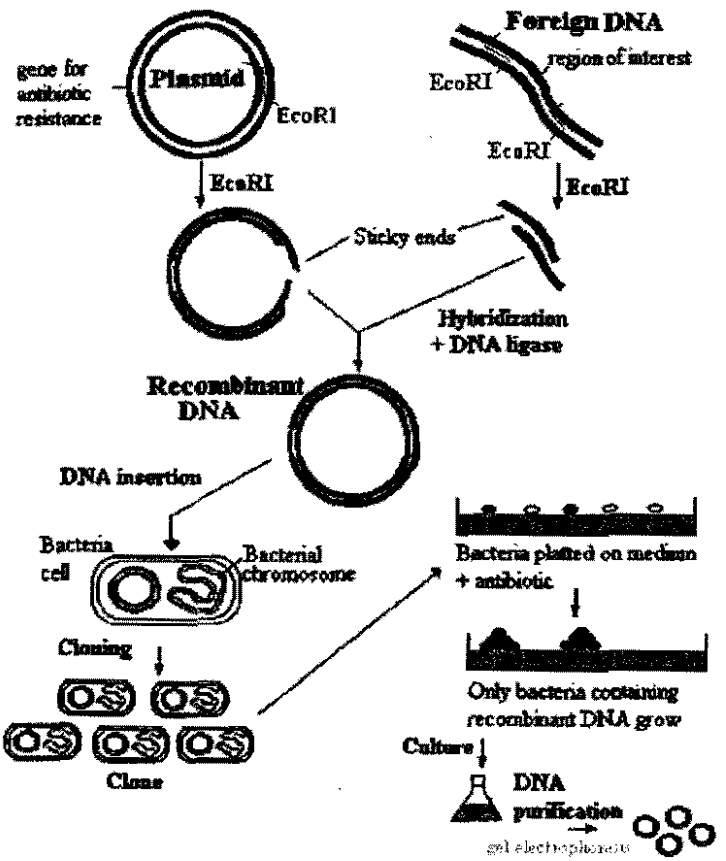
Restriction Endonuclease action[86]

[85] Fundamentals of molecular biology, The cell, A molecular Approach, second edition, Geoffrey M. Cooper

[86] http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/R/RestrictionEnzymes.html visited on 1st February, 2006

2. Vectors:

The role of a vector is to propagate and maintain the DNA fragments generated by restriction enzymes. Additional requisites of vectors are the efficiency and simplicity of inserting and retrieving the DNA fragments. The choice of vector depends on the objective of the experiment. A key feature of the cloning vector is the size of the DNA fragment insert that it can efficiently and reliably handle.



Cloning into a plasmid

Cloning[87]

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[87] http://www.nhgri.nih.gov/DIR/VIP/Learning_Tools/genetic_illustrations.html, visited on 17th January 2006

The figure above explains the process of gene cloning in a plasmid vector. The basic principle of cloning remains the same for all the vectors. Given below is a list of vectors and their insert size.

Vector	Insert size
Plasmids	0.5 - 2 kb
Lambda phage	10 - 20 kb
Cosmids /phagemids	40 - 50 kb
Bacterial Artificial Chromosomes (BACs)	100s of kb
Yeast Artificial Chromosomes (YACs)	1 Mb

3. Libraries:

Libraries are repositories of DNA fragments cloned in their vectors. Libraries can be classified based on the cloning vector. e.g. cosmid, BAC, or YAC. Alternatively, the library can be described in terms of the source of the cloned DNA fragments.

Genomic library

If total genomic DNA is digested and the fragments are cloned into an appropriate vector, the result is a genomic library. In principle, a library should consist of samples of the entire genomic DNA present in the organism, including

[87] http://www.nhgri.nih.gov/DIR/VIP/Learning_Tools/genetic_illustrations.html, visited on 17th January 2006

both coding and non-coding sequences. Ideally, every copy of every gene should be represented somewhere in the genomic library.

Expression libraries:

Expression libraries are also called as cDNA (complementary DNA) libraries. A cDNA library is generated from mRNA transcripts, using the enzyme reverse transcriptase, which can create a DNA complement to mRNA template. Since the cDNA library is based on mRNA, the library will represent only the genes that are expressed in the tissue that are sampled.

