Proteomics: Priniciples and Techniques
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Indian Institute of Technology, Bombay

Module No.# 01
Lecture No. # 01
Introduction to Proteomics Course

I am Madhuri Gopal Digmurti, a student of Proteomics Course at the I IT Bombay and this is my pleasure to introduce my professor Sanjeeva Srivastava.

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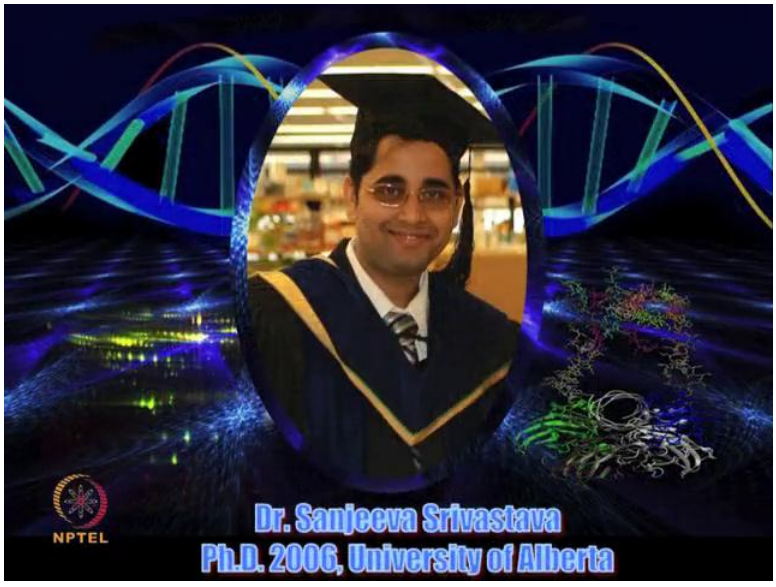

He obtained his doctorate degree from the University of Alberta, Canada in 2006.

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As a graduate student, he was a recipient of over 20 awards, including National Young Scientist Footsteps Award, in Canada.

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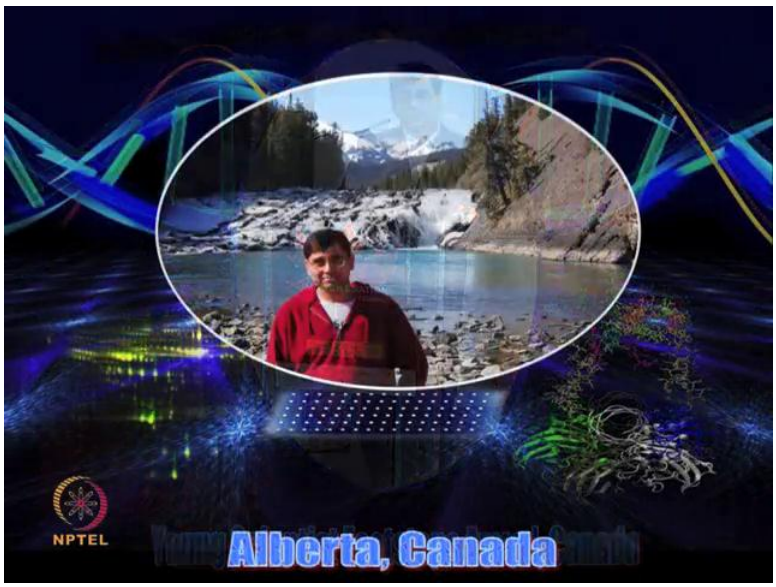

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From beautiful Alberta, he moved on to educational hub Boston, United States to pursue his post doctoral research at the Harvard Medical School in 2007.

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Supervision from his mentors, Professor Nathcav and George (( )) training in discovery and functional proteomics; experience of conducting proteomics course at the cold spring harbor laboratory, New York.

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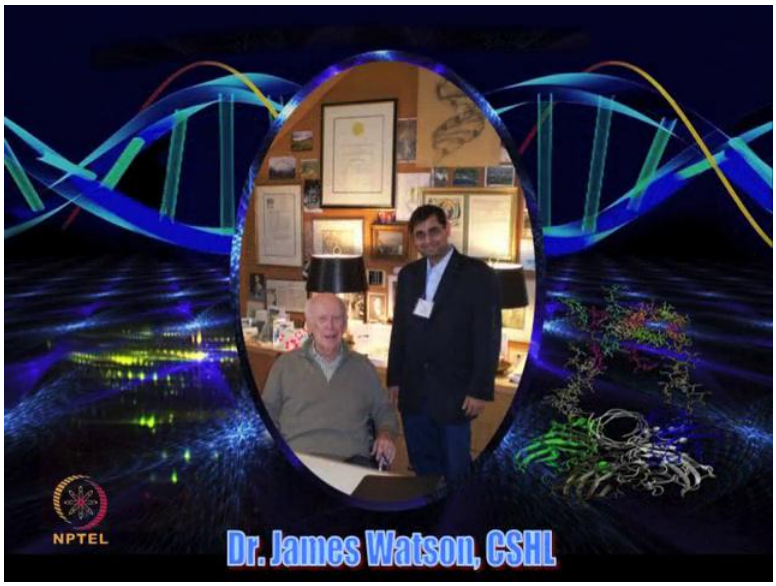

And interaction with leading scientist including Doctor James Watson, prepared him for his academic career in proteomics. He gave invited lectures in several world renowned institutes such as Max Planck Institute Germany, Oxford University UK, M I T Harvard, University College Dublin Monash University Australia, Estonia, china and many other countries.

Professor Srivastava joined I I T Bombay in 2009as an assistant professor, additionally he is a visiting professor in China and visiting scientist in United States. Current research in his group centres on using high throughput proteomics for bio marker discovery in cancer and infectious diseases, to study protein protein interactions and drug target discovery. His group has also developed e learning resources such as virtual laboratory at I I T Bombay, as a community resource to advance this knowledge frontier for the benefit of global health.

Doctor Srivastava has published several papers in high impact scientific genres; he serves on editorial board of several international genres .He is recipient of young scientist award from department of science and technology and department of atomic energy, India and apple research technology support award U K. Very recently; he has received the young investigator of year 2011 in I I T Bombay. Professor Srivastava has highly motivated us for the proteomics research atI I T Bombay. In N P T E L course, he

will take you to a journey of proteins, facilitate the knowledge and stimulate for proteomic research, thank you.

Welcome to N P T E L course on proteomics, my name is Sanjeeva Srivastava and I am in department of bio sciences and bio engineering of I I T Bombay. In this introductory lecture, I will discuss about proteomics, and provide an overview of the entire course, which consists of 40 lectures.

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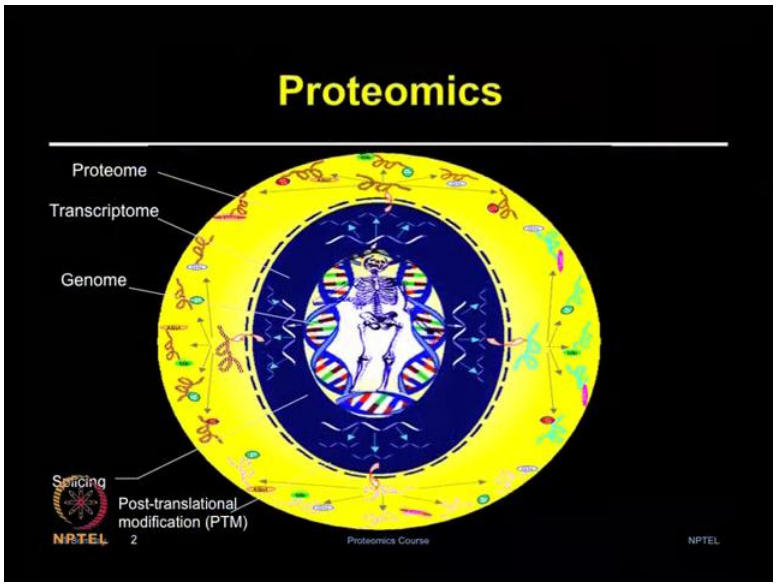

So first of all, what is proteomics? The proteome describes the protein compliment expressed by the genome or more precisely we can say, the protein compliment of a given cell at a given time including the set of all the protein isoforms, and its modifications. The study of entire compendium of proteins, which are encoated by the genome, is known as proteomics.

In this slide, I have illustrated the complexity of human proteome as compared to the genome or transcriptome. The extent of diversity and complexity due to alternatively splicing and post translational modifications is tremendous. Therefore, the studying proteins and proteome are very important.

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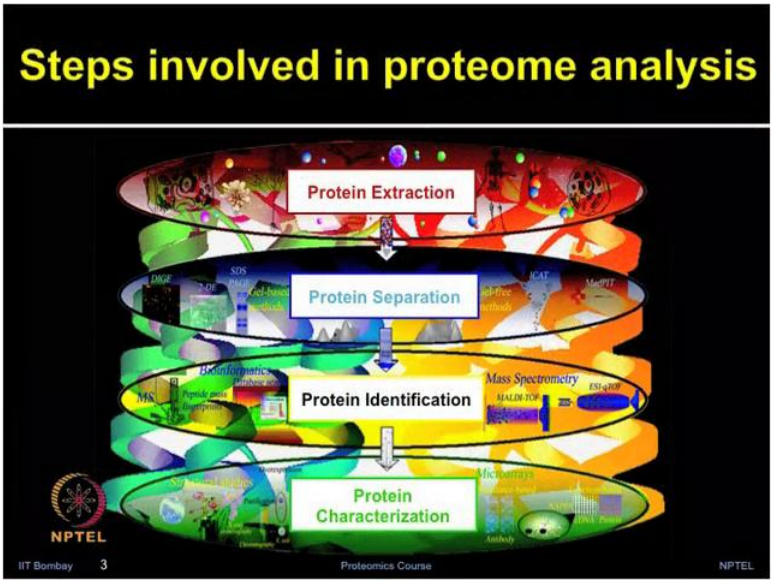

What are different steps involved in the proteome analysis? As shown here, the protein extraction, protein separation, protein identification and protein characterisation, these are the major steps, which are involved in proteome analysis. The protein extraction from whole cells, tissue or organism is first requirement for proteome analysis. Protein separation and quantification is achieved by various proteomic techniques, including gel based techniques such as two dimensional electrophoresis and gel free techniques such as (( )) mass spectrometry based techniques.

The functional characterisation of proteins using novel proteomic platforms open new horizon for exploration in biology. The proteomic discipline can be grouped under two major disciplines, abundance and function based proteomics.

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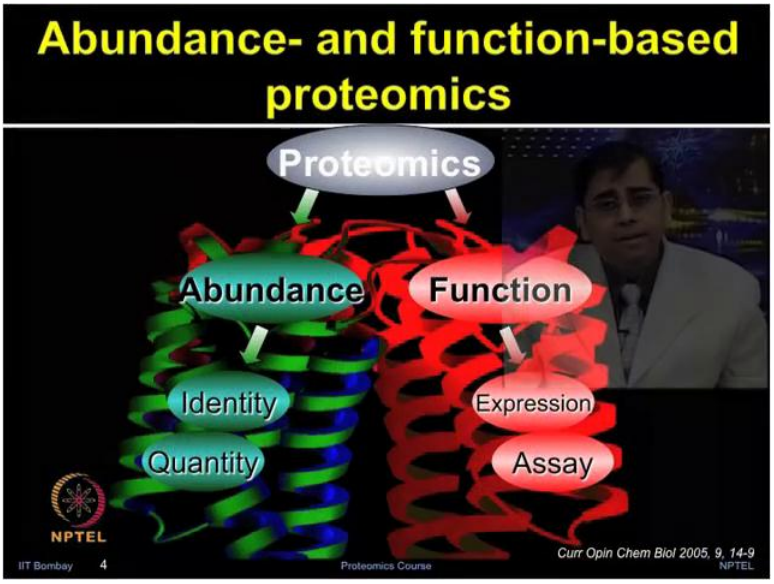

The abundance based proteomics aims to measure the abundance of protein expression whereas, the functional proteomics aims to determine the role of proteins by addressing protein interactions and their bio chemical activities.

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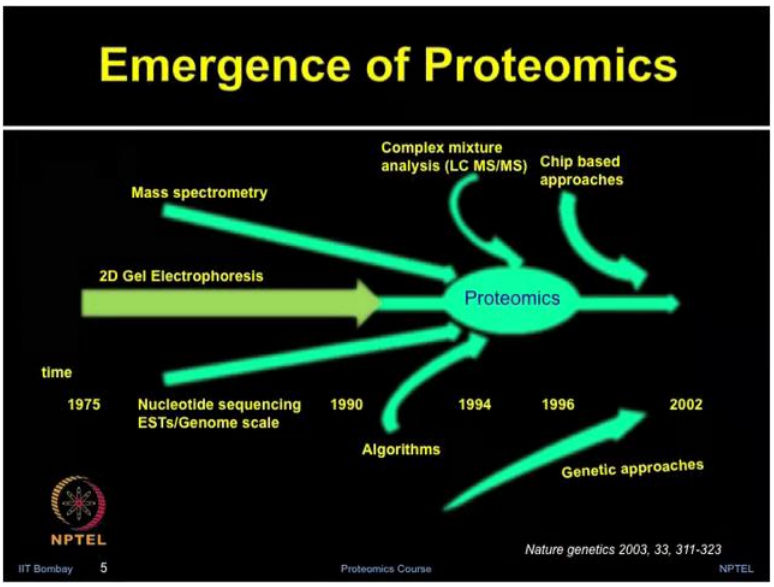

So, how did proteomics field emerge? As you can see in the time scale here, shown in the slide; advancement of various techniques such as two dimensional electrophoresis, and mass spectrometry, genome sequencing in formation and computational algorithm, together led to the emergence of proteomics field.

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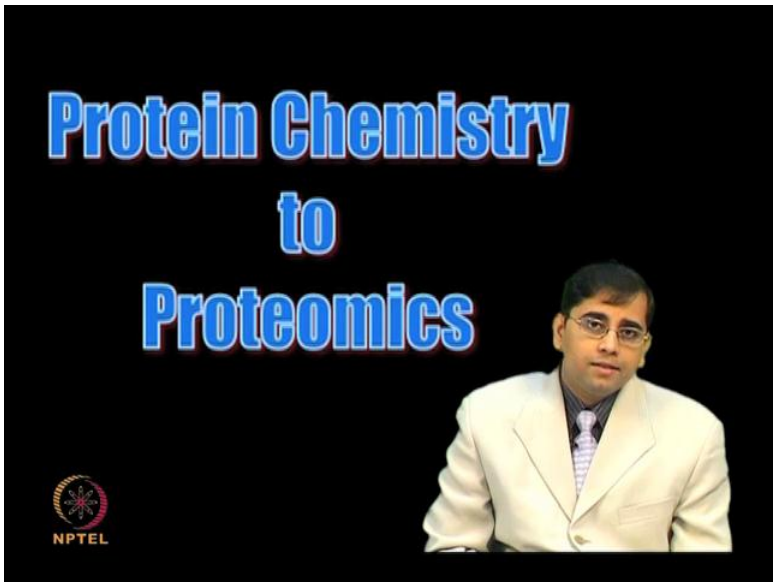

Proteomics research, originates from classical protein chemistry and it has embraced new high throughput techniques to analyse complex samples. Many of the techniques used under the modern proteomic umbrella. For example, two dimensional electrophoresis, mass spectrometry have actually originated several years ago.

So, what is new? The technological advancements in protein analysis with increase sensitivity resolution and capability to carry out, high throughput (( )) has led to the transition from protein chemistry to new field of proteomics. Protein analysis by mass spectrometry was challenging, due to complete degradation of samples with available hard ionisation techniques.

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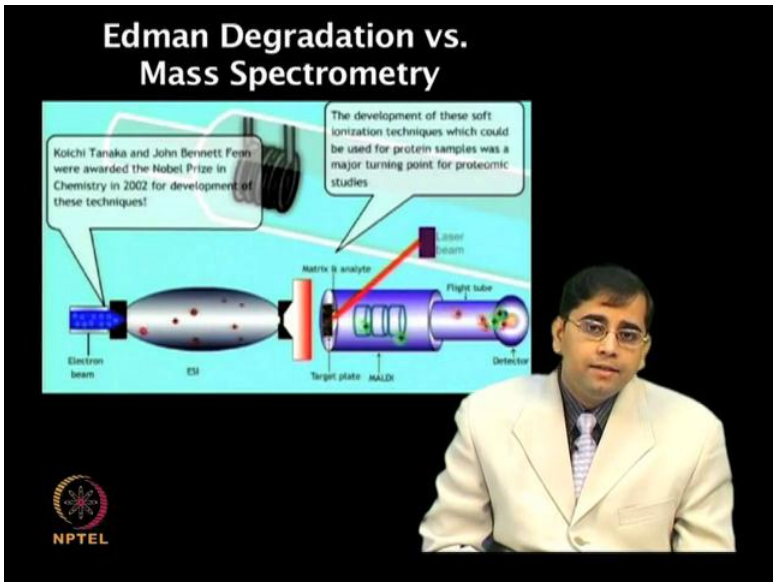

This limitation was overcome by soft ionisation techniques such as MALDI and electrospray ionisation, these techniques have greatly improved the proteomic studies as they facilitated mass spectrometry analysis of protein samples.

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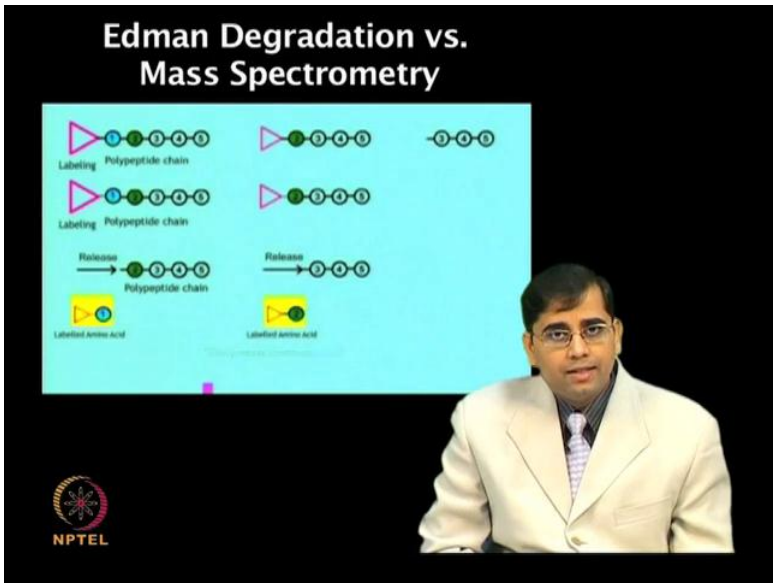

Protein sequencing by Edman degradation is time consuming and cumbersome. Several hours of sequencing are required for analysis of polypeptide chains.

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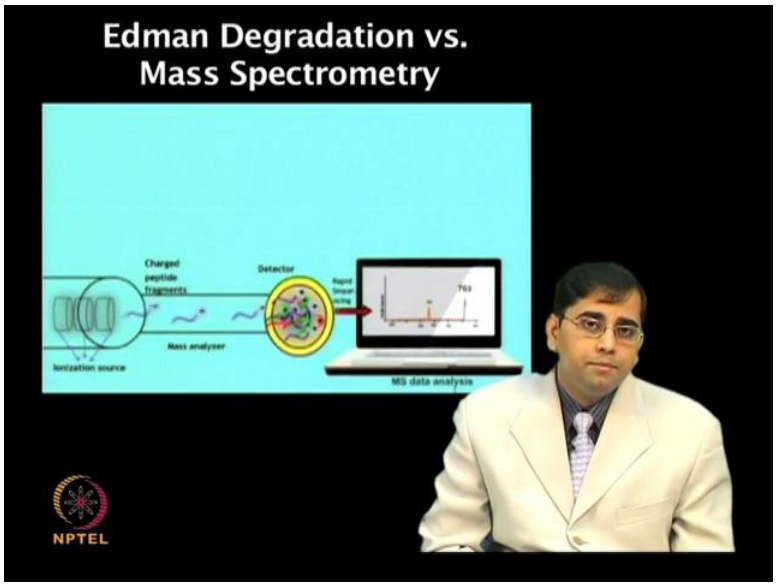

However, peptide sequencing by mass spectrometry is much faster and allows, large number of samples to be analysed in a short time.

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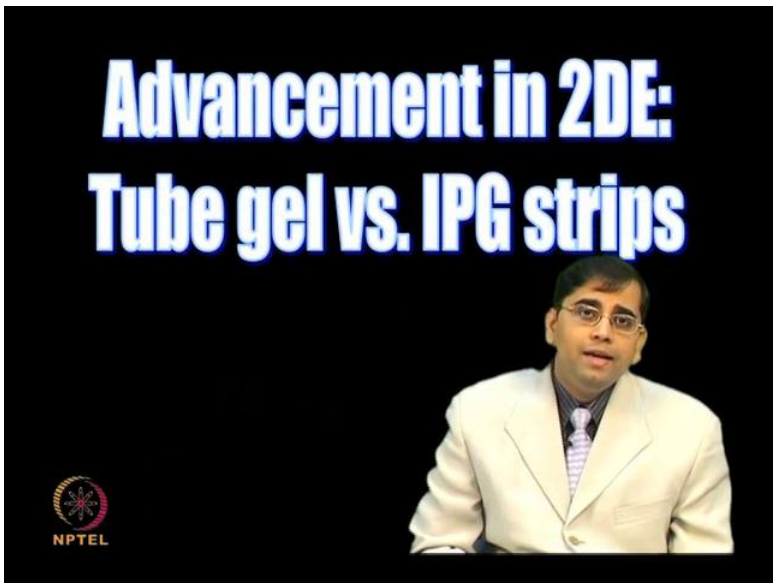

Another aspect, development of immobilised p H gradient strips, facilitates proteomic analysis using two dimensional electrophoresis. The p H gradient in tube gels are established by (( )), which are not always very stable.