4. Focusing on specific sequences (DNA manipulations[88])

The two ways to track or find specific DNA sequences are hybridization and amplification. Hybridization is based on the pairing affinity of complementary single stranded DNA molecules and amplification is based on the Polymerase Chain Reaction, a technique capitalizing on the directed and repeated replication of a specific DNA sequence.

Hybridization

Single strand nucleic acids have a natural tendency to find and pair with other single strand nucleic acids with a complementary sequence. An application of this affinity is to label one single strand with a tag of radioactive and fluorescent dyes. A labeled strand is used as a probe to find complementary sequences in a population of single stranded nucleic acids. For example, a known clone can be used as a probe to look for a homologous sequence in another DNA sample. By denaturing the DNA in the sample, and using labeled single stranded probe the complementary sequences can be searched for[89].

[88] http://cropandsoil.oregonstate.edu/classes/css430/notes visited on 31st Jan, 2006
[89] Recombinant DNA and Genomics. Chapter 7, Molecular cell Biology, Lodish, Berk et al.

The concept of hybridization can be applied to pairing events involving DNA: DNA, DNA: RNA and protein: antibody. The following techniques are used to find specific targets in populations of targets separated by electrophoresis.

Hybridization procedure	Nature
Southern blotting	DNA: DNA
Northern blotting	DNA: RNA
Western blotting	Protein: antibody

Amplification -

DNA replication technique can be utilized to increase the abundance of specific sequences in a process called Polymerase Chain Reaction (PCR). The selectively amplified sequences thus formed can be identified by the use of the appropriate label, e.g. radioactivity or fluorescence[90].

PCR is a process based on a specialized thermostable polymerase enzyme called Taq polymerase, which can synthesize a complementary strand to a given DNA strand in a mixture containing the 4 DNA bases and 2 DNA fragments (primers, each about 20 bases long) flanking the target sequence. The mixture is heated to separate the strands of double-stranded DNA containing the target sequence and then cooled to allow (1) the primers to find and bind to their complementary sequences on the separated strands and (2) The polymerase to extend the primers into new complementary strands. Repeated heating and cooling cycles multiply the target DNA exponentially, since each new double strand separates to become two templates for further synthesis. In about 1 hour, 20 PCR cycles can amplify the target by a million fold.

[90] Recombinant DNA and Genomics. Chapter 7, Molecular cell Biology, Lodish, Berk et al.

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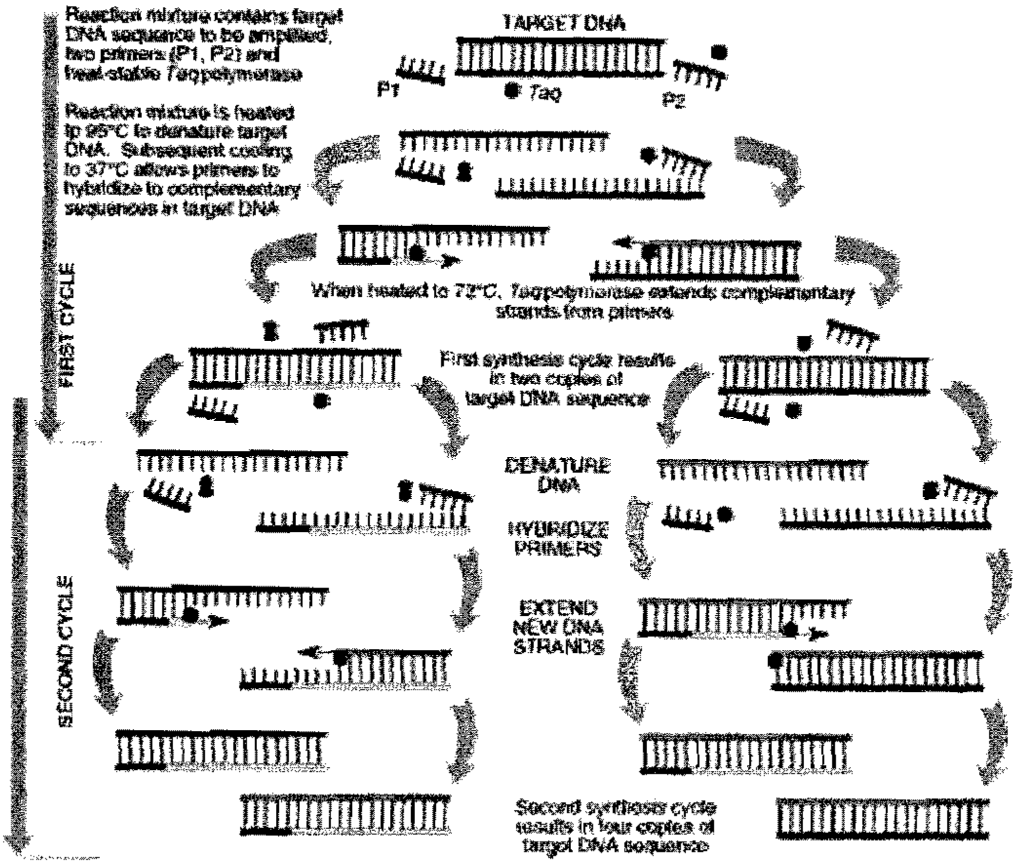
DNA Amplification Using Polymerase Chain Reaction