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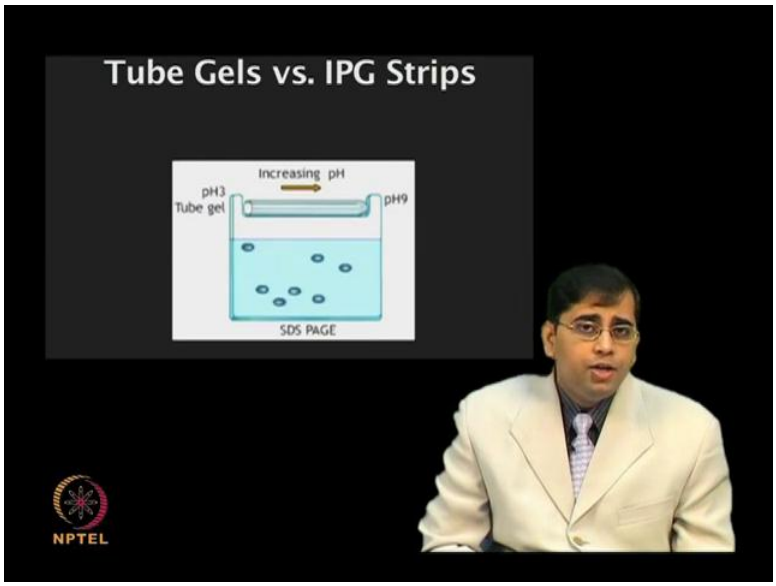

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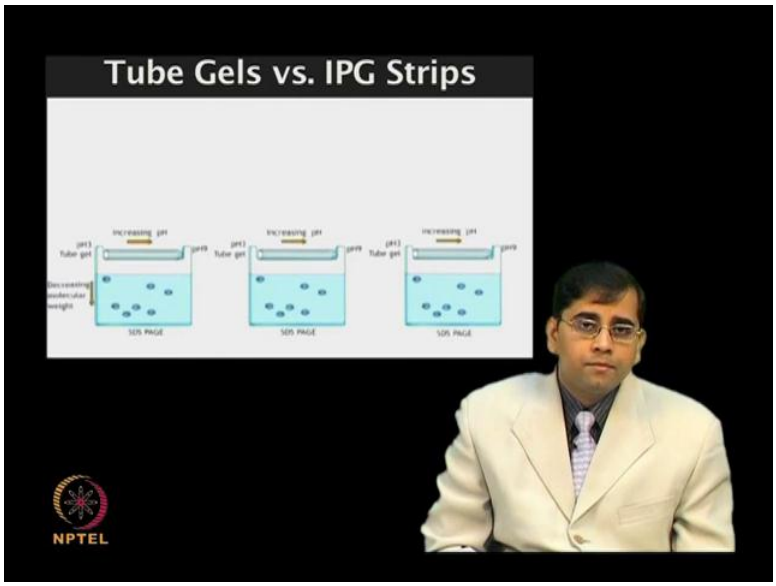

And tend to break down upon addition of the concentrated samples. Analysis of protein mixture by two dimensional electrophoresis using tube gels, often resulted into variations in the gels.

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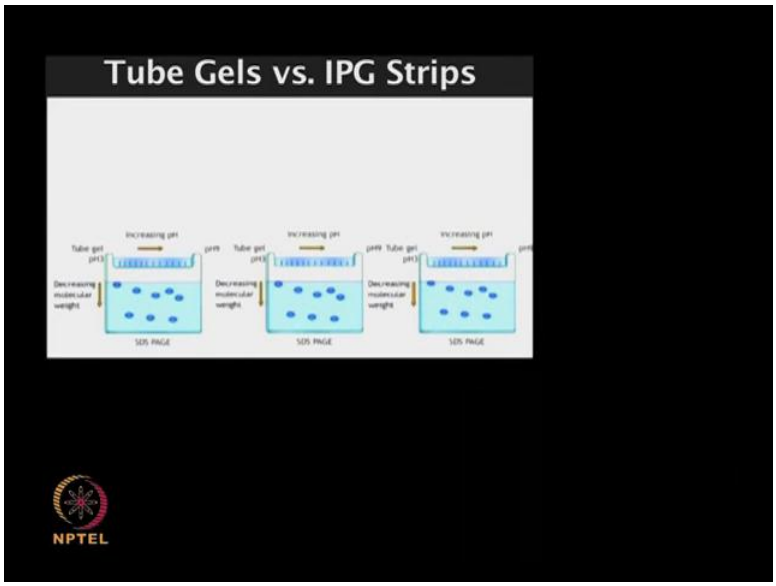

The problem of reproducibility was overcome to a large extent by the development of immobilised p H gradient strips or I P G strips. Minimal gel to gel variation was observed (( )) samples (( )) two dimensional electrophoresis employing I P G strips, which made this technique suitable for the large scale proteomic applications.

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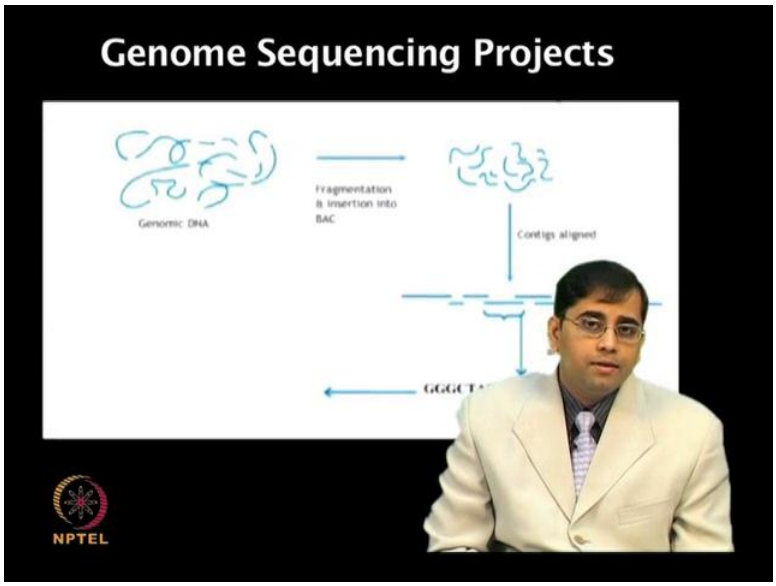

Completion of several genome sequencing projects, genome sequencing of several organisms, including humans have been successfully completed.

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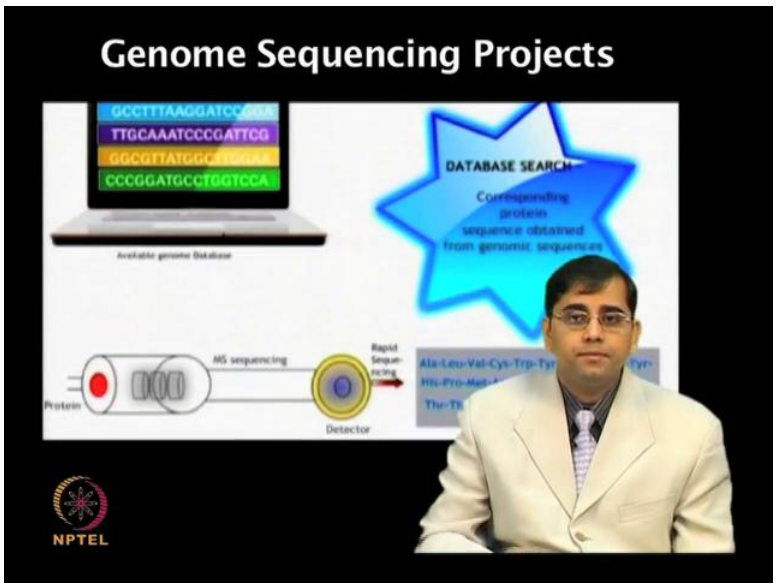

And these genome databases are extremely useful in correlation of gene and protein sequences. Several databases are now (( )) available which can easily help in identification of gene sequences of a protein, which has been sequenced by mass spectrometry. Genome represents an important starting point towards understanding complexity of biological functions. However, proteins provide a much more meaningful

insight into the mysteries of essential biological processes to obtain better understanding of cellular processes and regulation, there has been an increasing interest in studying proteome.

There are several reasons, why one needs to study proteomics? First, the genomic D N A contains large stretches of non coding regions, the pre-mRNA synthesised from the genomic D N A by the process of transcription.

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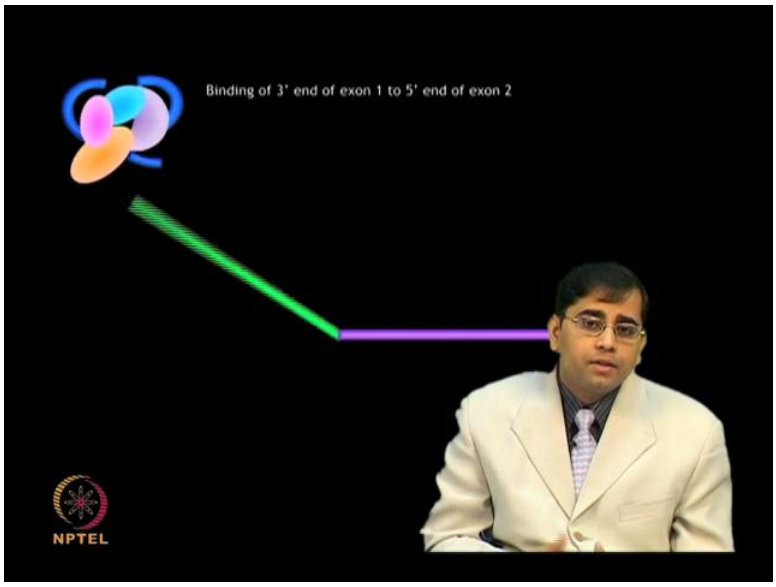

mRNA contains both exons, the coding sequences as well as introns, which are intervening, non coding sequences; by involving series of steps finally, the free 3 prime hydroxyl group of the 1 exon attacks the 5 prime end of the 2 exon such that, they are joined together to give the mature mRNA.

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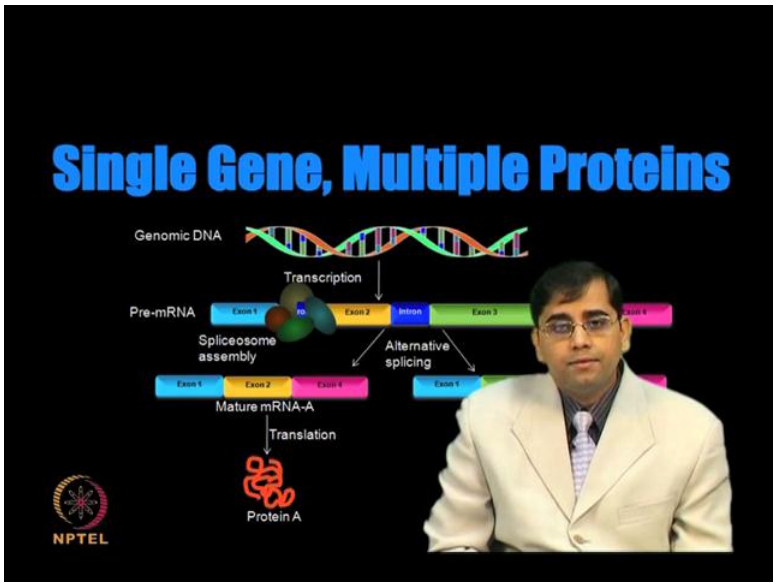

Second important factor is single gene can give rise to multiple proteins.

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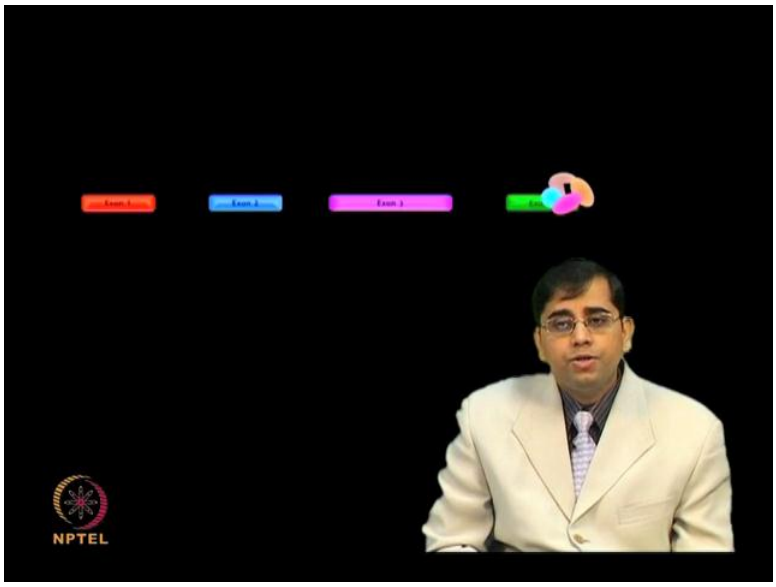

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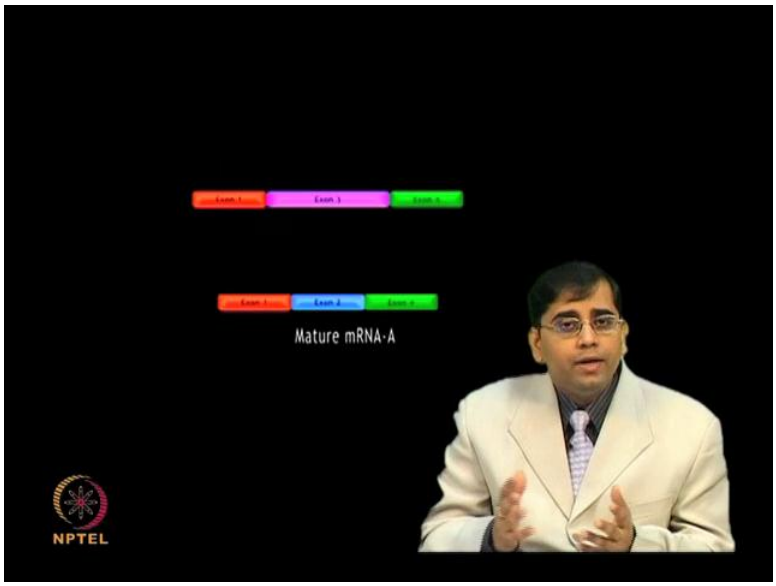

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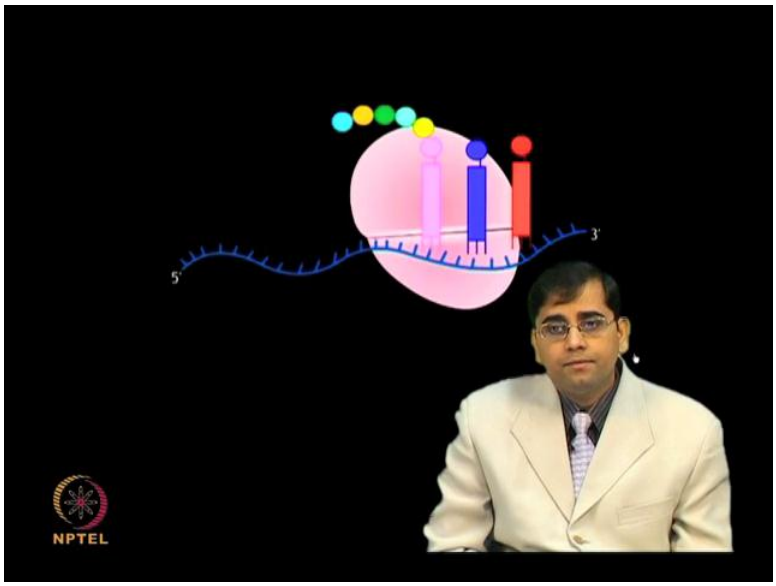

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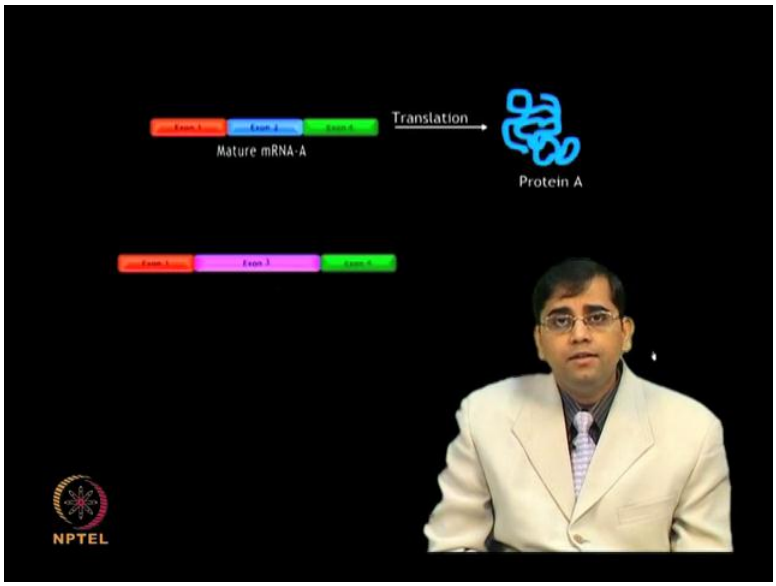

The alternative splicing is a process by which exons or coding sequences of pre-mRNA produced by transcription of a gene are combined in different ways during R N A splicing; resulting mature mRNA, give rise to different protein products by translation most of which are isoforms of one another. The diversity of proteins encoated by a genome is greatly increased, due to alternative splicing.

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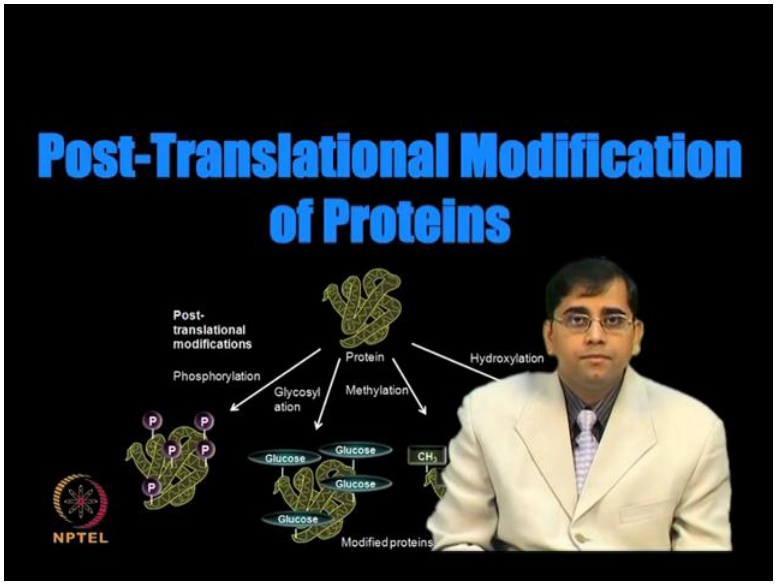

Third important factor is post translational modification of proteins.

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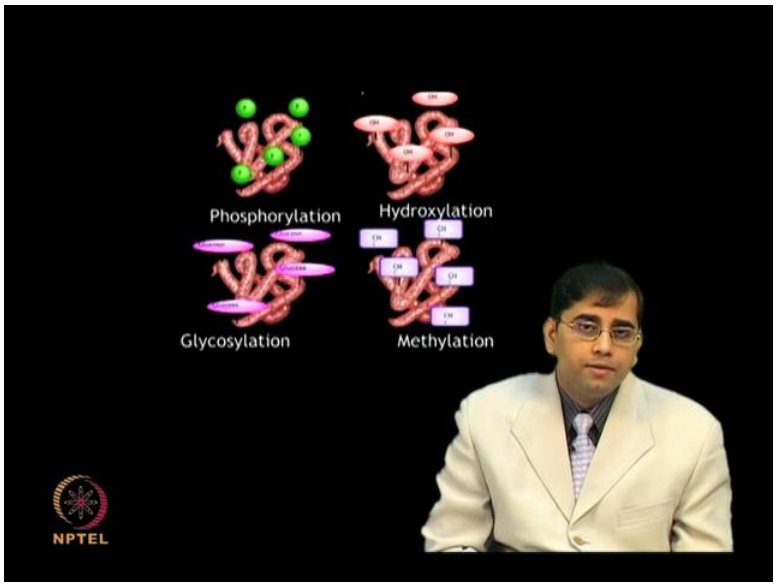

The protein obtained by translation undergoes folding and various post translational modifications such as phosphorylation, glycosylation, alkylation, hydroxylation etcetera to give the final functional protein.

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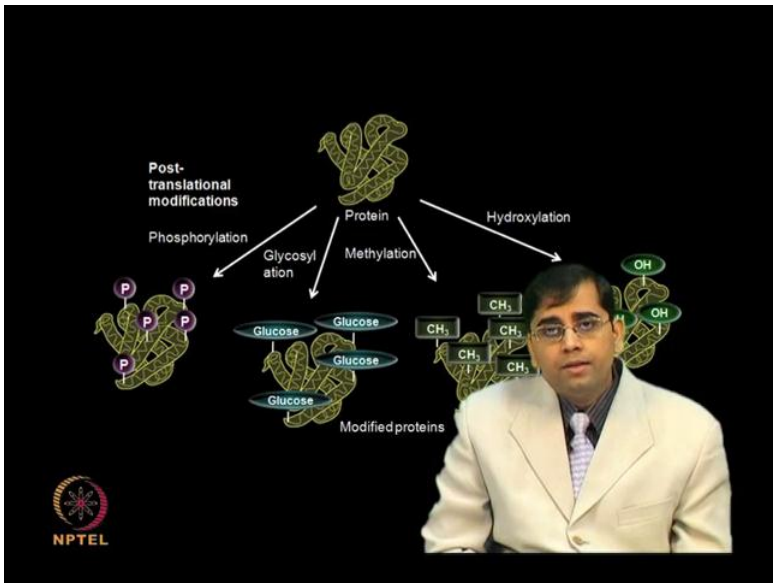

The p t m's generate diversity, complexity and heterogeneity of gene products and its functional consequences can be modulation in protein dynamics, and alteration of its functional activity.

During the last decade, we have witnessed the revolution in biology as the discipline has fully embraced omics tools. The emergence of genome wide analysis to understand cellular D N A, R N A and protein content by employing genomics, transcriptomics and proteomics at systems level has revolutionised, our understanding of control networks that, mediate this cellular processes, these concepts will be discussed in 1 module.