Source: DNA Science, see Fig. 13.

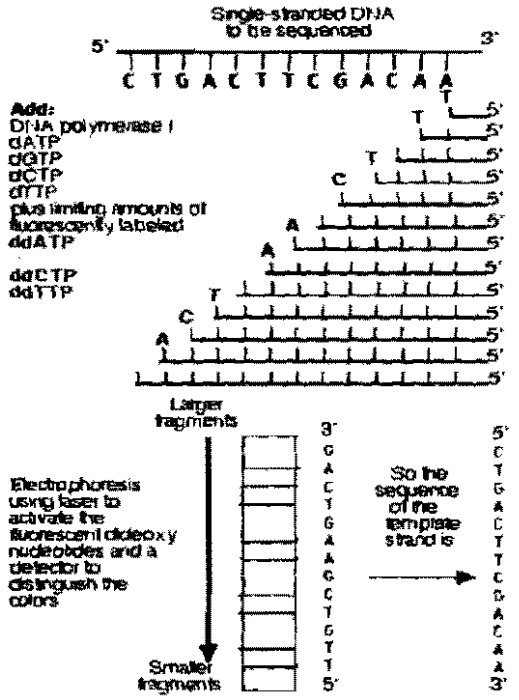
5. DNA sequence analysis

The purified DNA segment obtained either by cloning or amplified by PCR can be studied further to determine its nucleotide sequence. The common approach is to use chemical analogs of nucleotides to inhibit the enzyme DNA polymerase as it synthesizes the complementary strand to the original template to be sequenced. DNA synthesis is primed by a short oligonucleotide. DNA polymerase proceeds along the template sequence, incorporating either P32 or S35 labeled nucleotides into the newly synthesized sequences. Four inhibitory analogs, which compete with their respective deoxynucleotides, are used. For example, in a reaction to define the location of A (adenine) residues, an A analog is used, and DNA synthesis of individual molecules stop when an analog

substitutes regular A in the chain. So in the newly synthesized strands some stop at the first A while some at some the second A and so on.

Studies of this strand give the details of the positioning of A. Similar studies with the other bases are carried out. The reaction is further studied on electrophoretic gel, which yields a ladder of DNA sequences and is read as radioactive bands. The total study thus gives the nucleotide sequence of the DNA under study[91].

The figure below demonstrates an advanced version of the sequencing technique based on the same principle. Here 4 fluorescent dye tags are used to label the 4-deoxynucleotide analogs. This simplifies the reading of the sequence.[92]



Gene mapping

[91] Sangers Sequencing, Essentials of Human genetics

[92] http://users.rcn.com/jkimball.ma.ultranet/BiologyPages/D/DNAsequencing.html, visited on 25th Jan 200