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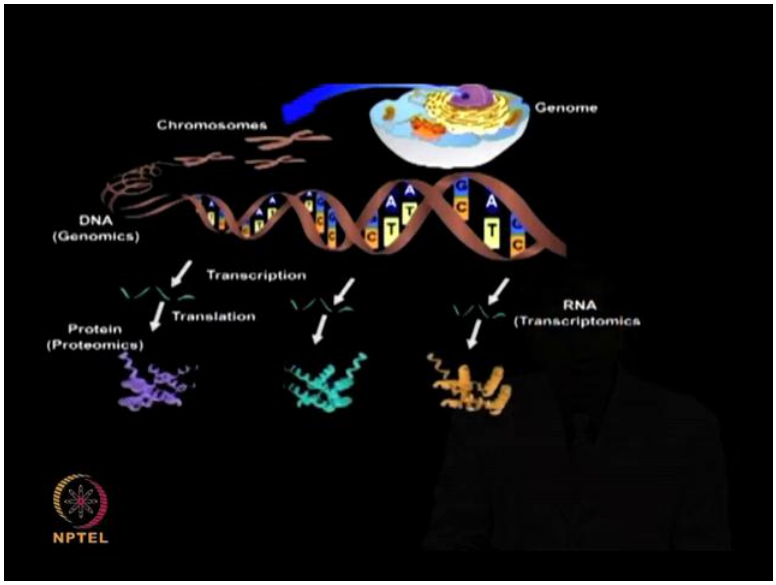

Genes are the blue print for life, and proteins are the effective molecules, due to this fact, the central dogma has guided research at the systems level. After completion of human genome sequence, number of genes around 25,000 are surpassed by an estimated number of proteins in millions. Studying large scale study of protein structure and function requires a thorough understanding of protein composition and their various structural levels by employing high throughput tools. Proteins play an important role in essential characteristic of living systems, how they function and replicate themselves through intricate molecular interactions.

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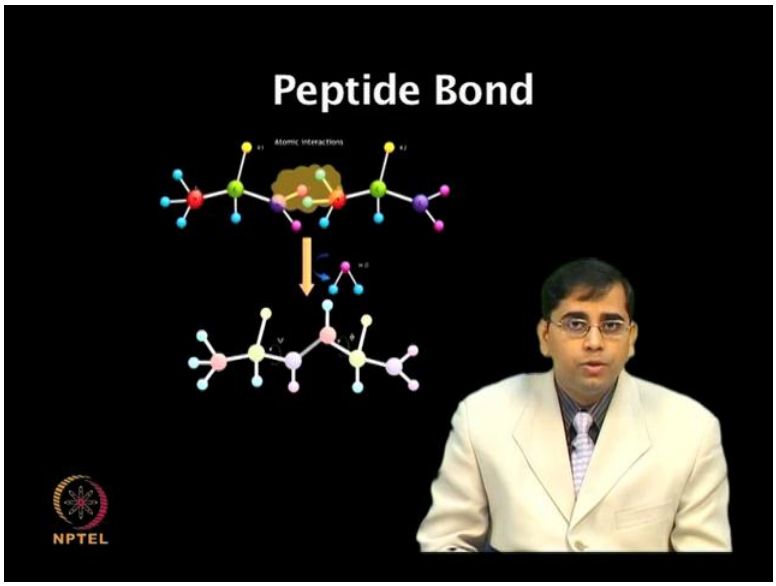

Amino acid constitutes the basic monomeric units of proteins, which are joined together by peptide bonds.

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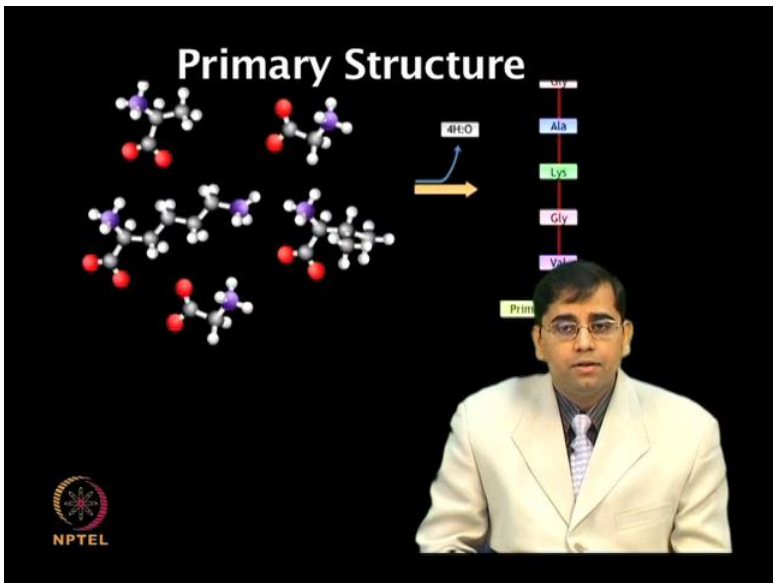

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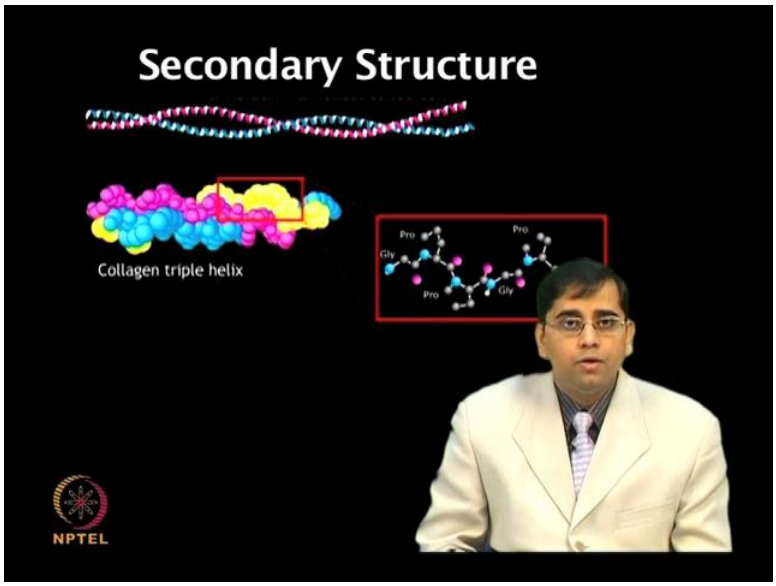

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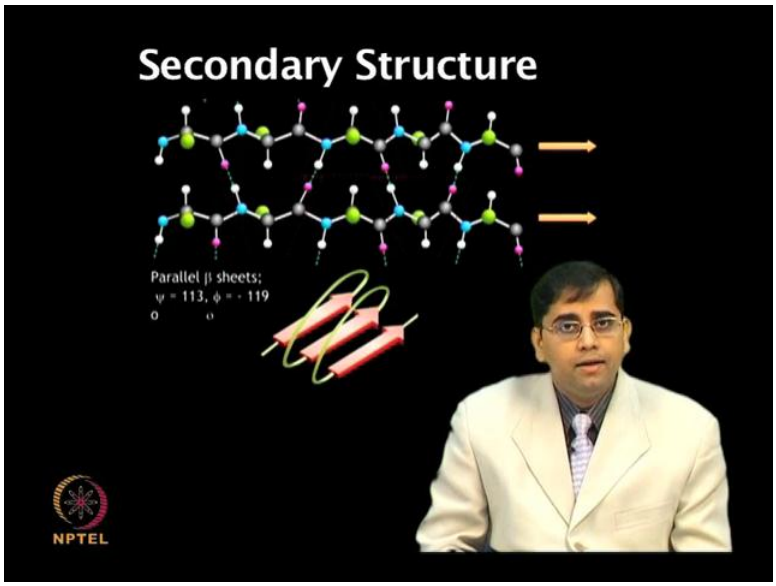

The linear sequence of amino acids constitutes the primary structure, holding of polypeptides or protein chain into regular structures like, alpha helices, beta sheets turns and loops, give rise to secondary structure.

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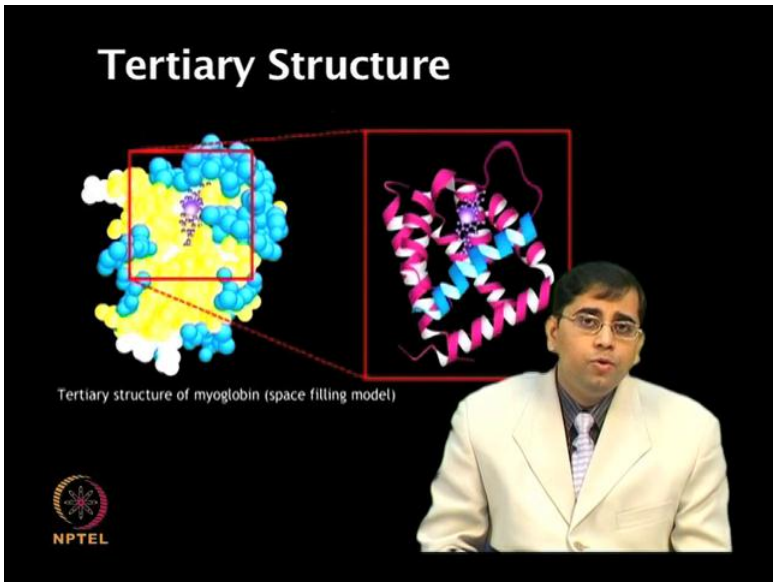

The three dimensional compactly folded a structure of proteins makes tertiary structure, which represents overall organisation of secondary structural elements in three dimensional space.

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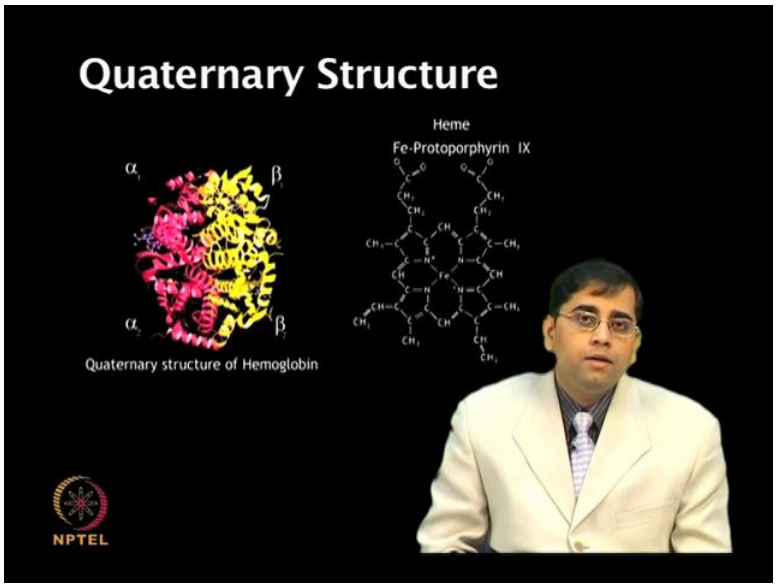

The quaternary structure refers to the interactions between individual proteins, sub units in a multi sub unit complex.

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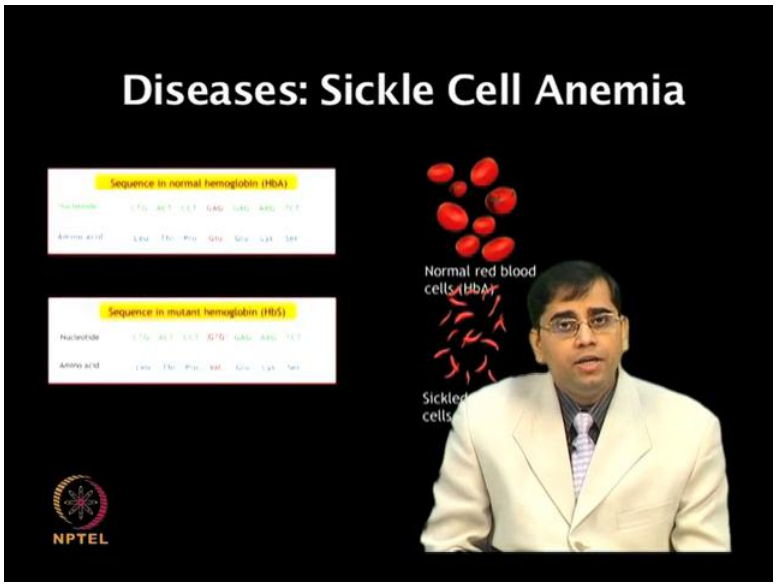

Sickle cell anemia is caused due to a single nucleotide substitution, which converts a glutamic acid residue to valid in the beta chain of hemoglobin.

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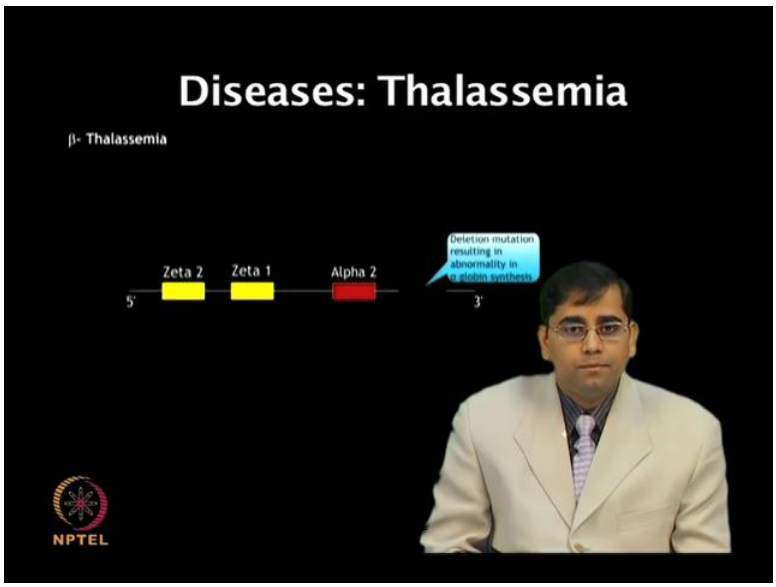

Thalassemia is caused due to abnormalities in hemoglobin synthesis.

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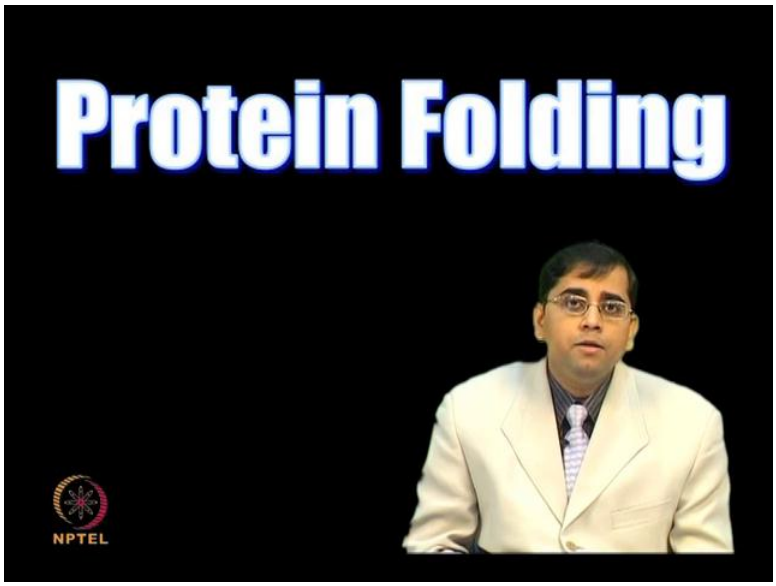

Protein folding is an elegant example of biological self-assembly, understanding the mechanisms through which protein folding takes place remains challenging for the scientific community.

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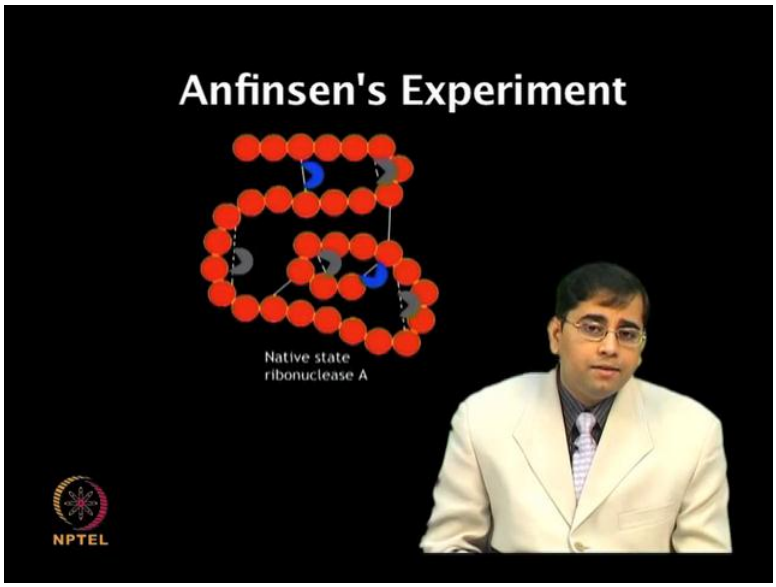

Anfinsen tested the ability of reduced and unfolded protein to a spontaneously fold into its (( )) by using protein ribonucleaseA. This study established that, the primary amino acid sequence of a protein content all of them formation necessary for the proper folding into its native form.

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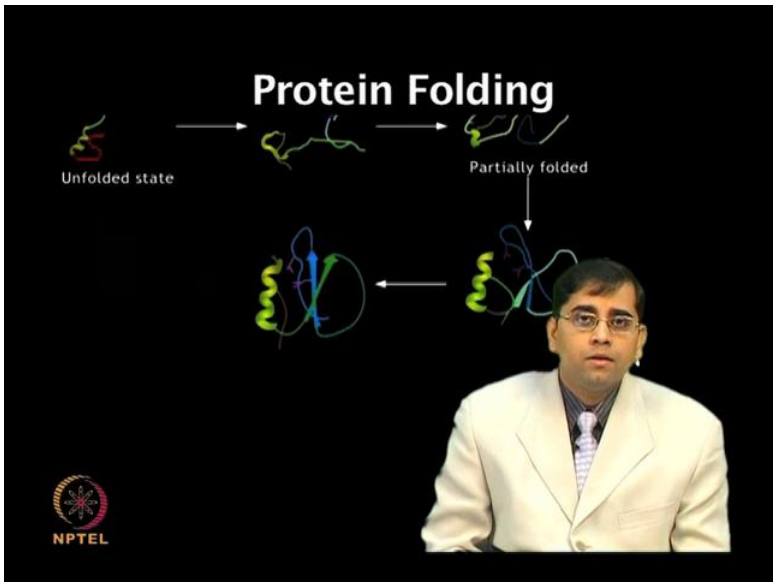

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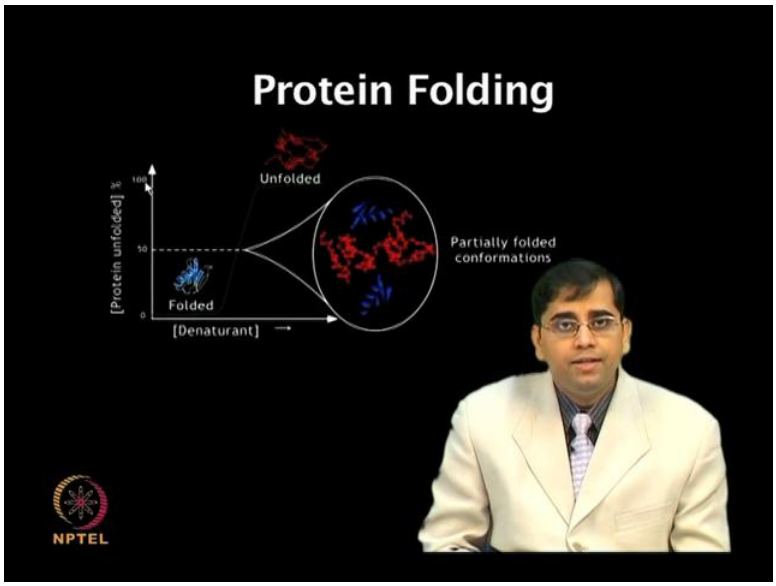

Protein folding is a cooperative process, which arises from simultaneous formation of multiple interactions within a polypeptide chain.

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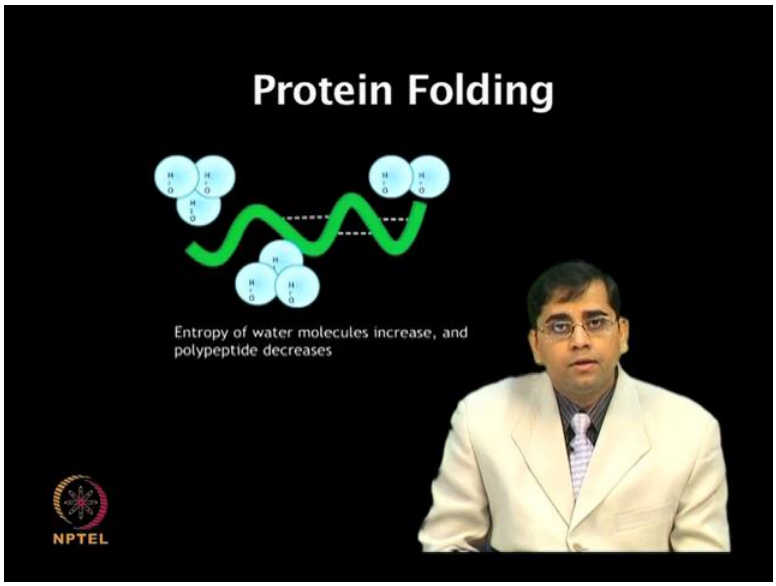

Protein folding is thermodynamically favourable, and a spontaneous process.

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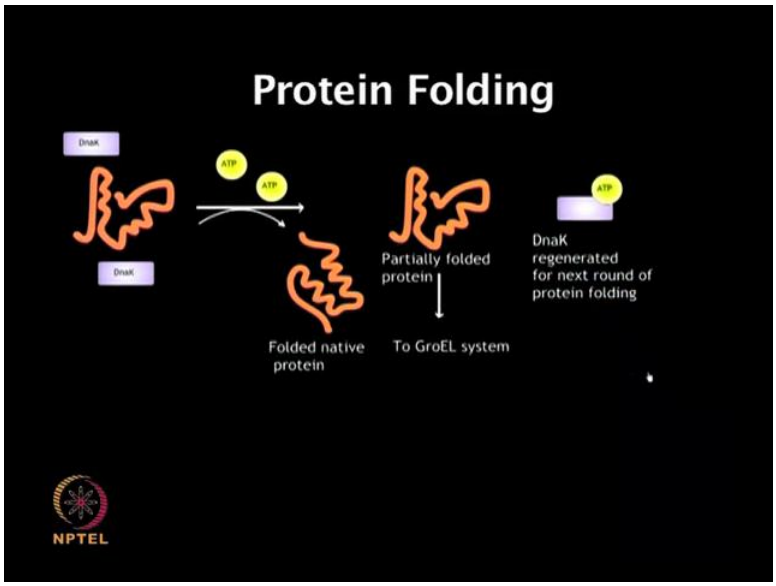

The folding efficiency could be limited by processes such as protein aggregation. The molecular chaperones are designed to assist in protein refolding.

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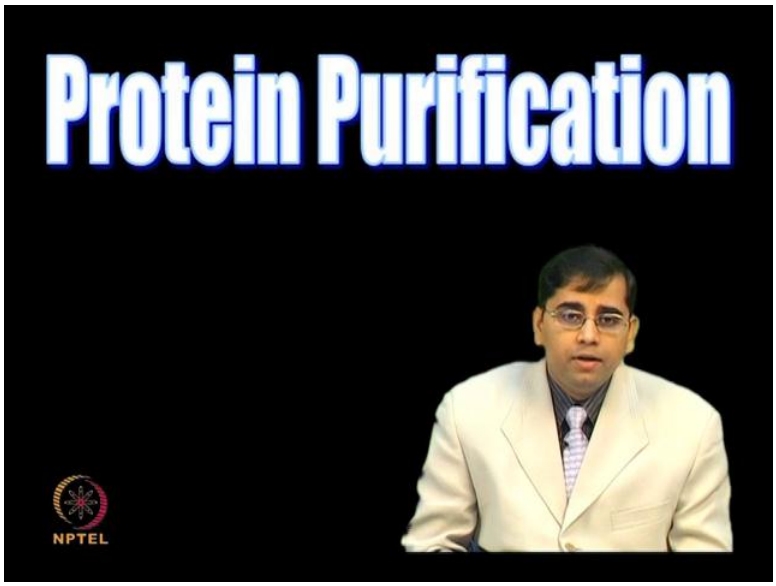

Some complex proteome it is challenging to purify a protein in a single chromatographic strip. Therefore, sequential prefractination (( )) involving different modes of chromatography becomes necessary.

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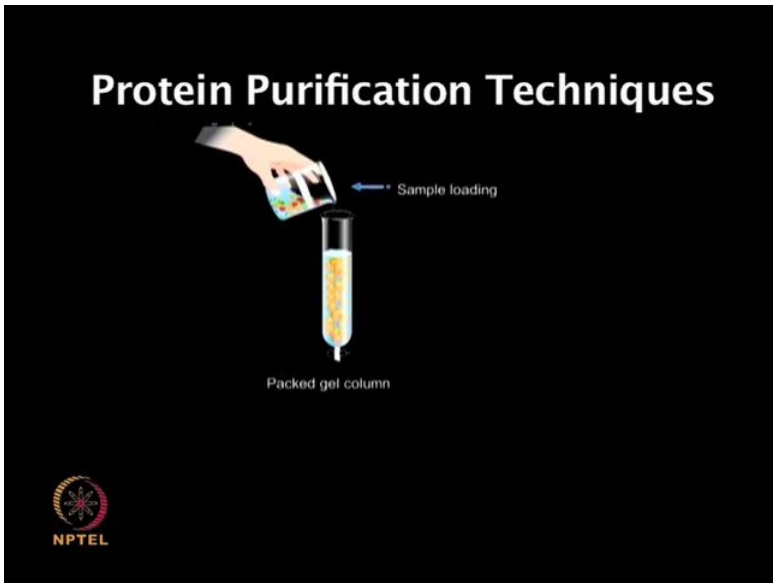

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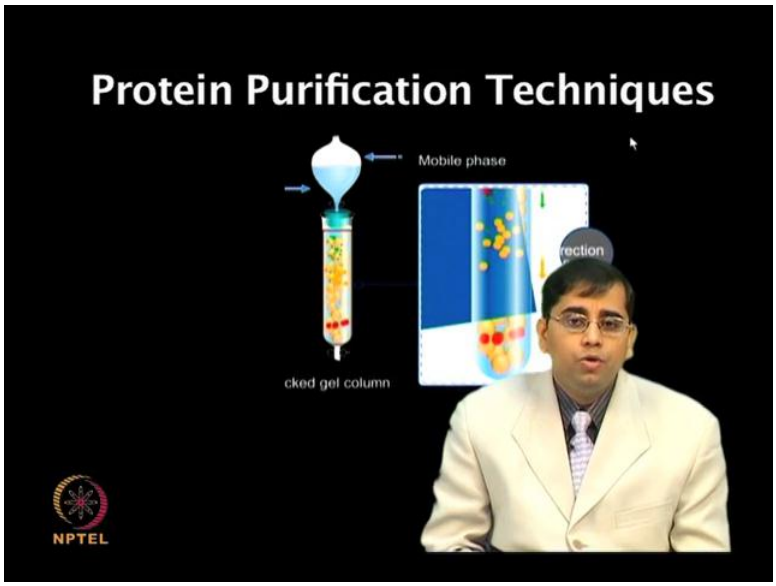

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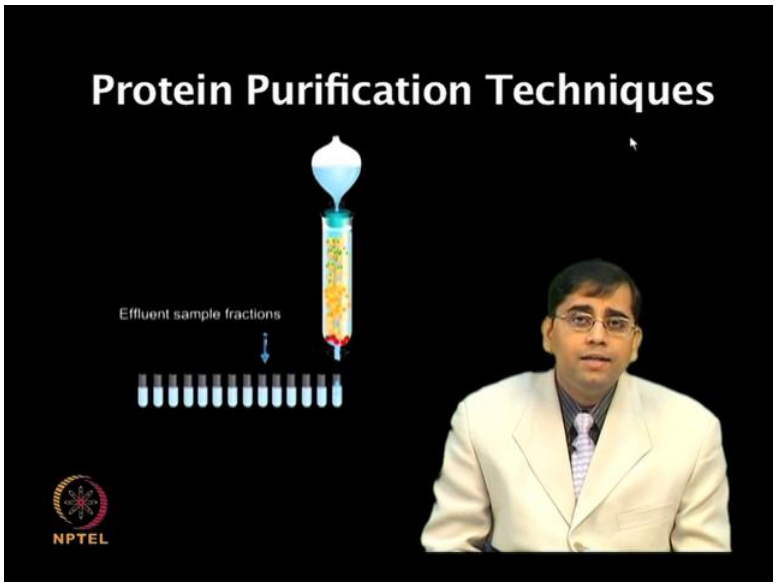

The gel filtration chromatography separates protein on the basis of difference in size, where a protein sample is applied to the column, the small proteins passes from the ports of the (( )), while the larger proteins are excluded. Therefore, this technique is also known as molecular exclusion technique.

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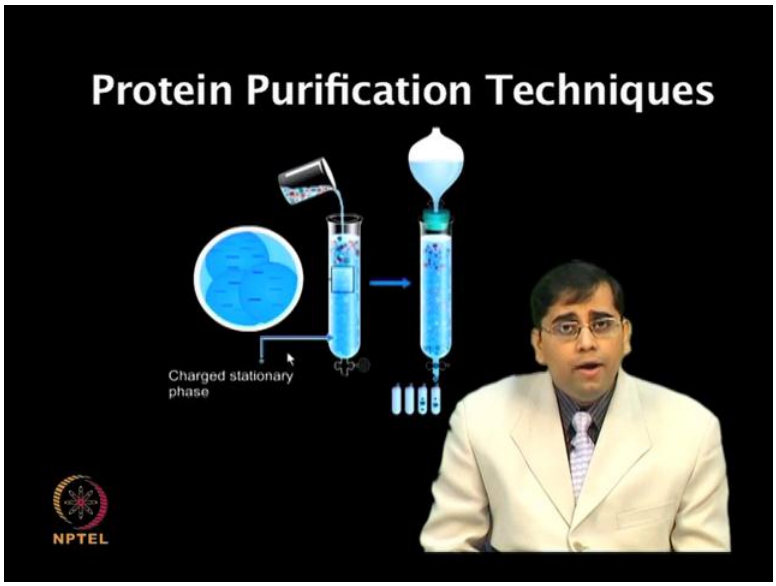

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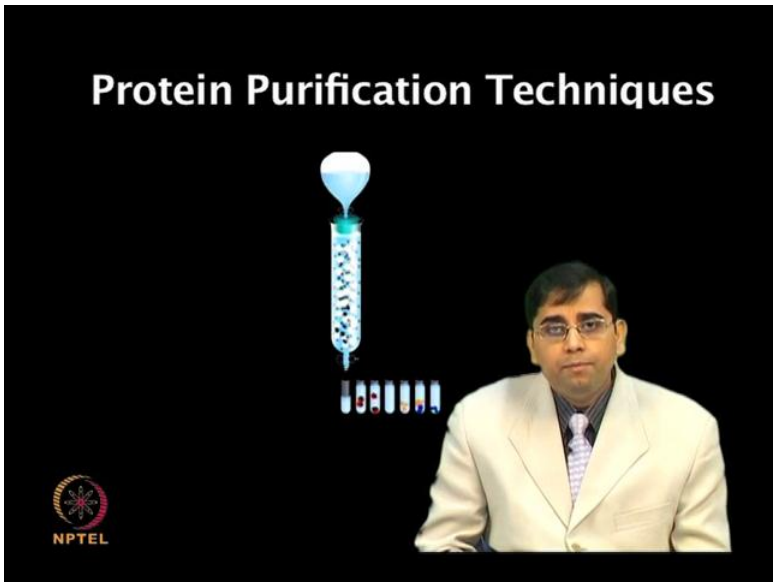

The ion exchange chromatography relies on differences between number of charges and distribution of charge groups in (( )) p H and solvent solutions.

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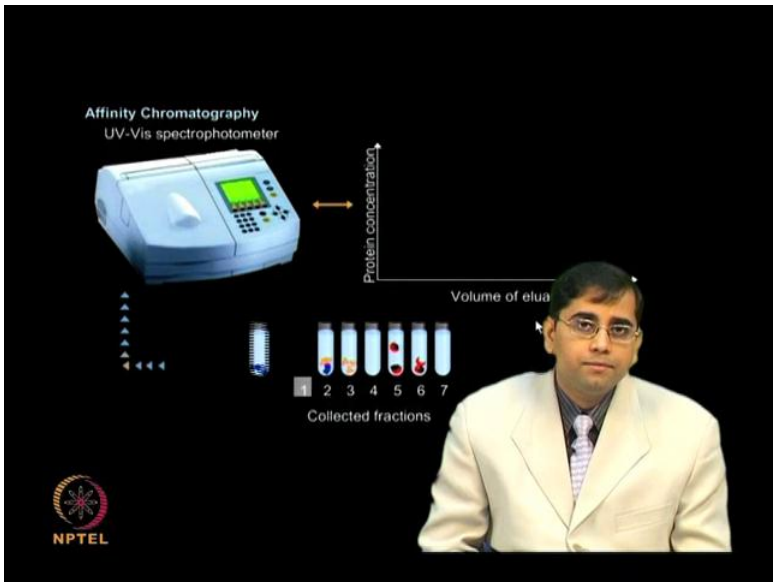
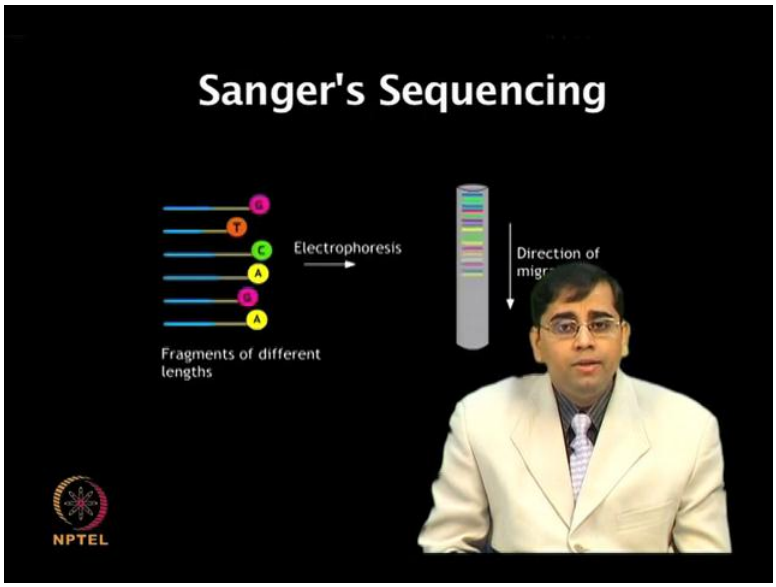


Affinity chromatography is based on affinity of protein to other bio molecules, where protein of interest binds through its specific interaction. We studying genome of an organism by employing sequencing, and genome mapping is known as genomics.

(Refer Slide Time: 22:19)



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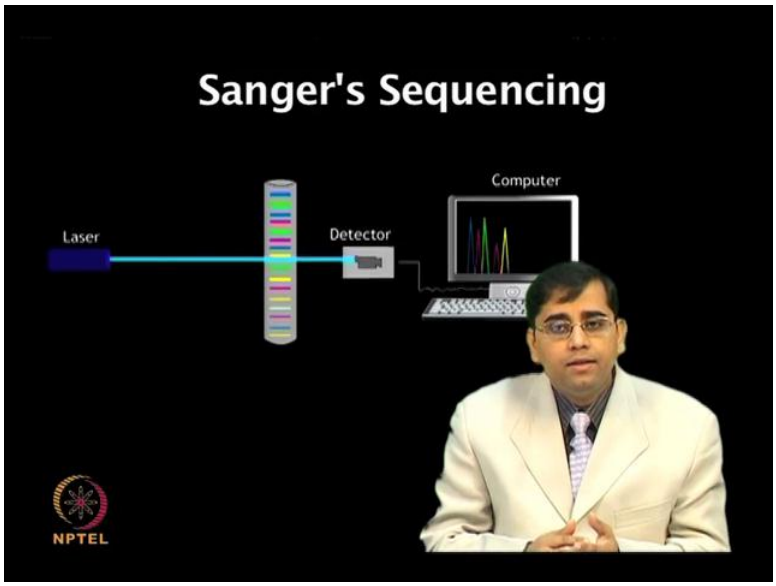

Several genome sequencing projects that aim to elucidate the complete genome sequence of organisms have been undertaken by several research groups, all over the world.

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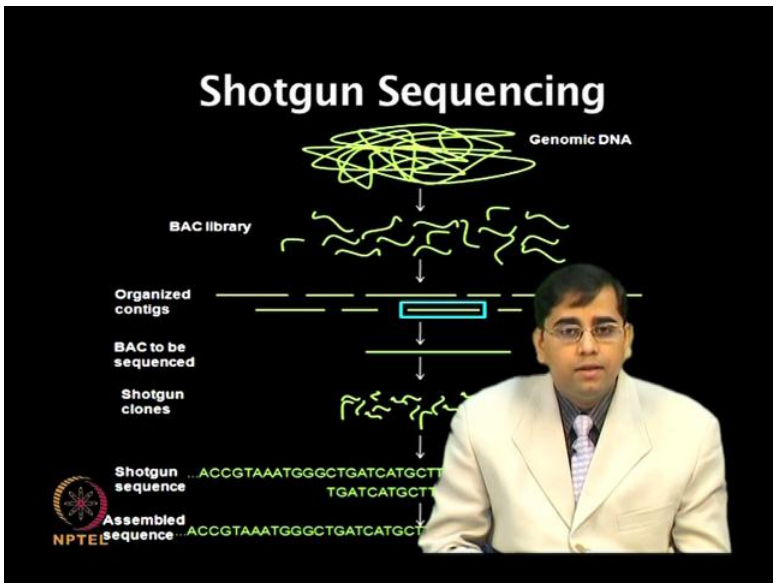

From a genomic library closed well isolated and ordered into a detailed physical map. Further, individual clones were sequenced by shotgun sequencing to provide the complete genome sequence.

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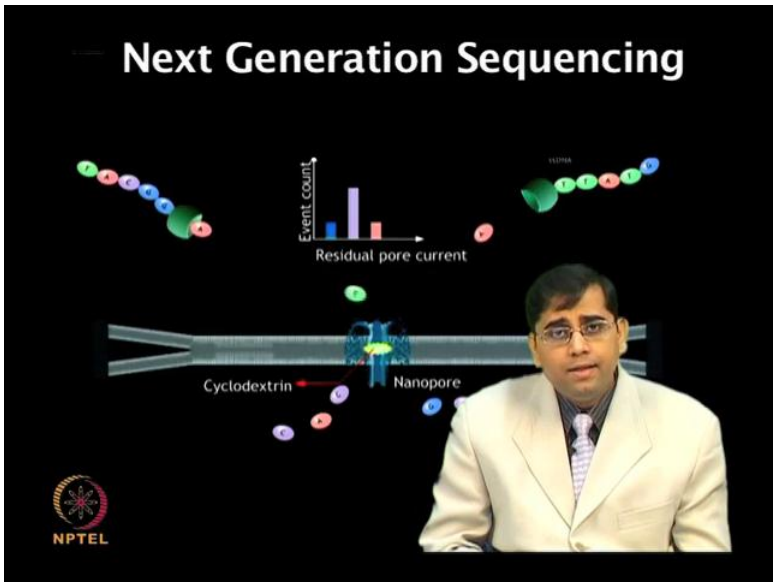

Recently, Next Generation Sequencing N G S is strategies have dramatically increased the phase of sequencing by several order of magnitudes. Next generation sequencing based on nanopore structures is known as nanopore sequencing.

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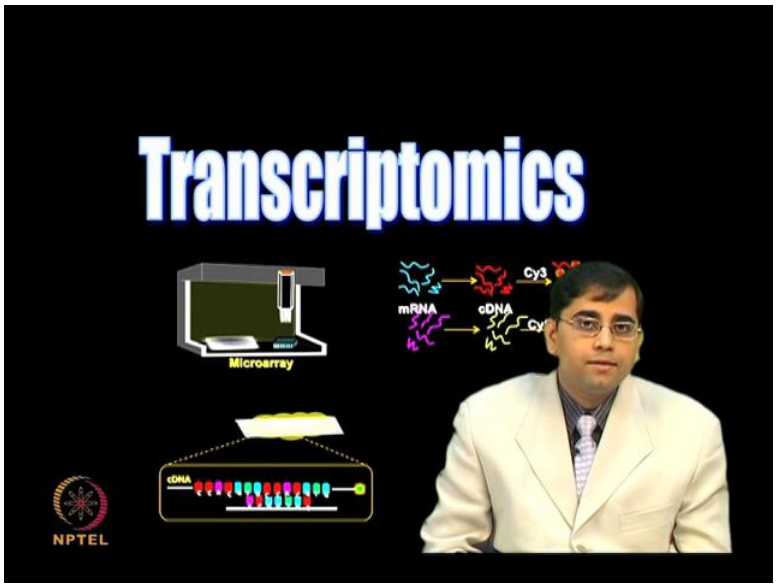

Transcriptomics is study of all the mRNA molecules expressed by a particular cell type of an organism is known as transcriptomics. The transcriptomics analysis, measures the genes that are been actively expressed at any given time and varies significantly with

external environmental condition. Various techniques such as microarray,(( )) etcetera have been widely used for transcriptional analysis.

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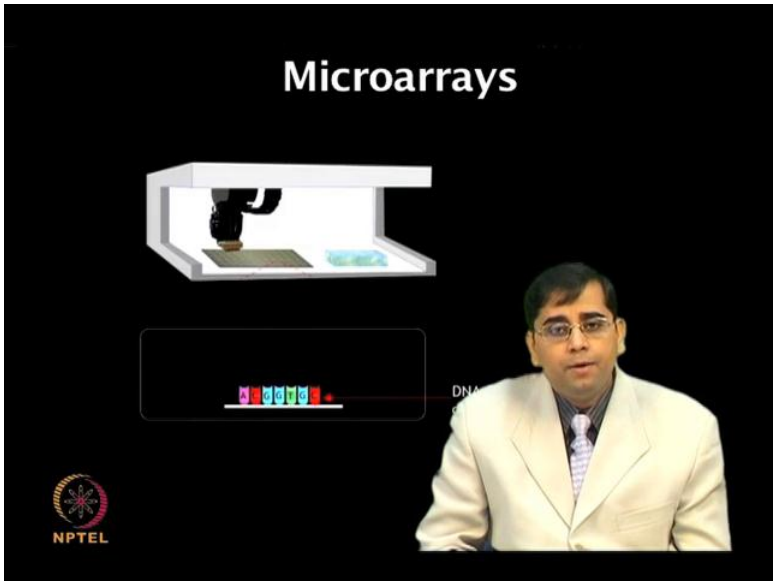

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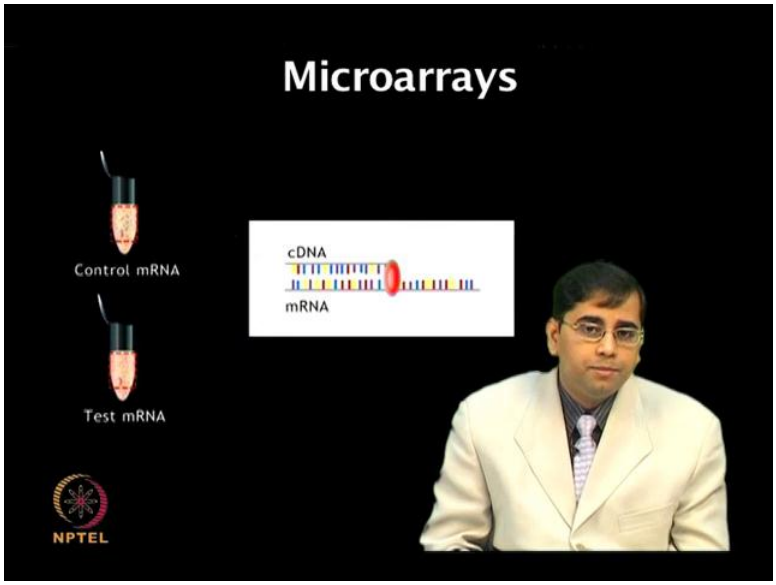

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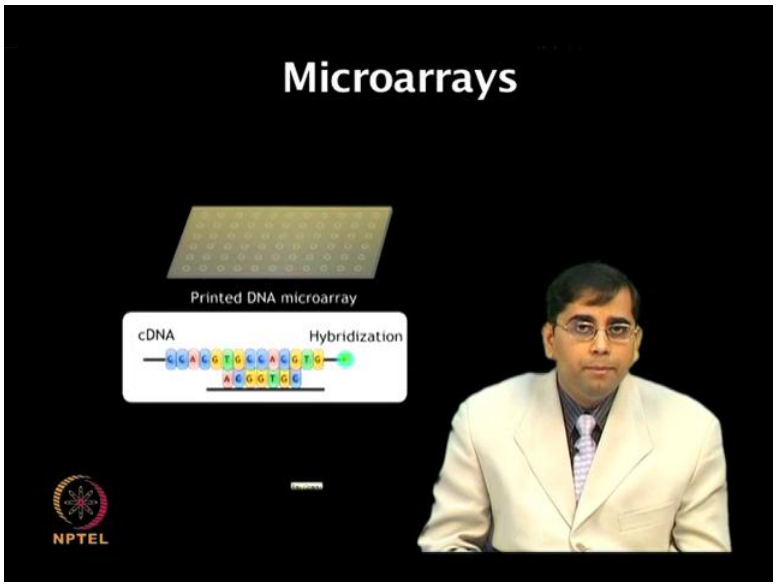

In a microarray experiment, the mRNA from control and test samples are extracted and reverse transcribed into its corresponding cDNA. The cDNA samples are labeled with (( ))and (( )) and mixed cDNA sample is incubated on printed D N A microarray. This allows hybridization to occur between the probe oligonucleotide on array surface and the labelled cDNA sample of interest. In this manner expression level of 1000sub genes can be measured and analyzed simultaneously.