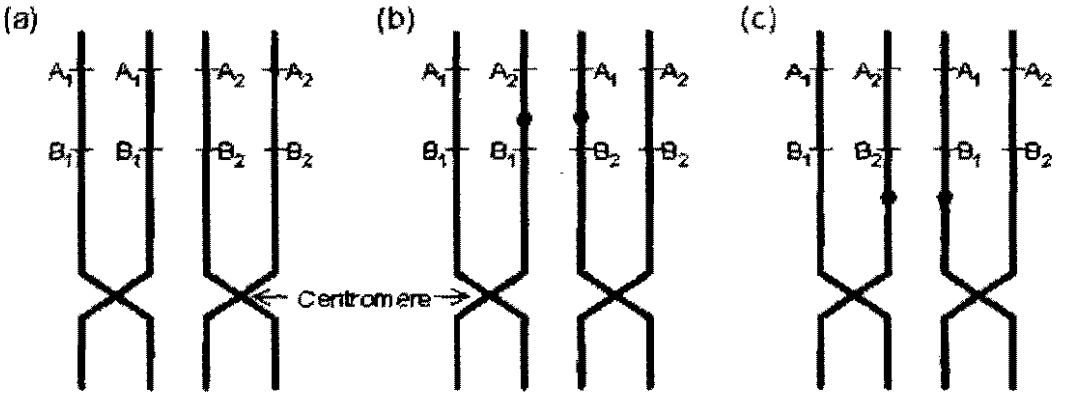
Gene mapping refers to the mapping of genes to specific locations on chromosomes. It is a critical step in the understanding of genetic diseases. The ultimate goal of gene mapping is to clone genes, especially disease genes. Once a gene is cloned, its DNA sequence can be determined and the protein products can be studied.

There are two types of gene mapping:

Genetic Mapping

Genetic mapping uses linkage analysis to determine the relative position between two genes on a chromosome.

The genetic mapping is based on the linkage between "loci" (locations of genes). If two loci are usually inherited together, they are said to be "linked". Two loci on different chromosomes are not linked, because they are usually separated by independent assortment.



As per this figure, the DNA crossover may cause recombination of loci A and B. A_1 and B_2 (or A_2 and B_1) are located on the same chromosome. The recombination frequency depends on the distance between the two loci and the position of crossover, which is called the chiasma. The closer they are, the less likely the recombination will occur, because recombination occurs only when the chiasma is located between the two loci.

To apply this basic principle to map a disease gene, one must analyze the pedigree and estimate recombination frequency.

The recombinant DNA tools reviewed in the preceding section can all be applied to development of markers for mapping. The principal types of DNA-based markers currently used for linkage mapping are ESTs, RFLP, SNPs, STS etc

Sequence tagged site (STS)

A sequence tagged site (STS) is a short DNA sequence about 200 to 500 base pair long, that has a single occurrence in a genome and whose location and base sequence are known . STSs are useful for localizing and orienting the mapping and sequence data reported from many different laboratories and serve as landmarks on the developing physical map of a genome.

Expression Sequence Tags

An expressed sequence tag or EST is defined as a short sub sequence of a transcribed protein coding or non-protein coding DNA sequence. ESTs can also be defined as STSs derived from cDNAs.

ESTs have been instrumental in identifying gene transcripts, gene discovery and sequence determination. These tags are also a useful resource for designing probes for DNA micro arrays used to determine gene expression.

Single Nucleotide polymorphism (SNP)

Single Nucleotide polymorphism or SNP is a DNA sequence variation occurring when a single nucleotide in the genome differs between the genomes of the members of the same species. SNPs are generally thought to be a form of point mutation which has evolved enough to recur in significant proportions in a species.

Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism is the identification of specific restriction enzymes that reveal a pattern difference between the DNA fragment sizes in individual organisms. To discover RFLPs, restriction enzymes are used to cut DNA at specific 4-6 bp recognition sites. Sample DNA is cut with one or more restriction enzymes and resulting fragments are separated according to molecular size using gel electrophoresis. Differences in fragment length result from base substitutions, additions, deletions or sequence rearrangements within restriction enzyme recognition sequences.

Although two individuals of the same species have almost identical genomes, they will always differ at a few bases. Some of these differences will produce new restriction sites and the banding pattern seen on a southern blot will thus be affected. For any given probe or gene, it is often possible to test different restriction enzymes until you find one which gives a pattern difference between two individuals. The less related the individuals, the more divergent their DNA sequences are and the more likely you are to find a RFLP.

RFLP is most suited to studies at the intraspecific level or among closely related taxa. Presence and absence of fragments resulting from changes in recognition sites are used for identifying species or populations.