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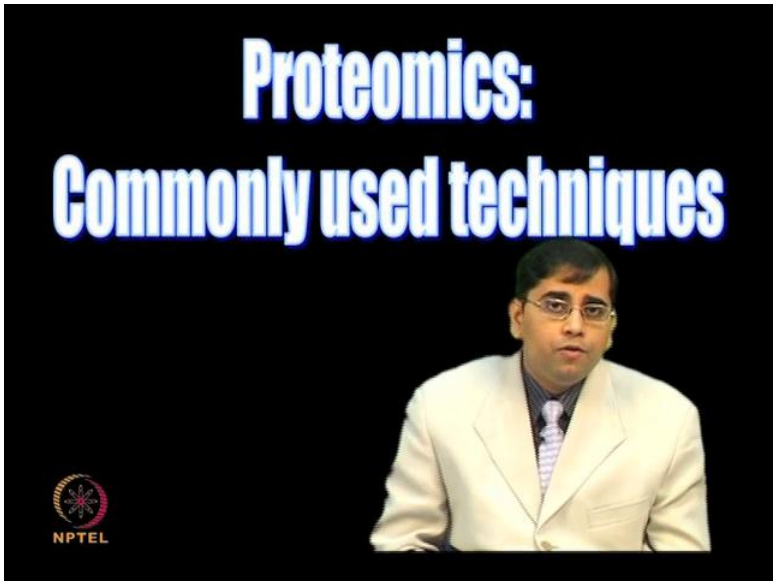

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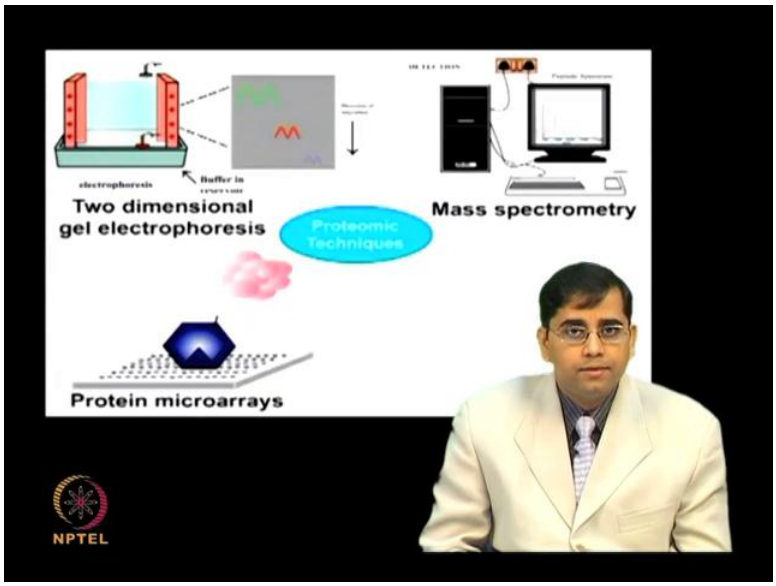

Different type of proteomic technologies; such as, two dimensional electrophoresis, mass spectrometry, micro arrays and label free technique will be discussed in more detail later.

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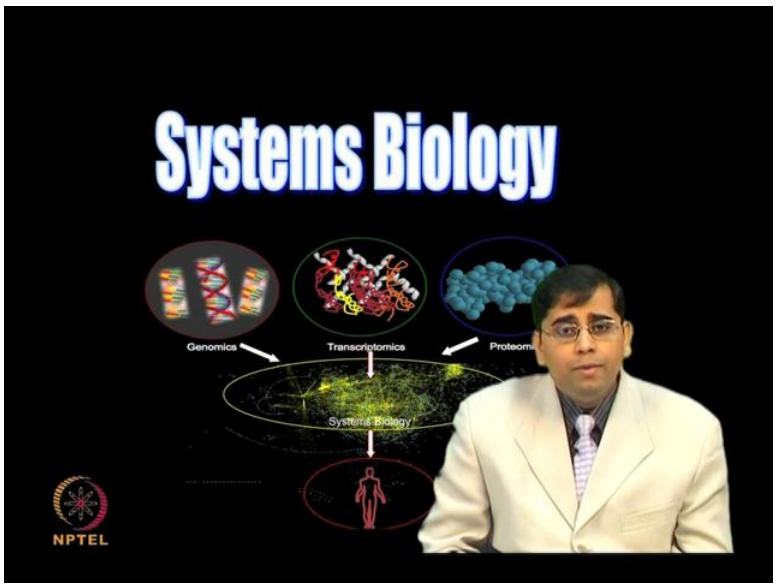

In (( )) technological advancement in genomics, proteomics and metabolomics have generated larger scale data sets in all the aspects of biology. These large datasets have motivated the computational biology and systems approaches with objective of understanding the biological system as a whole.

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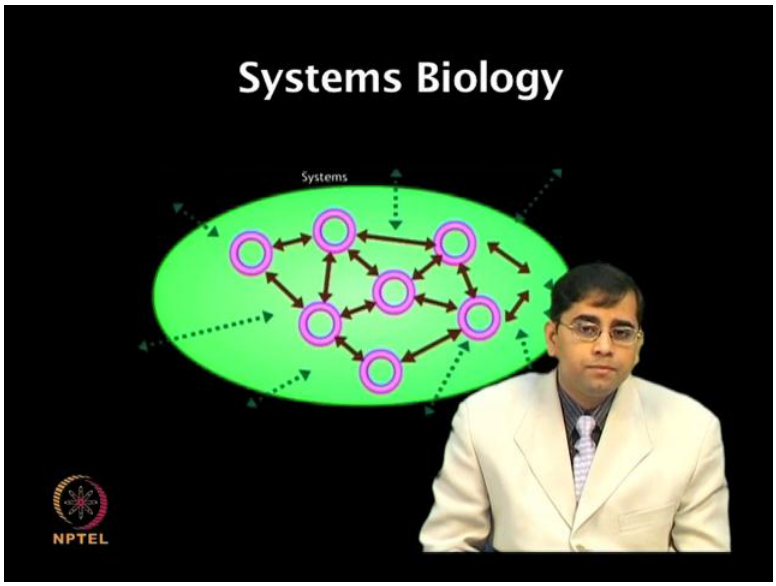

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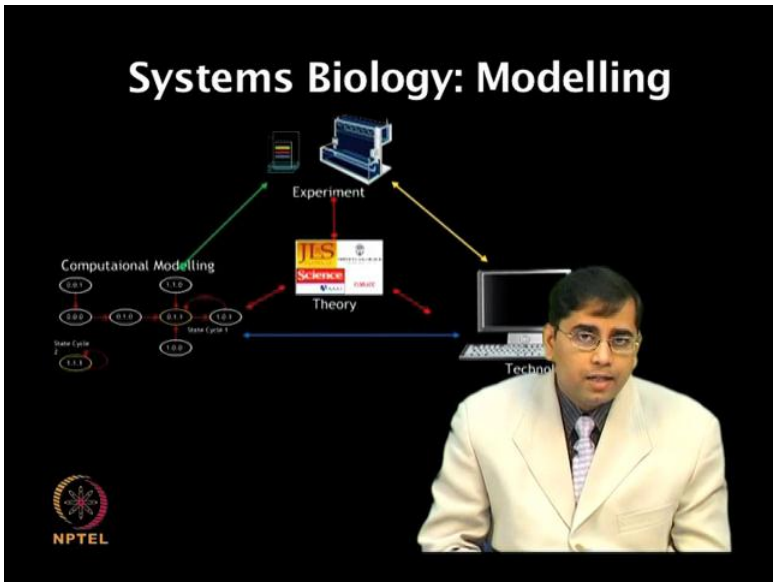

The system biology and biological network modelling aims to understand the biological processes as whole system rather than, the isolated parts by synergistic application of experiment, theory, technology and modelling.

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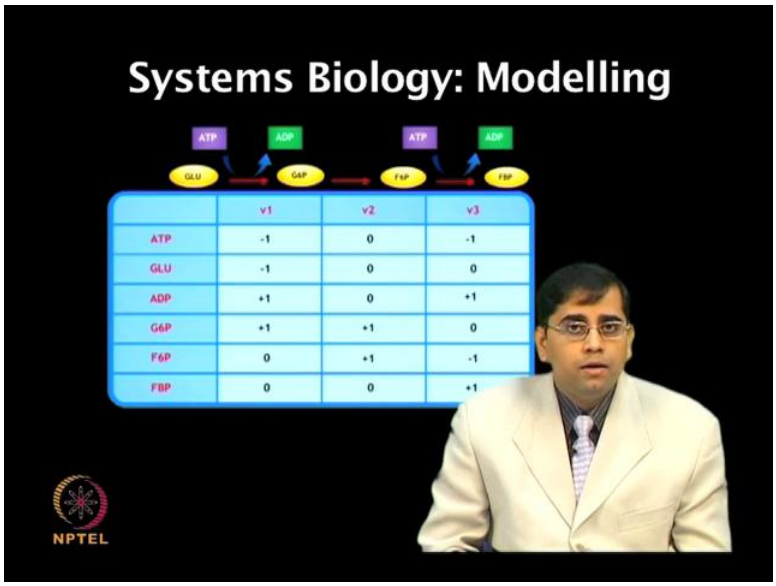

The systems level studies aim to develop computationally efficient and reliable models of underlying gene regulatory networks.

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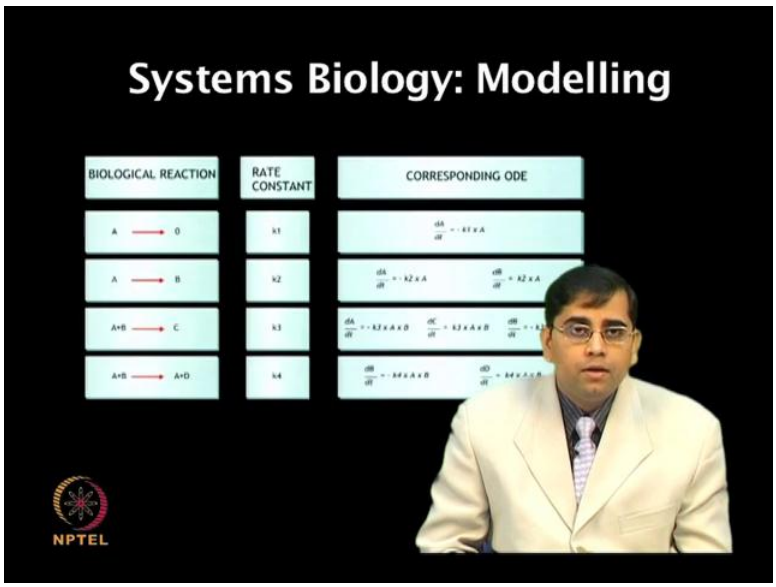

The quantitative analysis measures and aims to make models for precise kinetic parameters of a systems network component, it also uses properties of network connectivity.

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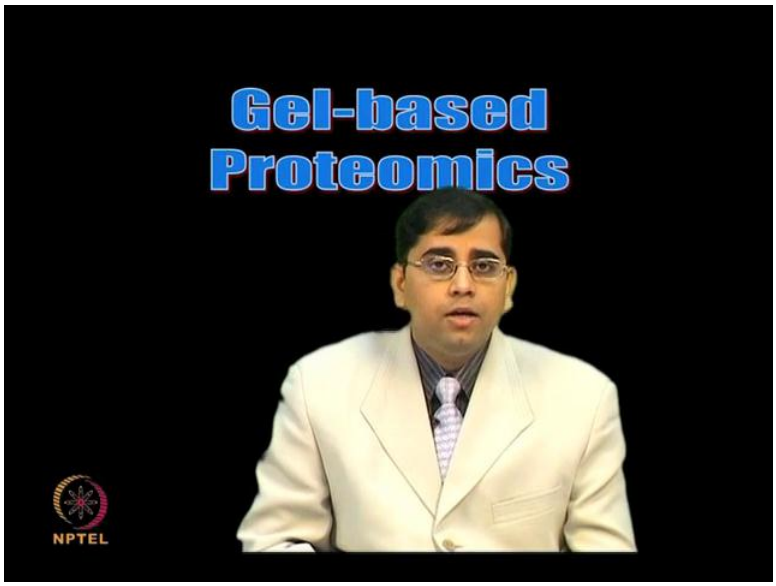

Several techniques used in proteomics, typically aim to elucidate the expression localization interaction and cellular function of proteins, SDS-PAGE, two dimensional electrophoresis, difference in gel electrophoresis are various commonly used gel based proteomic techniques. Protein extraction is the first step for the proteomic analysis. The protein extraction methods aim that most, if not, all the proteins in a cell or its organelles are extracted by the procedure; and the presence of interfering components are reduced or minimized, different biological systems, different biological samples pose different type of challenges.

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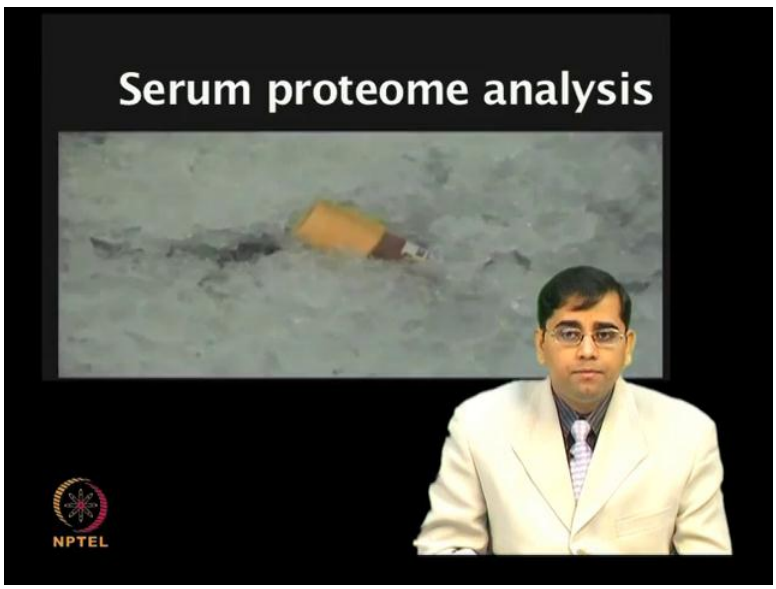

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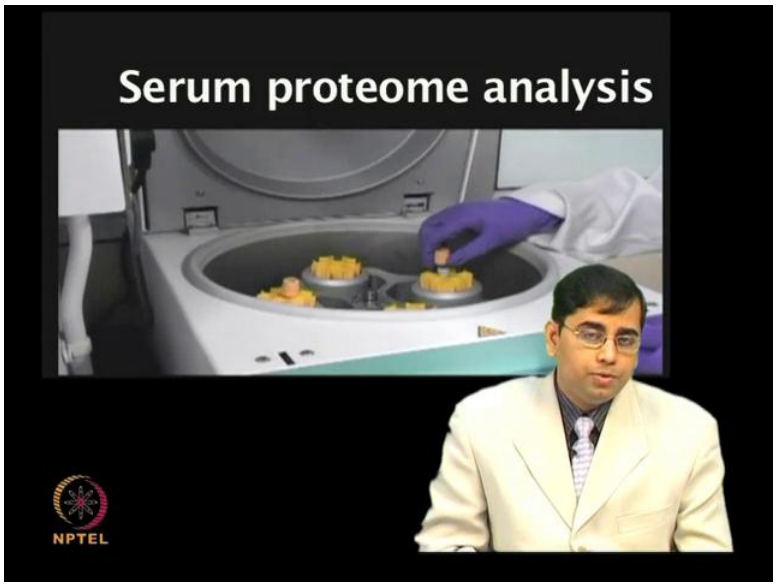

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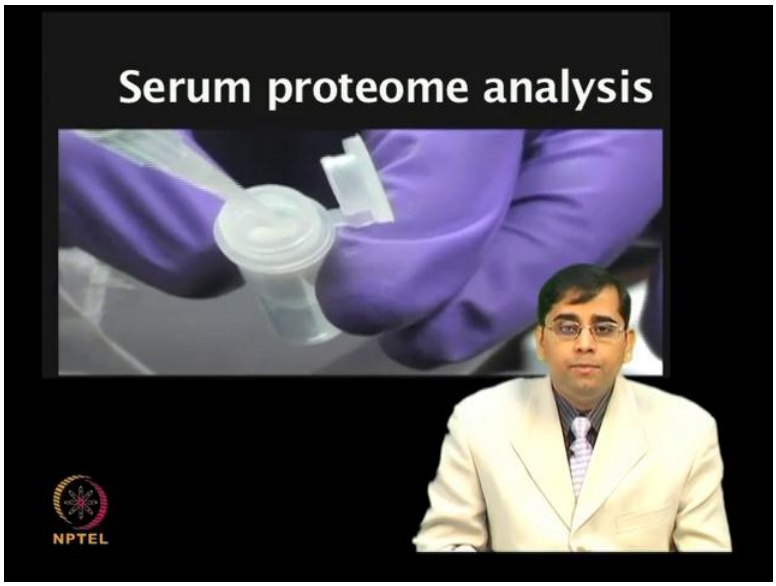

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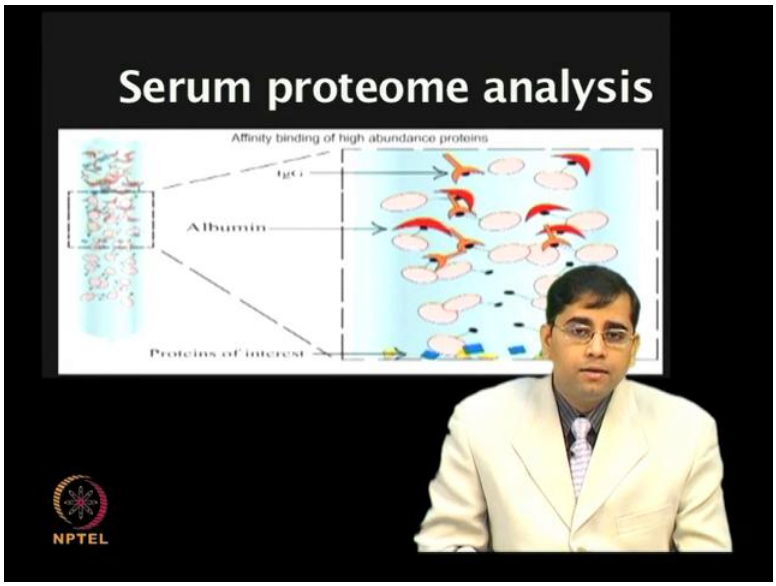

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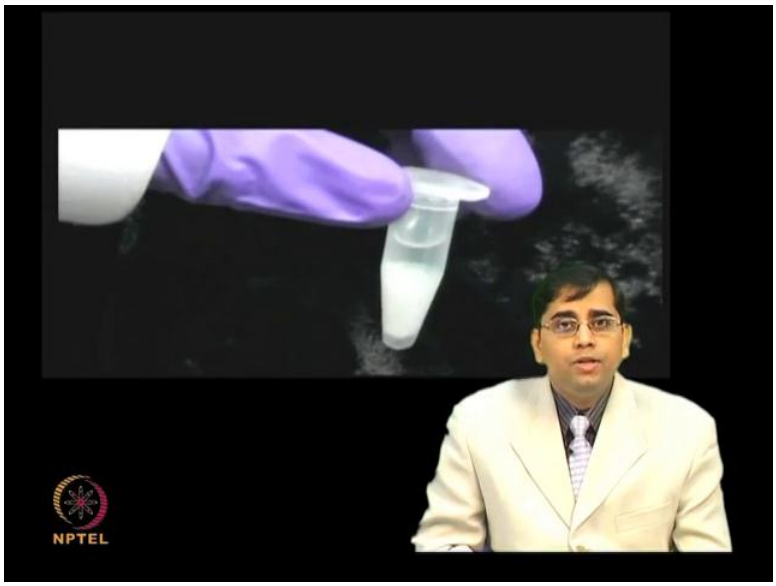

For example, serum protein analysis shown here, illustrates that proteins in biological systems such as serum, may have difference of several order of magnitude, albumin and immunoglobulin are the most abundant proteins. In serum, which mask other low abundant proteins, which are present in the lower concentration. It is therefore, referred to remove this high abundant proteins by using affinity chromatography methods, while the serum has been processed using this chromatography methods, this proteins can be extracted.

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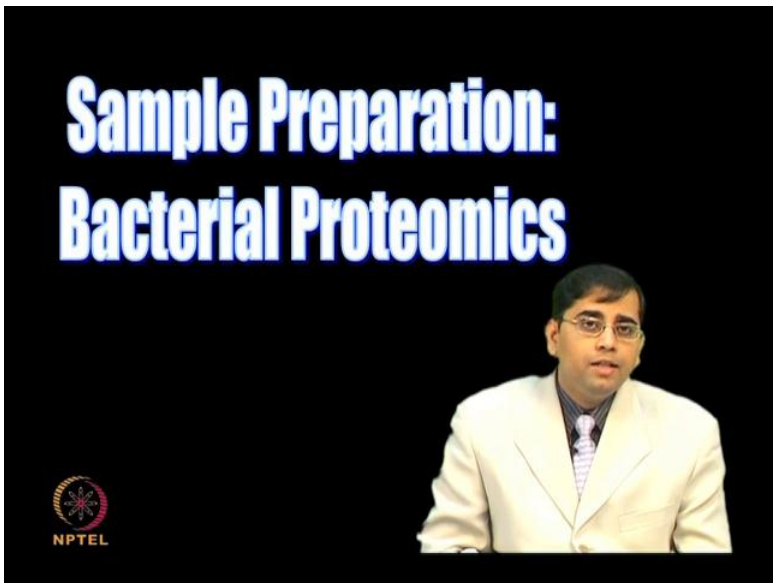

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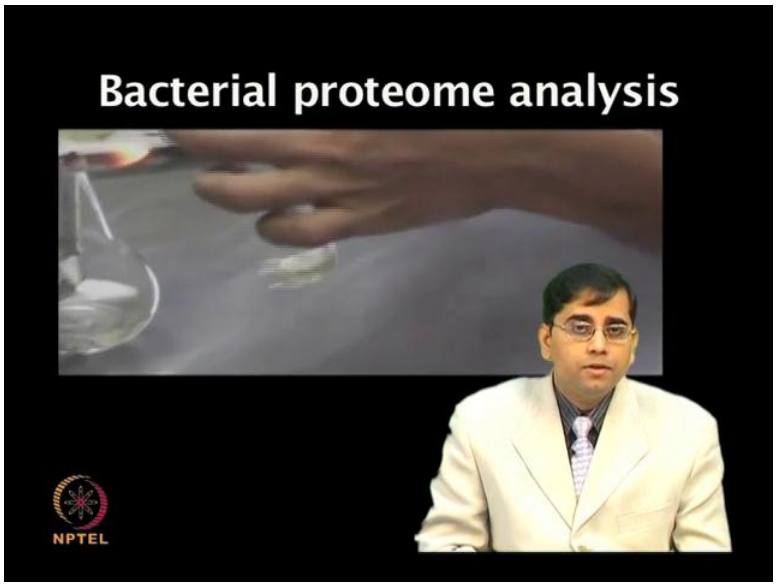

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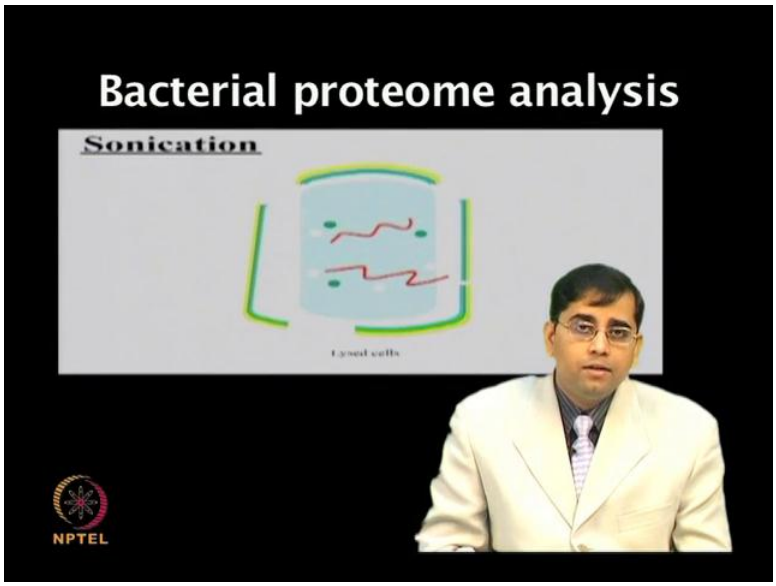

In bacterial protein sample preparation, sonication is an important strip to disrupt the bacterial membrane. Sonication breaks open the cellular contents, cellular membranes to relief the intra cellular contents. Protein extraction can be performed by using different methods and protein pellets are reconstituted in lysis buffer for proteomic analysis.

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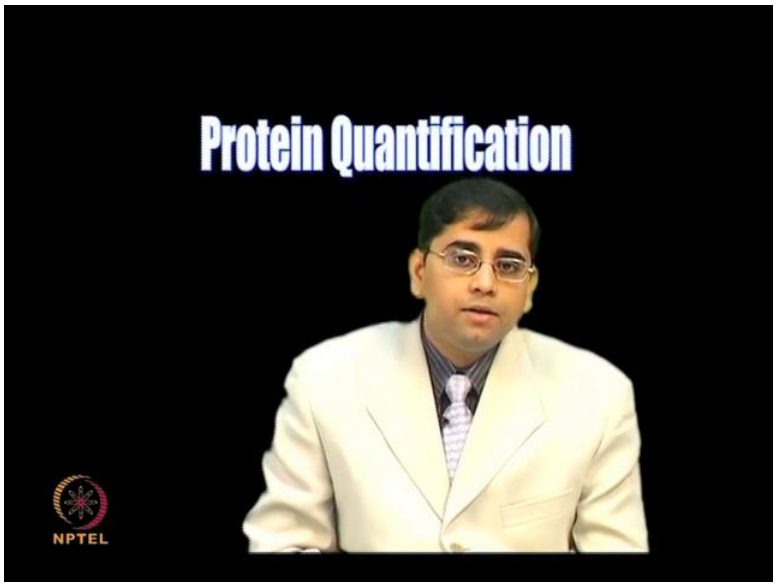

Protein quantification it is sensitive to detergent or certain ions therefore, it is crucial select the correct quantification method.

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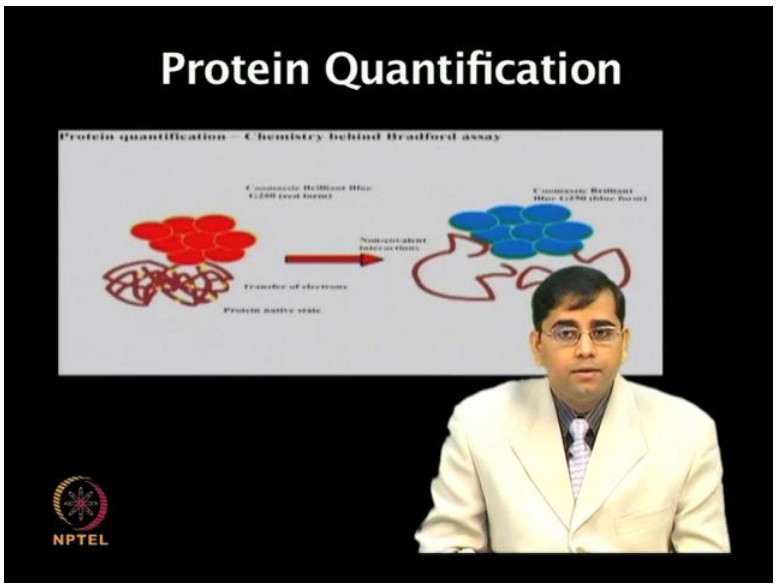

In Bradford,(( )) transfer of electron, converge the (( )) towards the blue form, there by giving the solution blue color.

Absorbance of a standard and unknown protein sample can be measured at (( )) nanometers, and protein concentration can be determined from the standard plot of the absorbance values.

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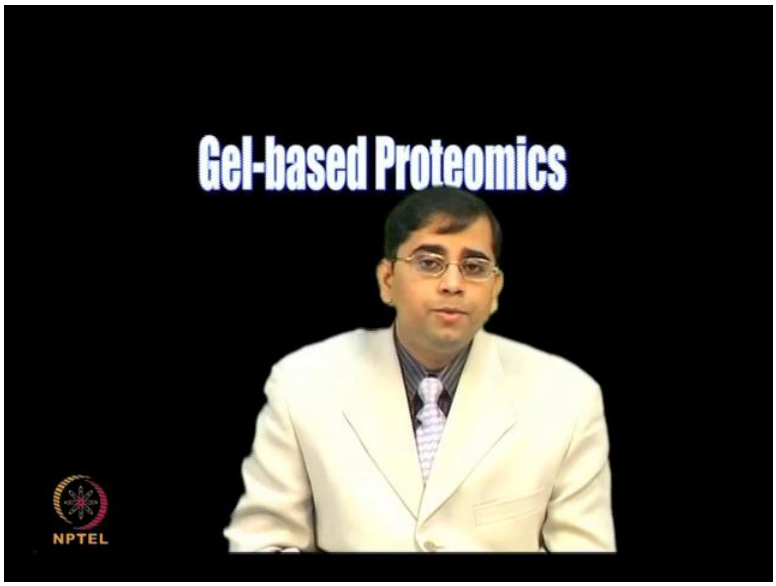

In gel based proteomics, proteins are commonly analyzed using SDS-PAGE and two dimensional gel electrophoresis.

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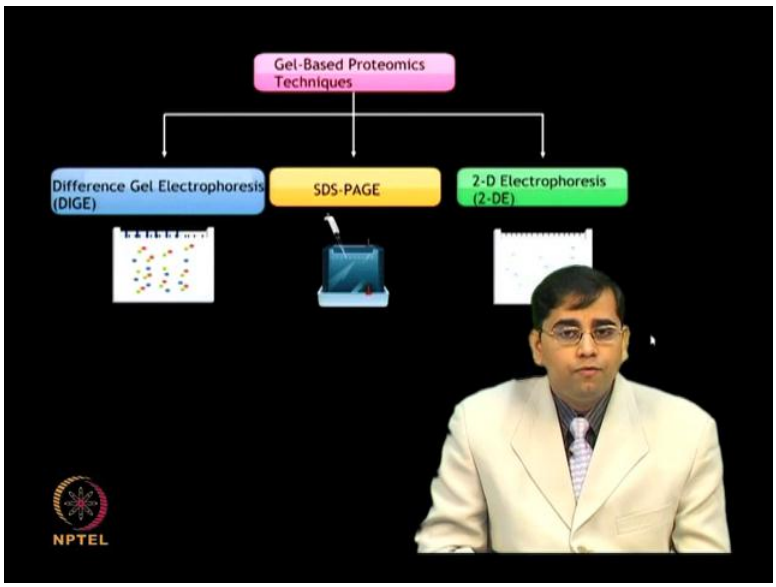

Separation in SDS-PAGE occurs almost accesively on the basis of molecular weight, whereas in in 2-DE the first dimensional separation is based on isoelectric point and second dimension separation based on molecular weight.

Some of the limitations of two dimensional electrophoresis can be overcome by difference gel electrophoresis or DIGE technique. 2-DE or DIGE in combination with

mass spectrometry has been the standard technique for proteome analysis. The two dimensional electrophoresis involves protein separation on p H gradient, based on their isoelectric point (( )) isoelectric focusing followed by separation in second dimension using SDS-PAGE.

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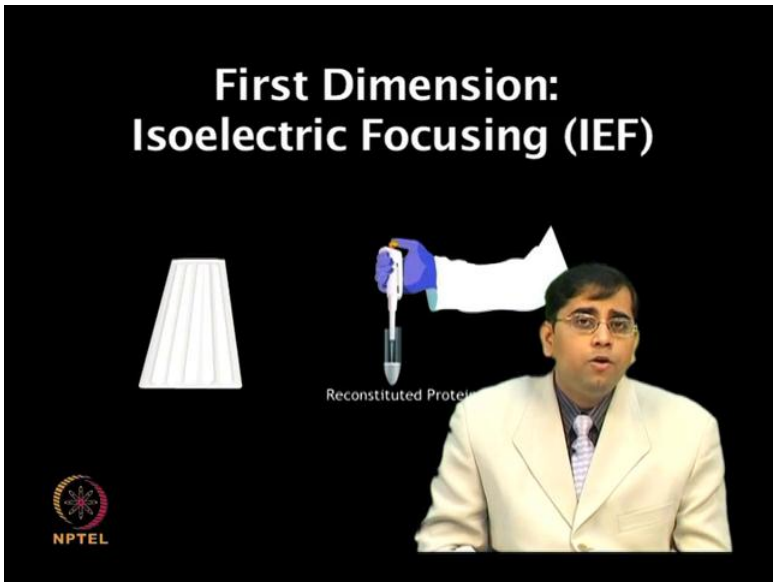

To perform 2-DE at the reconstituted protein sample to the rehydration tray and place the I P G strip for rehydration.

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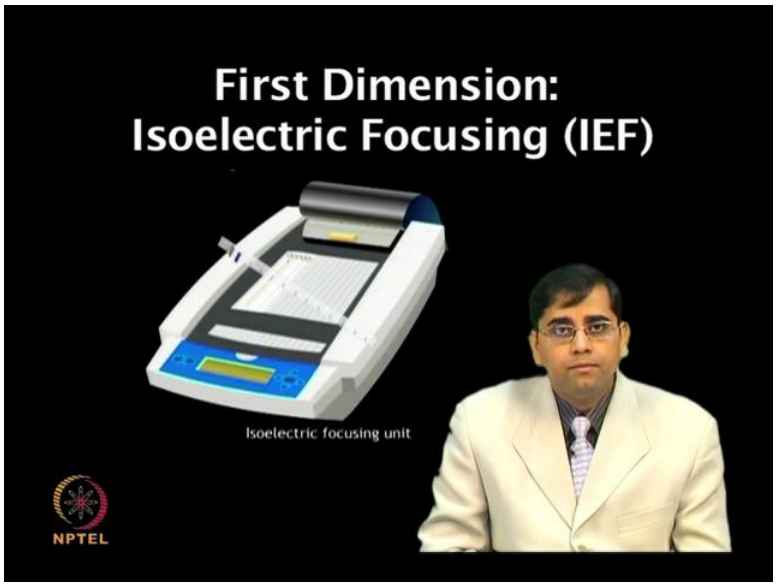

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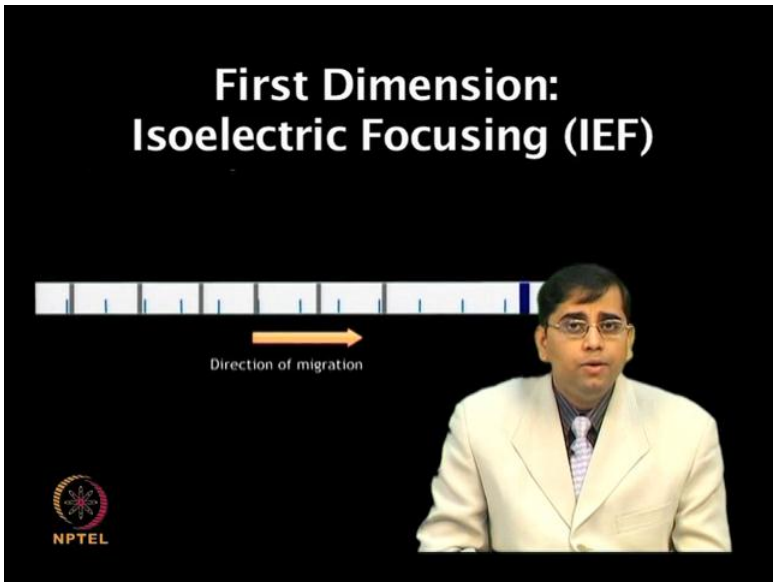

Isoelectric focusing involves the application of an electric field, which causes the proteins to migrate to the position on the p H gradient strips that matches, the p I of a specific protein after which it does not move in the electric field owing to the lack of charge. The proteins migrate along the strip and come to a rest at a point, where the net charge becomes 0 known as isoelectric point.

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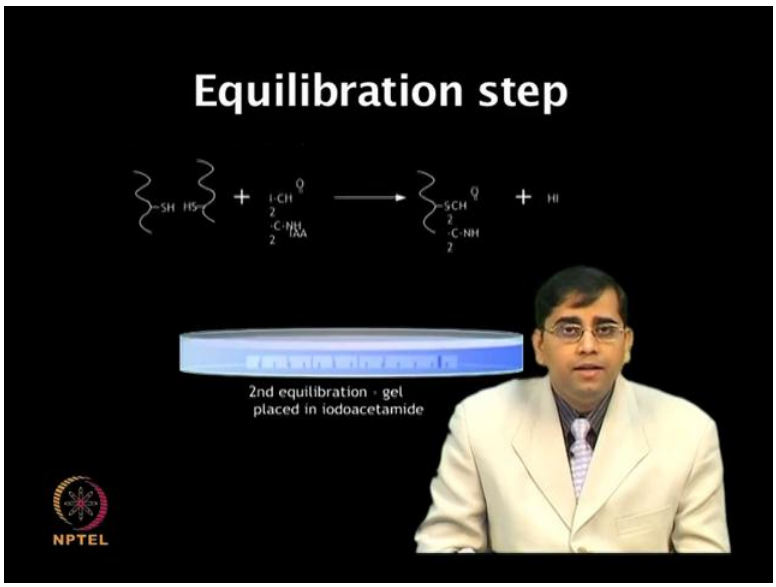

Prior to the second dimension separation an equilibration step is required. In equilibration, dithiothreitol brings about cleavage of the protein disulphide bonds, while iodoacetamide prevents reformation of these bonds by binding to the sulfhydryl groups.

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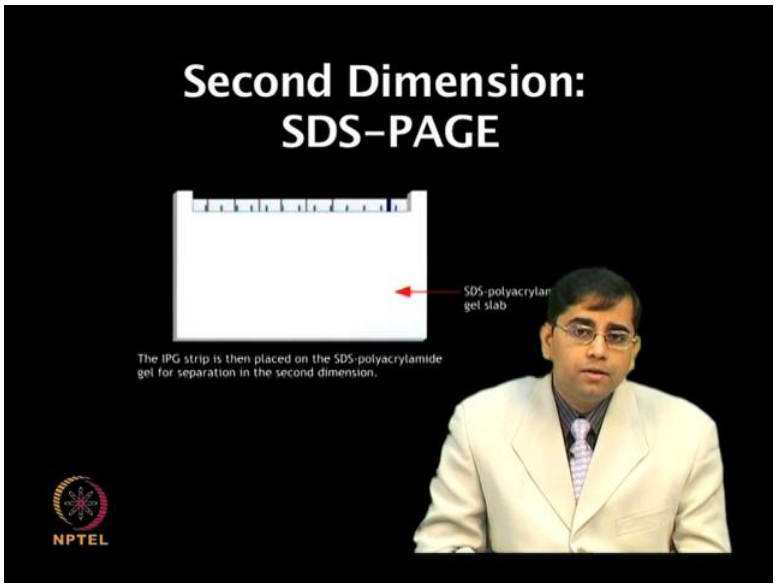

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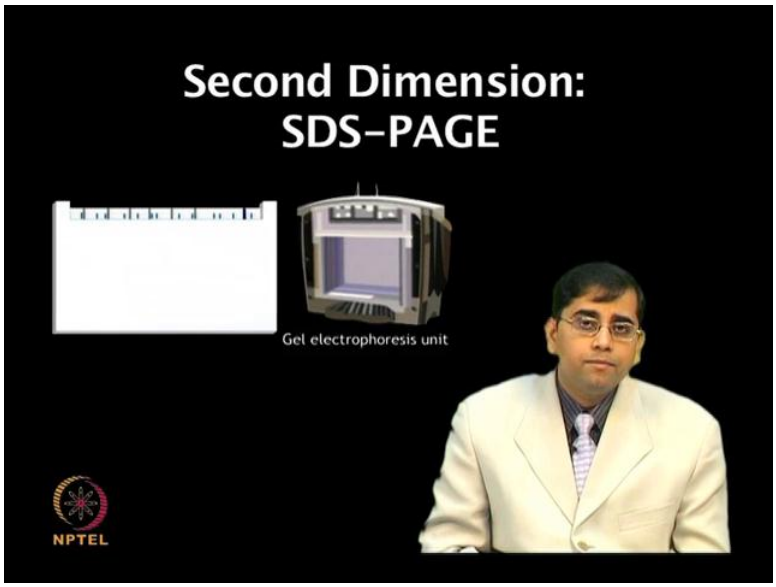

On SDS-PAGE gel, proteins get separated on the basis of their molecular weight with the low molecular weight proteins having high mobility and migrating further through the gel and the high molecular weight proteins remains close to the point of sample application.

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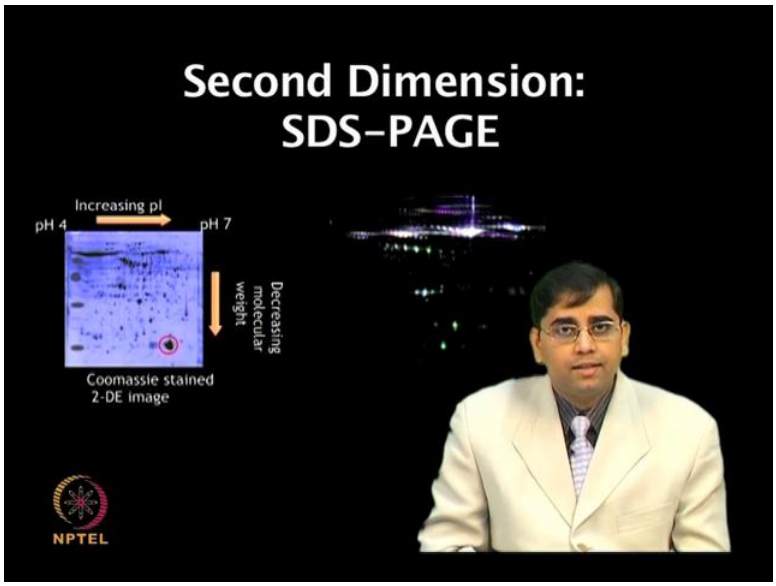

Gels can be visualized by different staining methods such as coomassie staining, silver staining and shining (( )).

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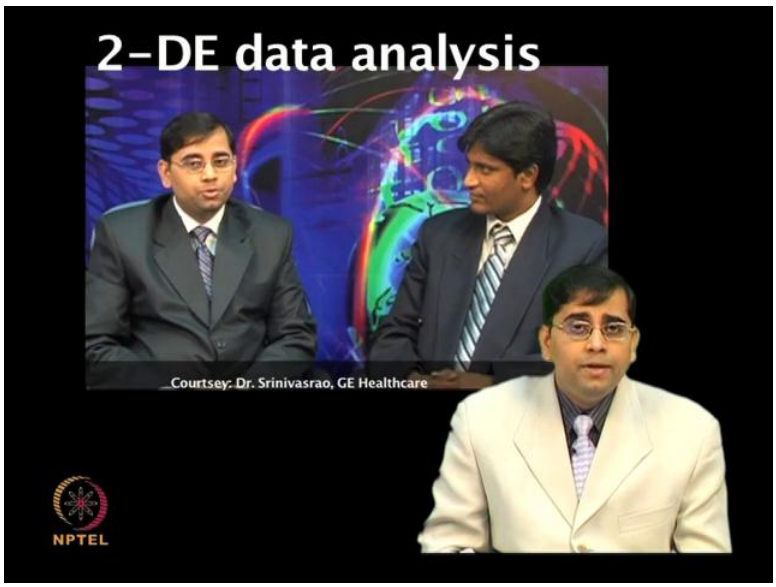

The gel data analysis will be discussed with an application expert of GE Healthcare.