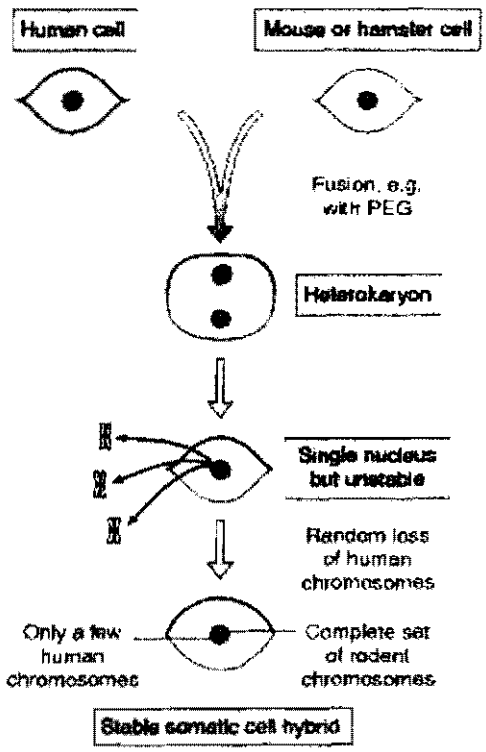
Physical Mapping

Physical mapping uses somatic cell hybridization or fluorescent in situ hybridization to determine the absolute position of a gene on a chromosome.

Somatic Cell Hybridization

Under certain experimental conditions, cultured cells from different species can be induced to fuse together, thereby generating somatic cell hybrids. In human genetic mapping, hybrid cells are typically constructed by fusing human

cells and mouse or hamster cells. The initial fusion products are described as heterokaryons because the cells contain both a human and a rodent nucleus. Eventually, heterokaryons proceed to mitosis, and the two nuclear envelopes dissolve. Thereafter, the human and rodent chromosomes are brought together in a single nucleus. However the hybrid cells thus formed are unstable. For reasons that remain unknown, most human chromosomes fail to replicate in subsequent rounds of cell division and are lost giving rise, eventually, to a variety of stable hybrid cell lines, each with full sets of rodent chromosomes plus a few human chromosomes. The loss of the human chromosomes occurs essentially at random, but can be controlled by selection.



The example in the figure shows how stable human-rodent somatic cell hybrids can be generated following initial fusion using polyethylene glycol. For reasons that are not understood, human chromosomes are selectively lost from the initial fusion products. The loss occurs essentially at random so that eventually the stable products of a single fusion experiment will include a variety of cells with different complements of human chromosomes. The human chromosomes

can be cloned to establish individual cell lines with a specific complement of human chromosomes. The identity of the human chromosomes can be established by PCR-based typing for chromosome-specific markers.

Fluorescence in situ hybridization[93]

Fluorescence in situ hybridization (FISH) uses fluorescent molecules to vividly paint genes or chromosomes. FISH is particularly useful for gene mapping and for identifying chromosomal abnormalities.

FISH involves the preparation of short sequences of single-stranded DNA, called probes, which are complementary to the DNA sequence of interest. The probes hybridize, or bind, to the complementary DNA and, because they are labeled with fluorescent tags, allow one to see the location of those sequences of DNA. Unlike most other techniques used to study chromosomes, which require that the cells be actively dividing, FISH can also be performed on nondividing cells, making it a highly versatile procedure.

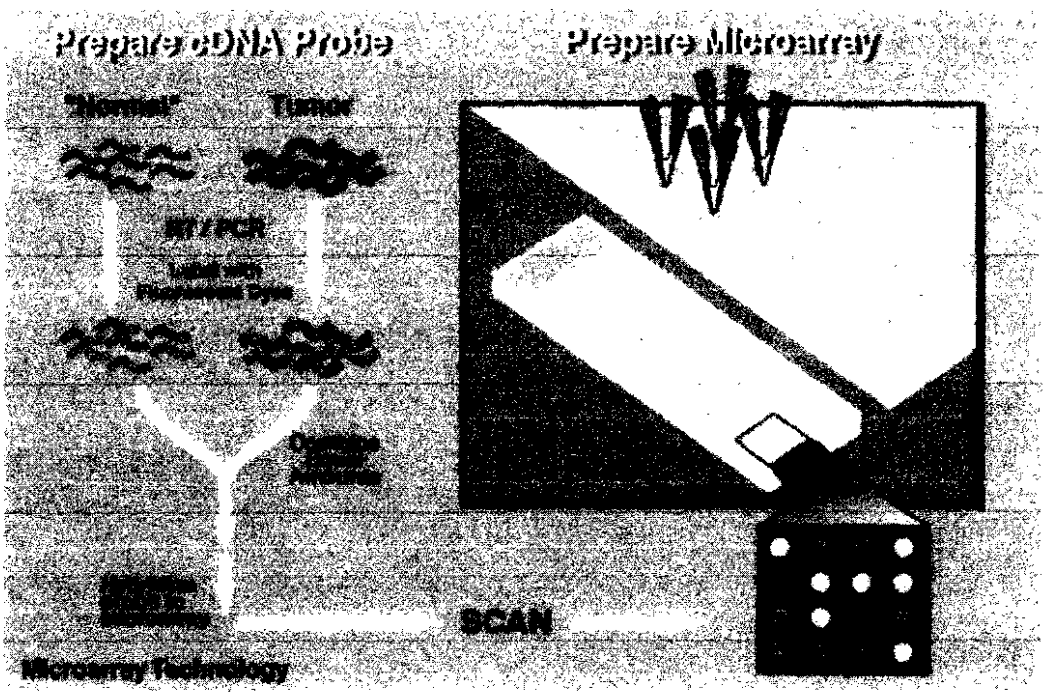
Fluorescence in situ hybridization (FISH)[94]

[93] NHGRI fact sheet, http://www.accessexcellence.org/RC/VL/GG/nhgri_PDFs/fish_TXT.pdf

DNA Microarray technology

Although all of the cells in the human body contain the same genetic material, the same genes are not active in all of those cells. Studying which genes are active and which are inactive in different kinds of cells helps one understand more about how these cells function and about what happens when the genes in a cell do not function properly.

In the past, scientists have been able to conduct genetic analyses on a few genes at once. With the development of DNA Microarray technology, however, one can now examine thousands of genes at the same time, an advance that will help them determine the complex relationships between individual genes.



[94] http://www.accessexcellence.org/RC/VL/GG/fish.html **visited on 27th January 2006**

Microarray is based on a database of over 40,000 fragments of genes called expressed sequence tags (ESTs). Minuscule amounts of hundreds or thousands of these ESTs are arranged on a single microscope slide by a robot. Next the genetic messenger molecules, which signal the production of proteins, from a particular cell are labeled with fluorescent tags and allowed to hybridize, or bind, to the ESTs on the slide whose sequences are complementary to those of the messengers. After a scanner measures the fluorescence of each sample on the slide, scientists can determine how active the genes represented by the ESTs are in the cell. Strong fluorescence indicates that many of the cell's messengers hybridized to the EST and that the gene is very active in the cell. Conversely, no fluorescence indicates that none of the messenger molecules hybridized to the EST and that the gene is inactive in the cell.

Gene Therapy

Gene therapy is a technique for correcting defective genes responsible for disease development. The technique aims to supplement a defective mutant allele with a functional one. Researchers may use one of the below mentioned approaches for correcting the defective gene[95].

- The most common approach involves the insertion of a normal gene into a nonspecific location within the genome to replace a nonfunctional gene.
- An abnormal gene could be swapped for a normal gene through homologous recombination.
- The abnormal gene could be repaired through selective reverse mutation, which returns the gene to its normal function.

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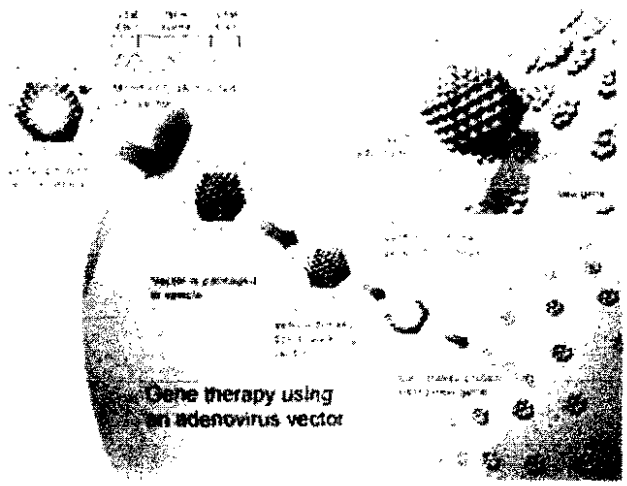
95 http://www.ornl.gov/sci/techresources/Human_Genome/medicine/genetherapy.shtml#whatis

- The regulation (the degree to which a gene is turned on or off) of a particular gene could be altered.