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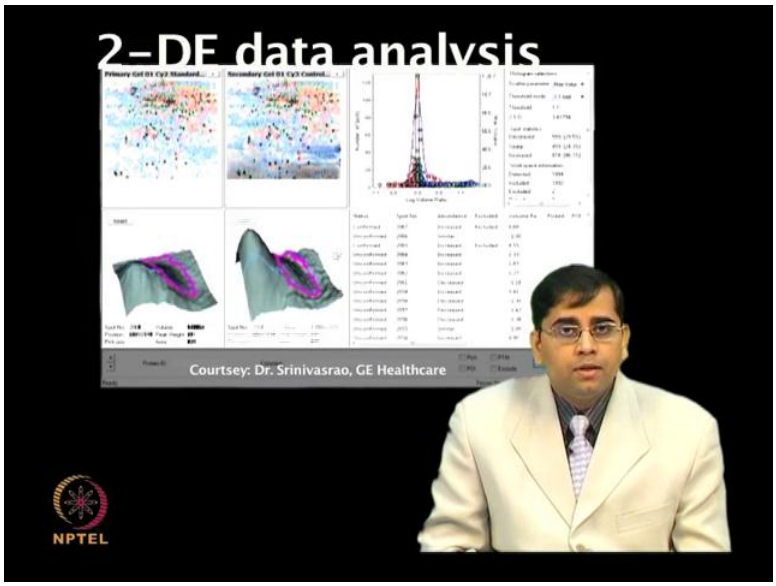

The gel analysis involves image processing, detection of spots making mashlets, landmarking, viewing histograms etcetera, various information regarding this spots such as the area, volume, intensity and statistical parameters such as standard deviations can also be calculated. Two dimensional electrophoresis has high resolving power, but it has several limitations such as staining artifacts, and reproducibility in gel to gel.

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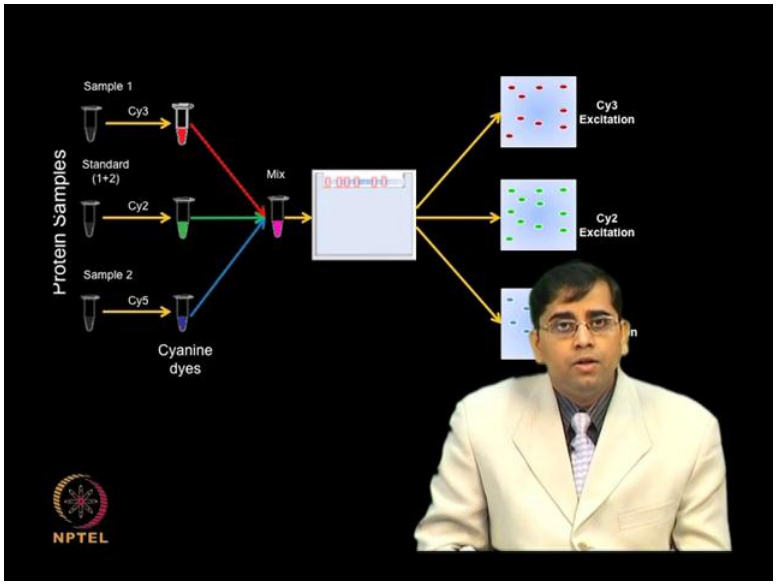

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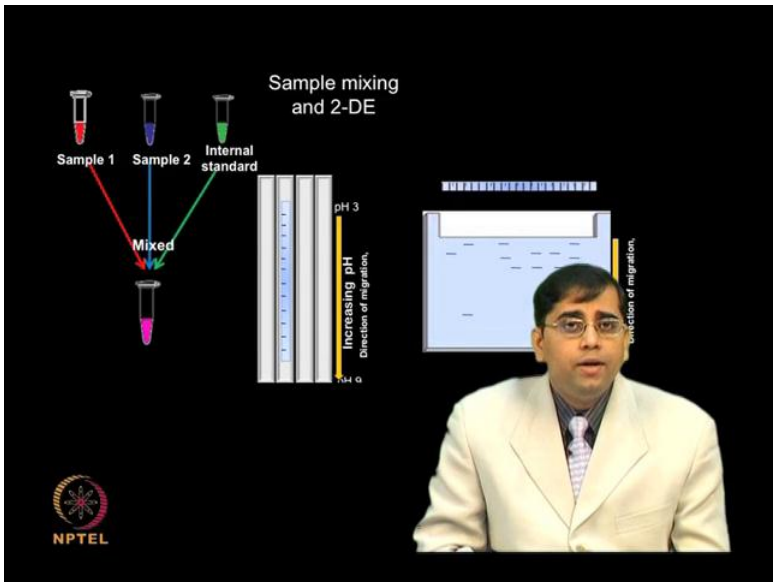

Fluorescence two dimensional difference in gel electrophoresis or 2 DE DIGE is an advanced 2 DE technique, that allows for accurate quantitation with a statistical confidence, while controlling the non biological variations.

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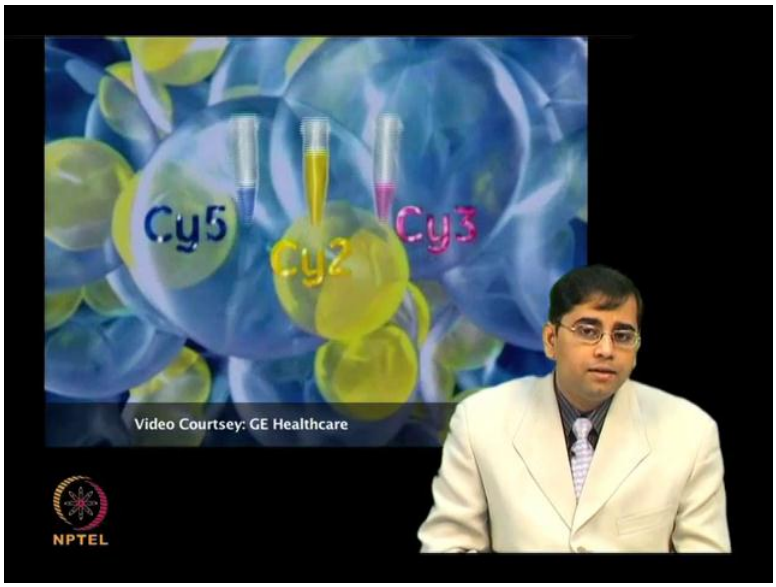

In DIGE, proteins extracted from different type of cells or tissue sample are lipid with different florescent reagents such as C y 2,C y 3 and C y 5 mixed and then separated by two dimensional electrophoresis on a single gel. The proteins are detected separately

using the excitation wavelength, specific to the different florescent reagents C y 2,C y 3 and C y 5.

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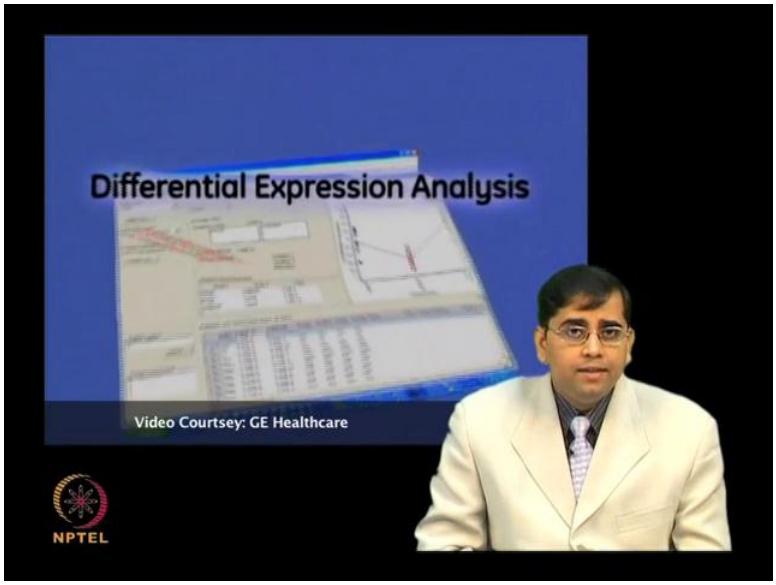

The commercial software such as decyder, facilitate the automated analysis of (( )) gelsand provide, differential expression analysis, principle component analysis, patterns and discriminant analysis.

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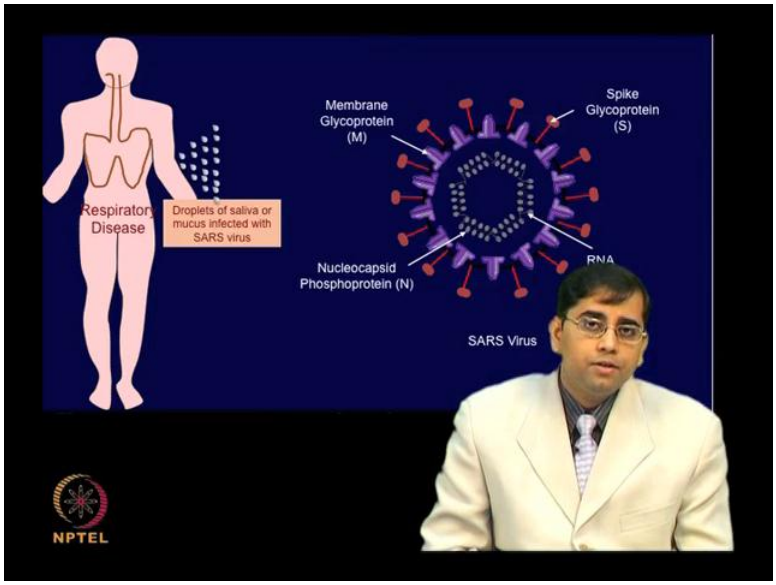

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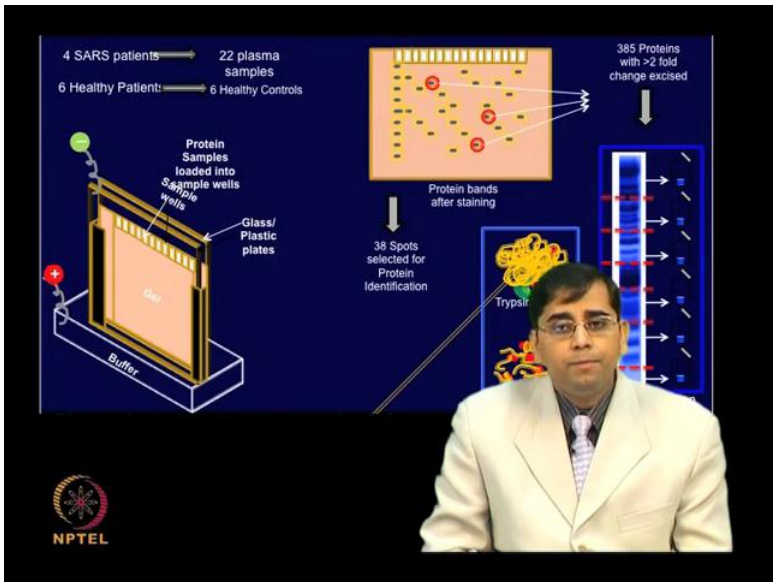

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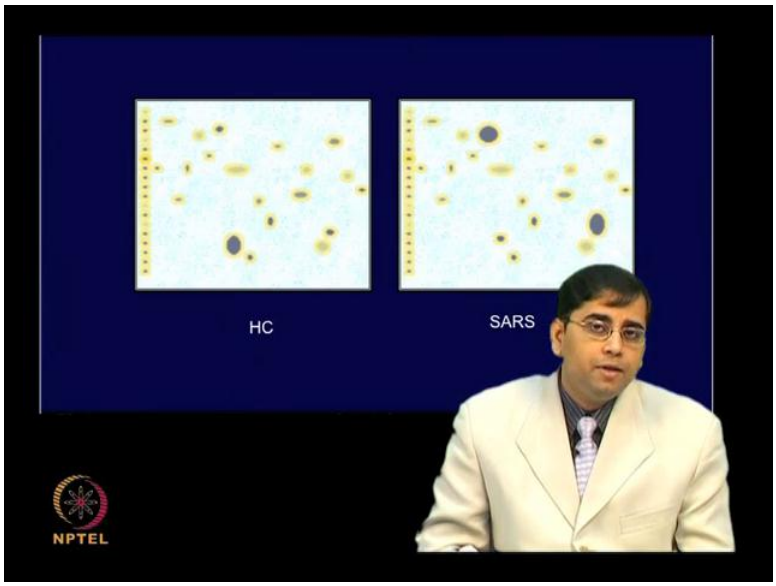

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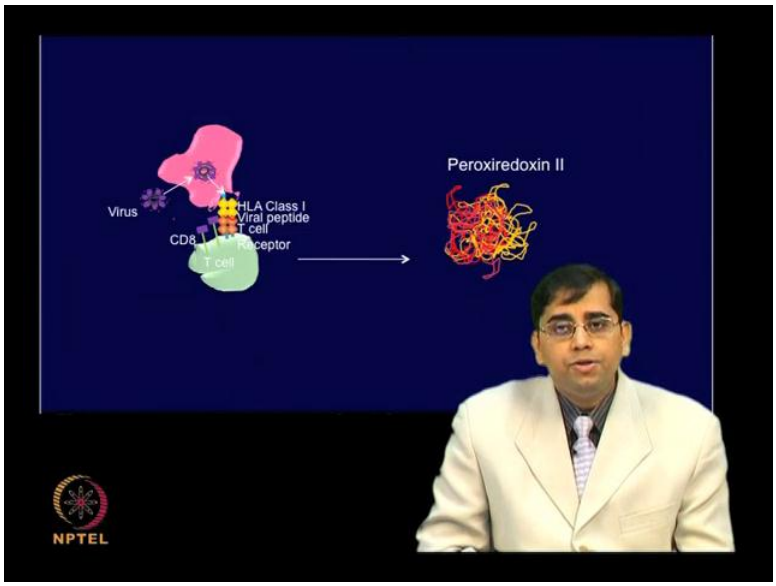

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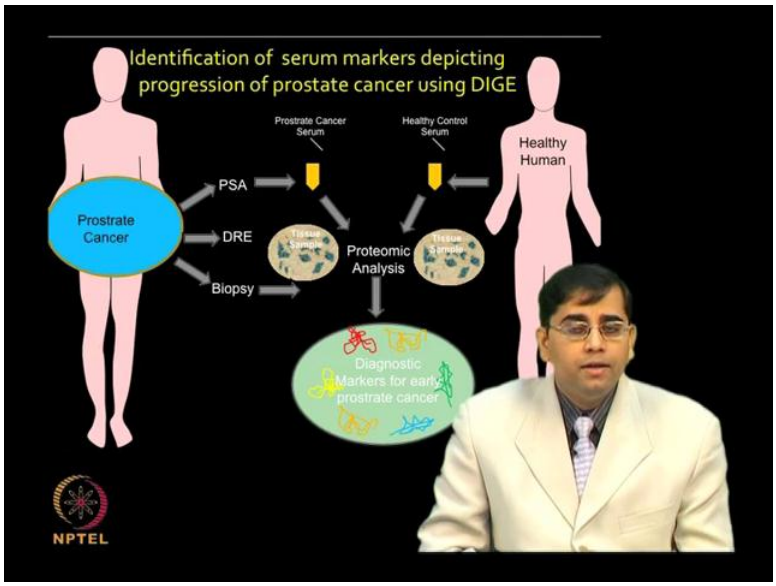

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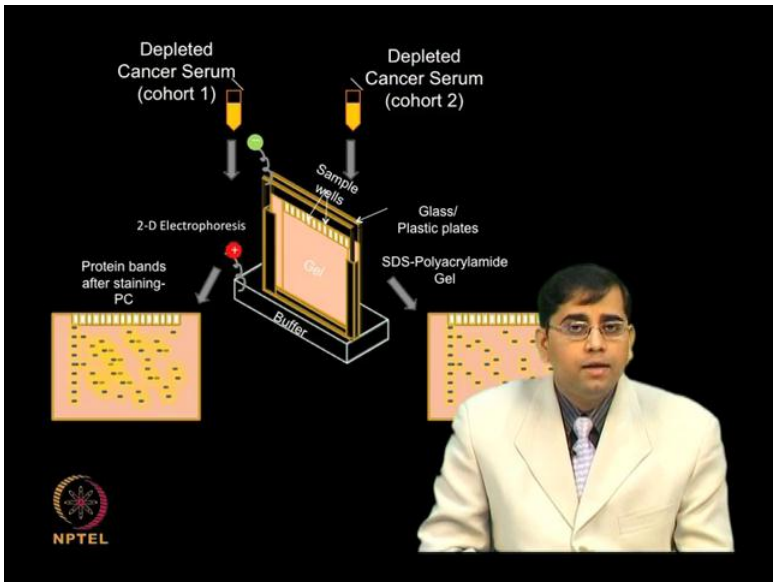

Two dimensional electrophoresis DIGE followed by mass spectrometry technique has been applied for many applications. Some of these applications will be discussed in this module of gel based proteomics.

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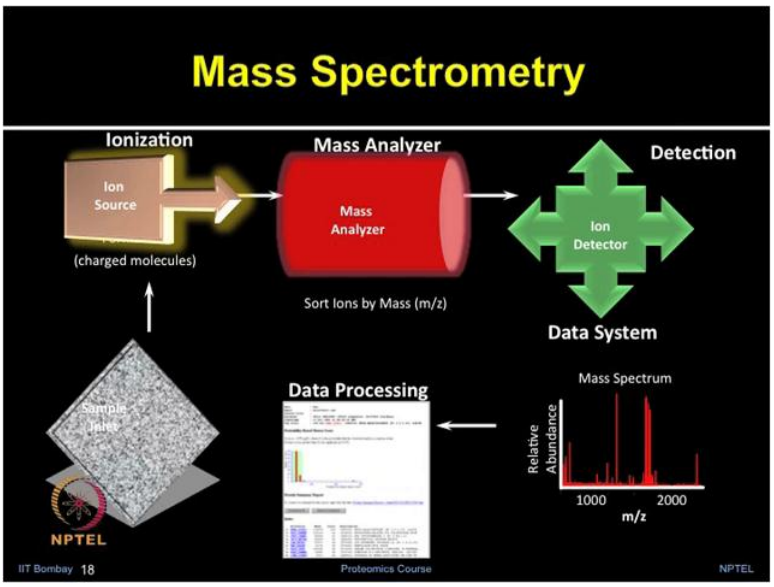

Mass spectrometry is technique for protein identification and analysis by production of charged molecular species in vacuum and its separation by magnetic and electric fields, based on mass to charge ratio. Mass spectrometry has become the method of choice for

analysis of complex protein samples in proteomic study, due its ability to identify thousands of proteins.

The gel based techniques typically resolve only products of a few hundred genes at best, had low throughput and low dynamic range to overcome such issues, mass spectrometry has become an important analytical tool in proteomics; and in biology in general, it offers high throughput, sensitive and specific analysis for many applications. In this module, we will discuss basic concepts of mass spectrometry, ionization sources, mass analyzers, hybrid m s configurations and quantitative proteomic technique such as SILAC and iTRAQ.

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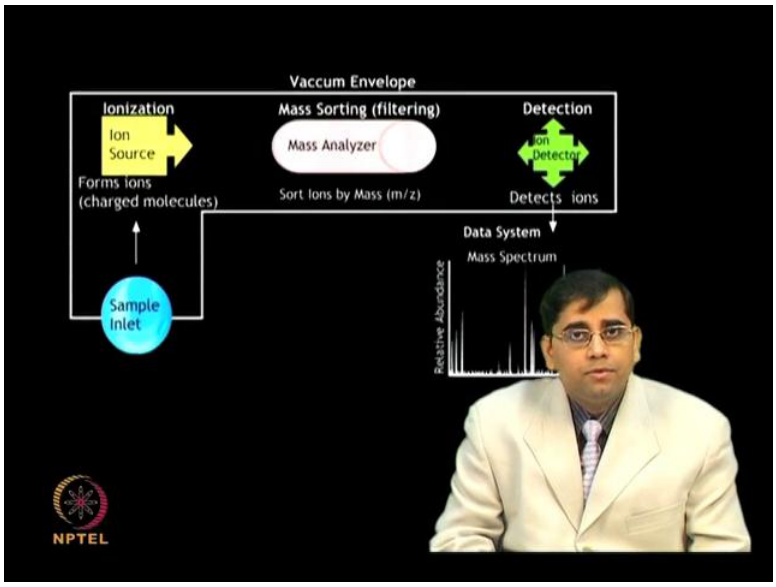

The basic components of mass spectrometry involve sample inlet to transfer sample into the ion source, ionization source which converts neutral sample molecules into gas phase ion. In mass analyzer to separate and analyze mass of ionic species; detector, which (( )) and amplifies ion current of mass reserve ion and data system to process and analyze data. Soft ionization techniques such as matrix assisted laser desorption, ionization MALDI and Electro SprayIonization,E S I are now widely used for proteomic applications.

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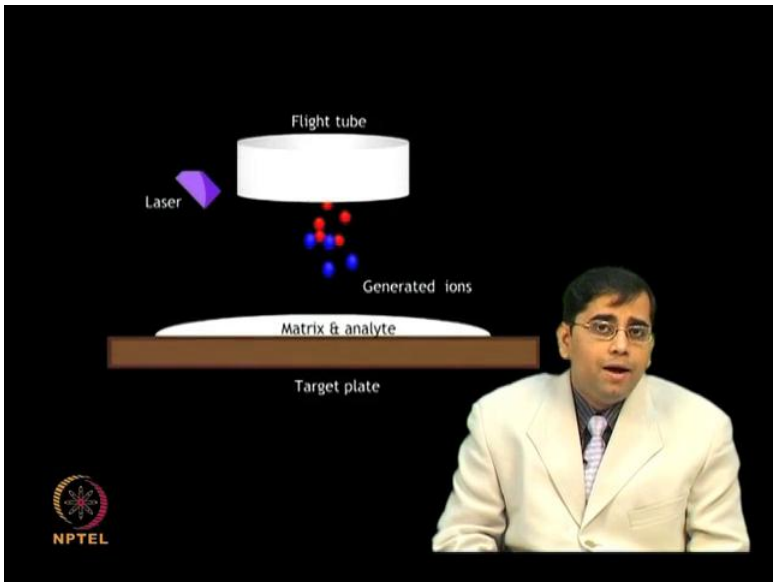

In MALDI, protein is mixed with matrix and laser beam ionizes matrix molecules, it is an efficient process for generating gas phase ions of peptides and proteins for mass spectrometric detection.