In most gene therapy studies, a normal gene is inserted into the genome to replace an abnormal, disease-causing gene. A carrier molecule called a vector must be used to deliver the therapeutic gene to the patient's target cells. Currently, the most common vector is a virus that has been genetically altered to carry normal human DNA. Viruses have evolved a way of encapsulating and delivering their genes to human cells in a pathogenic manner. Scientists have tried to take advantage of this capability and manipulate the virus genome to remove disease-causing genes and insert therapeutic genes.[96]



The figure above illustrates an example of Gene therapy using an Adenovirus vector. A new gene is inserted into an adenovirus vector, which is used to introduce the modified DNA into a human cell. If the treatment is successful, the new gene will make a functional protein.

Besides virus-mediated gene-delivery systems, there are several nonviral options for gene delivery. The simplest method is the direct introduction of

[96] US National Library of Medicine, visited on 26th January 2006

therapeutic DNA into target cells. The direct introduction method is limited in its application because it can be used only with certain tissues and requires large amounts of DNA.

Another nonviral approach involves the creation of an artificial lipid sphere with an aqueous core. The liposome, which carries the therapeutic DNA, is capable of passing the DNA through the target cell's membrane.

Therapeutic DNA also can get inside target cells by chemically linking the DNA to a molecule that will bind to special cell receptors. Once bound to these receptors, the therapeutic DNA constructs are engulfed by the cell membrane and passed into the interior of the target cell. The delivery system tends to be less effective than other options.

Researchers also are experimenting with introducing a 47th (artificial human) chromosome into target cells. The artificial chromosome would exist autonomously alongside the standard 46^{th} chromosome not affecting their workings or causing any mutations. The artificial chromosome would be a large vector capable of carrying substantial amounts of genetic code, and scientists anticipate that, because of its construction and autonomy, the body's immune systems would not attack it. However the difficulty lies in delivering such large molecules to the nucleus of a target cell.

Genetically Modified Organism (GMOs)

Genetically modified Organism or GMO is an organism whose genetic material has been altered using recombinant DNA techniques. The first GMO was created in 1973 by Stanley N. Cohen and Herbert Boyer.

GMOs include transgenic animals such as mice, transgenic plants or various microorganisms altered for the purpose of genetic research or for the production of pharmaceuticals.

Methods of Genetic Modification

Genetically modified Bacteria:

The genetic composition of bacteria can be altered by three different processes.

1. Transformation: Transformation is a process by which some bacteria are naturally capable of taking up DNA to acquire new genetic traits. This process facilitates foreign DNA uptake in presence of certain cations or by use of electric current.
2. Conjugation: In conjugation DNA is transferred from one bacterium to another via a temporary connecting tube of protein called pilus.
3. Transduction: Transduction refers to the introduction of new DNA into a bacterial cell by a bacteriophage.

Genetically Modified Plants

Genetically modified plants or transgenic plants are produced by adding one or more genes to a plant's genome, by a process called transformation.

The principal technique for the genetic modification of plants is based on a natural ability of the bacterium *Agrobacterium tumefaciens*. This bacterium infects plants and causes a tumor-like growth termed a crown gall. *A. tumefaciens* contains a plasmid that transfers from the bacteria into the infected plant and integrates into the plant's genome. The transferred genes cause the plant to form the gall, which houses the bacteria and produces nutrients that support the bacteria's growth.

Transgenic plants have been developed for a longer shelf life, disease resistance, herbicide resistance, and pest resistance. The first transgenic crop

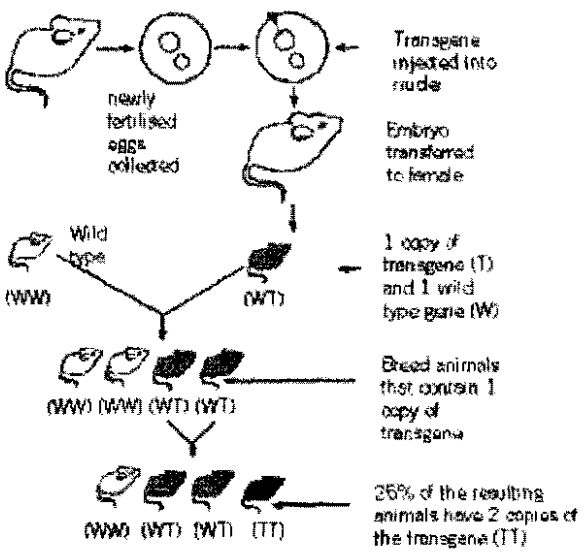
approved for sale in the US, in 1994, was the FlavrSavr tomato, which was intended to have longer shelf life.

Genetically Modified Animals

Like bacteria and plants, animals can be genetically modified by viral infection. However, the genetic modification occurs only in those cells that become infected, and in most cases these cells are eventually eliminated by the immune system.

In some cases it is possible to use the gene-transferring ability of viruses for gene therapy, i.e. to correct diseases caused by a defective gene by supplying a normal copy of the gene. The first genetically modified animals were transgenic mice, created in 1980.

Most transgenic animals have been produced using microinjection. Copies of a gene are injected into a newly fertilized egg with the aim of incorporating the gene into the egg's DNA. If this occurs, it will be copied each time the cell divides, and may be present in every cell in the body.



However the cells will contain only one insertion of the genetic material. Normally cells carry two copies of genetic sequences (one from each parent). Genetically Modified animals that carry two of the genetic insertions can be obtained through breeding. However, this method is inefficient because insertion into the DNA of the host cell is random. It can occur at any point. If it inserts into another gene then it may disrupt the gene's normal function. Such insertions would probably prove lethal before the animal was born, although in some cases the effect may only become apparent later in the animal's development.

Another approach is the genetic manipulation of stem cells isolated from embryos. New genes can be inserted into these cells or targeted genes can be 'knocked out'. The cells are introduced back into early stage embryos where they will give rise to mosaics animals where some cells are genetically altered and others are not. Breeding from those animals carrying the alteration in their sex cells will eventually produce GM animals that carry the alteration in all of their cells.

Human Cloning[97]

Cloning experiments involving plants and animal embryos have been performed for years. But experiments involving human beings have never been tried or thought possible, until "Dolly" the first mammal was cloned. This made the thought of human cloning realistic. However efforts in this direction have not progressed further due to moral and ethical issues related to human cloning.

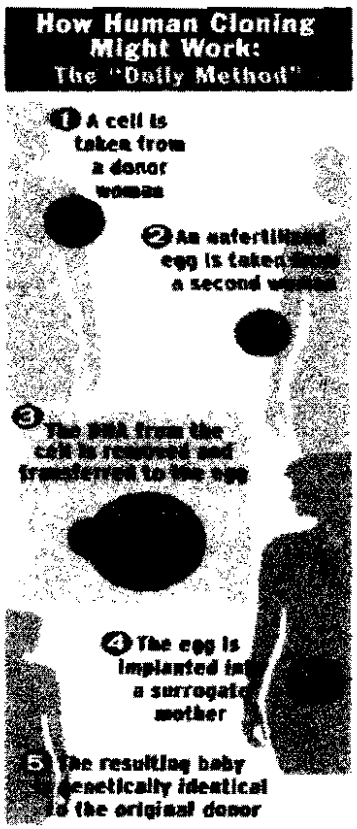
Dolly was cloned by a somatic cell nuclear transfer technique. This process includes fusing the DNA from a single cell taken from the ovary with the mammary cell. The fused cell then develops into an embryo, which is

[97] http://www.parliament.uk/post/pn157.pdf visited on 28th January 2006

implanted in the "surrogate" sheep. The embryo grows into a lamb, which is genetically identical to the donor sheep, thus giving rise to its clone.

Human cloning can be defined as the creation of a genetically identical copy of an existing, or previously existing human or growing cloned tissue from that individual.

Human cloning most probably can be done by a similar technique. A planned model for this process has been shown in the figure below.



When considering human cloning, it is important to think of how it could be used for the welfare of the society. A few advantages of human cloning are given below.

1. Never-ending supply of donor organs

2. Elimination of donor organ rejection