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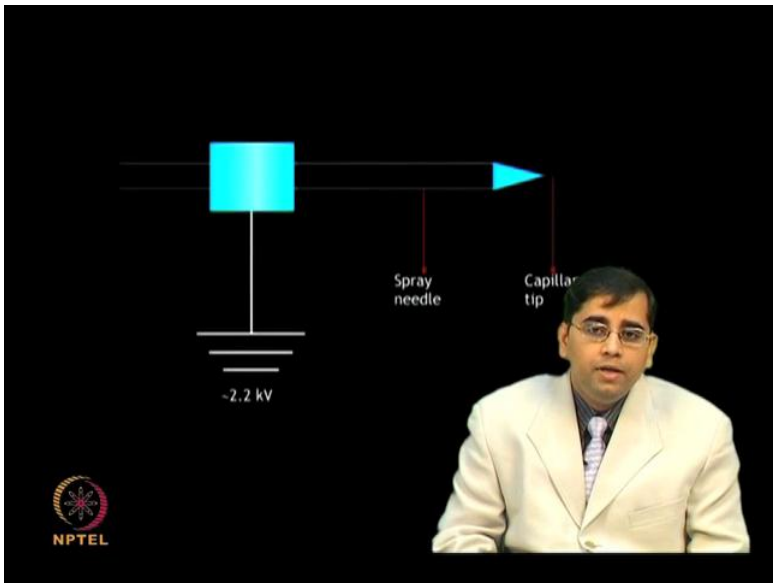

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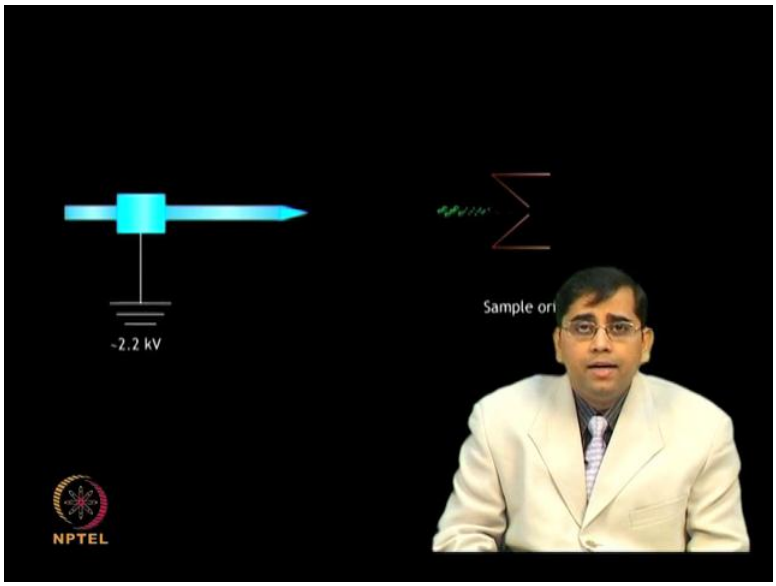

Electrospray ionization requires sample of interest to be in solution and produces gas phase ions from solution obtained from the chromatographic steps. The distinguishing feature of electrospray ionization is, its ability to produce multiply charged species. The mass analyzer disperses all the ions based on their mass to charge ratio, and focuses all the mass reserved ions at a single focal point and maximizes their transmission.

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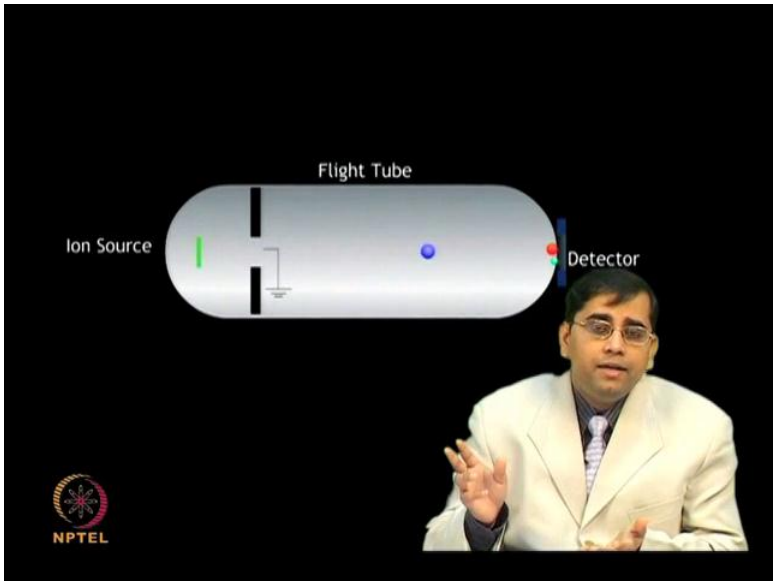

The time of light measures m by z ratio of ions based on the time it takes for ions to fly in the analyzer and strike to the detector.

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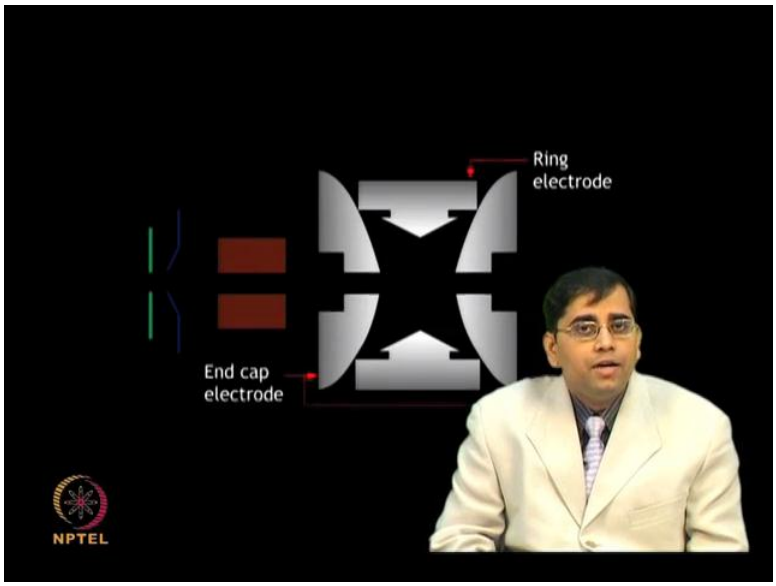

The ion trap it traps ions using electrical fields and measures mass by selectively ejecting them to the rejecter.

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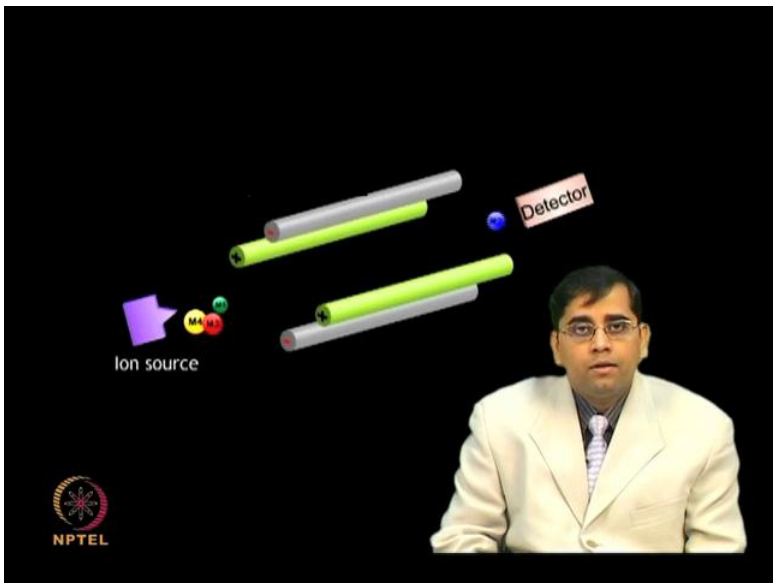

Quadrupole, it consist of our parallel metal rods and mass separation is accomplished by the stable vibratory motion of ions in a high frequency, oscillating electric field that is created by applying direct current and radio frequency potentials to these electrodes.

Some of the latest hybrid MS technologies will be discussed with application experts. The orbitrap technology will be discussed with application expert of thermo scientific

LTQ-Orbitrap technology shown in video, consist of both linear trap and orbitrap; it operates by trapping ions radially about a central a spindle electrode, unlike ion trap the orbitrap uses only electrostatic field to focus and analyze ions.

Another latest MS technology, Q-TOF and (( )) will be discussed with application expert of Agilent technologies, many such advancement in mass spectrometry during the last decade have provided new ways for protein analyses and facilitated proteomic analyses of various biological system. The Q-TOF l c m s system performs MS MS analysis using a quadrupole collision cell and time of flight, mass analyzer; the quadrupole, selects precursor ions, which are further fragmented in collision cell, the product ions move to detector and a spectrum is generated.

Protein labelling with a stable isotopes are effective methods for quantitative proteome profiling using mass spectrometry. A stable isotope labelling by amino acids in cell culture or SILAC, which is a metabolic labelling strategy to encode whole cellular proteome that is widely used method for quantitative proteomics.

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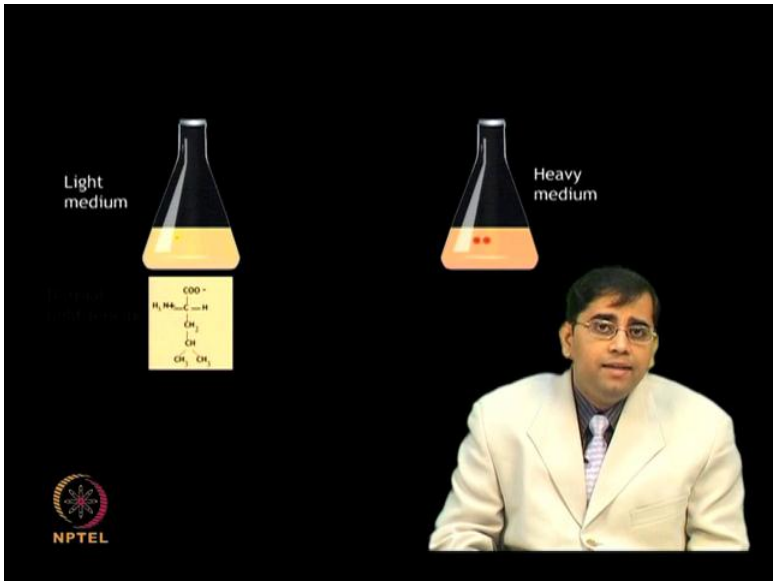

In SILAC, two group of cells are cultured in medium that are identical in all the respects, except that one contains a heavy isotopic analog of an essential amino acid, while the other contains the normal light amino acid. After a number of cell divisions, the grown cells are combined and digested using (( )).

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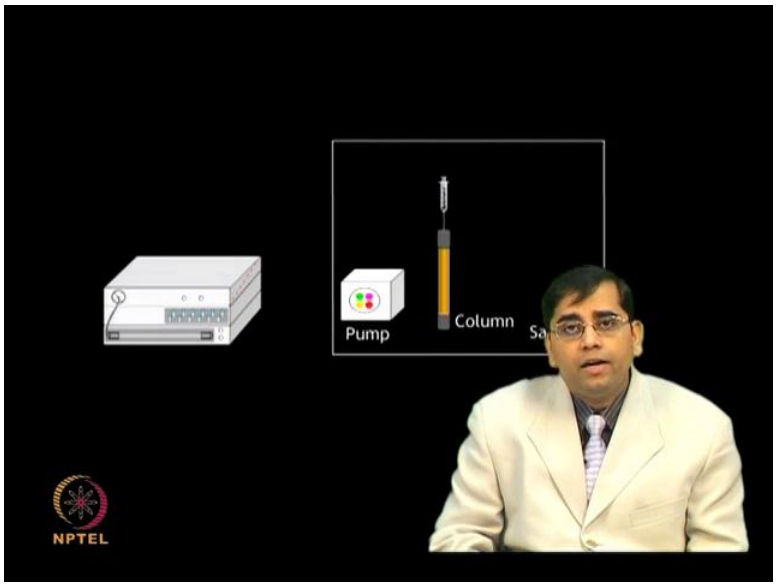

The complex protein mixture is further separated by SDS-PAGE to simplify the analysis. Further application is carried by liquid chromatography and purified peptide fragments are analyzed by M S M S.iTRAQ, it is aM S based technique for relative and absolute quantitation of protein.

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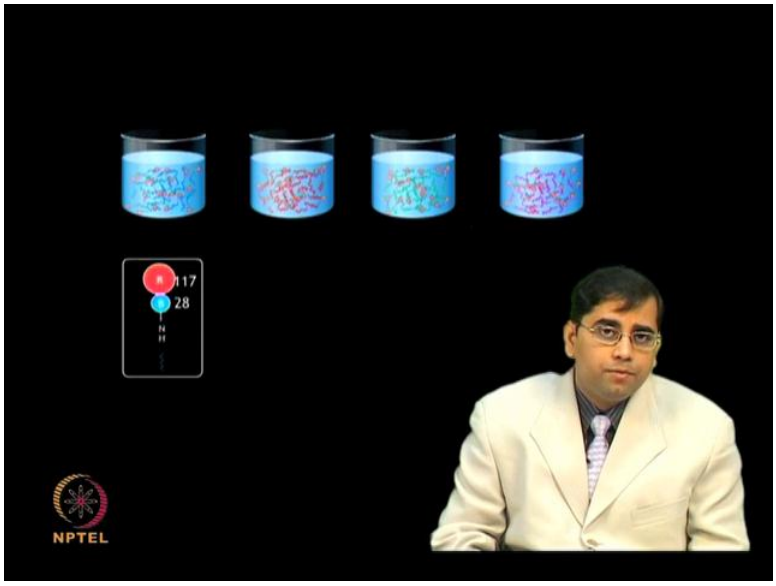

iTRAQ reagents are a set off our (( )) (( ))specific labouring reagents 1 14, 1 15, 1 16 and 1 17 and iTRAQ reagent consists of a reporter group, a balancer group and a peptide reactive group.

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Poor sample are purified on a strong cation exchange as (( )) column to remove the excess unboundary agent.

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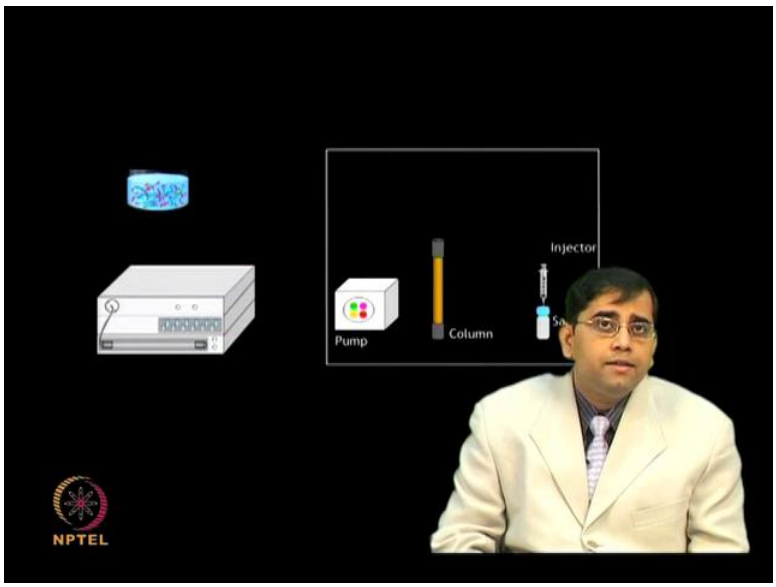

This isobaric label are detected upon fragmentation and release in mass spectrometry.

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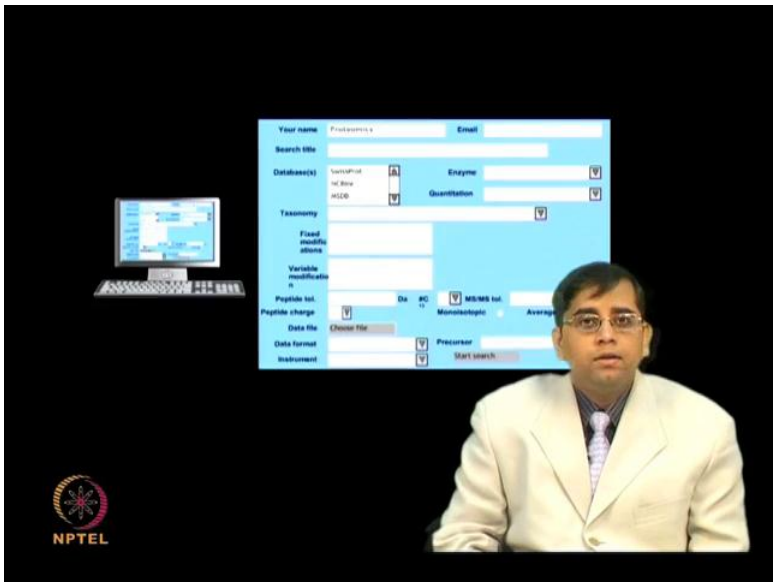

The data obtained from mass spectrometry can be analyzed by using search engines such as mascot, the analysis requires inputs regarding the experimental parameters such as enzyme cleavage, modifications, instruments used, peptide tolerance etcetera. The data files generated from M S is uploaded and the search carried out by employing databases such as NCBI,(( )) and Swiss-Prot.

Some of the widely recognized technologies that have been used to map, protein protein interactions at large scale such as yeast two hybrid ,immune precipitation with mass spectrometry, and different type of microarray platforms will be discussed in interactomics module.

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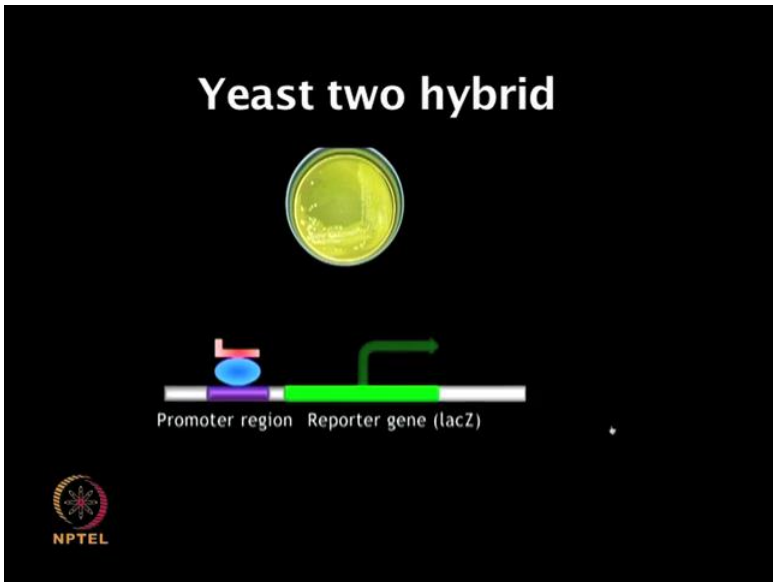

In yeast two hybrid the binding domain is fused with the bait protein, while activation domain is fused with the prey protein; binding of either one of the fusion protein to the promoter is insufficient to bring about transcription of the gene. When the bait protein bound with the binding domain interacts with the prey protein fused with activation domain, there will be (( )) of reporter gene, which can be easily rejected.

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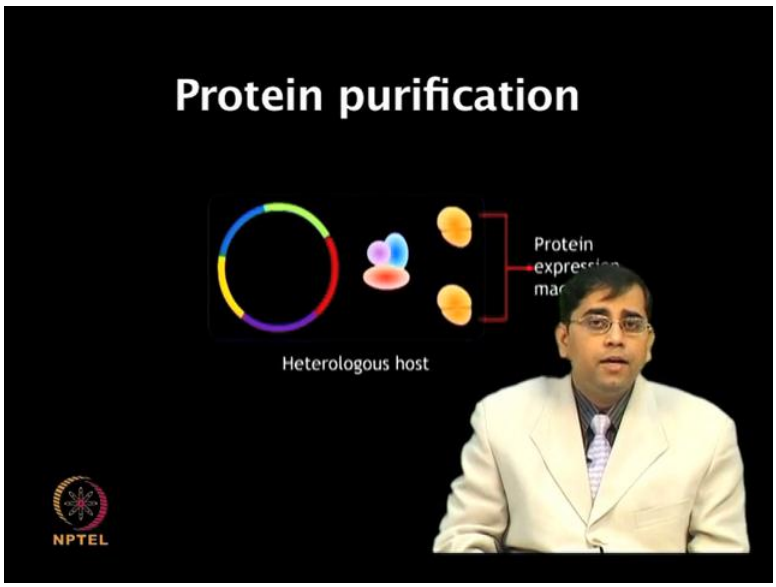

The gene coding for the protein of interest is expressed in heterologous system such as E.coli.

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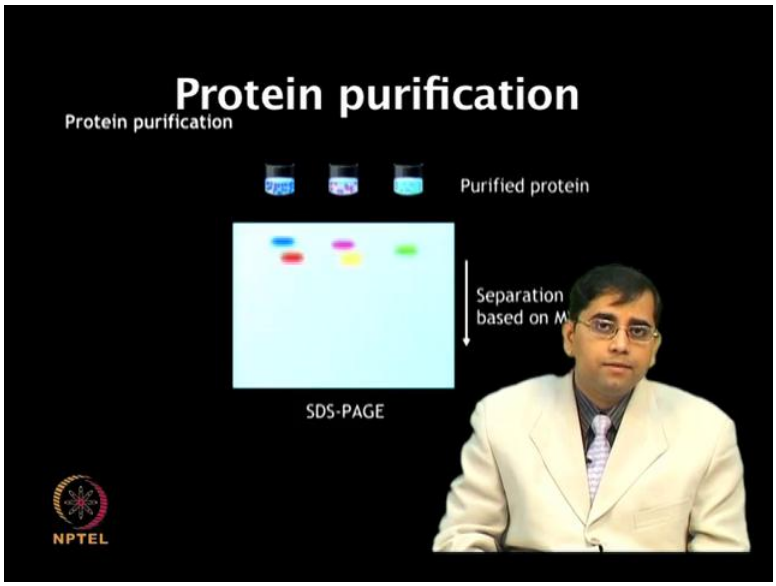

Protein purification can be done by chromatographic procedures to obtain the pure target protein and protein purity is tested on SDS-PAGE.

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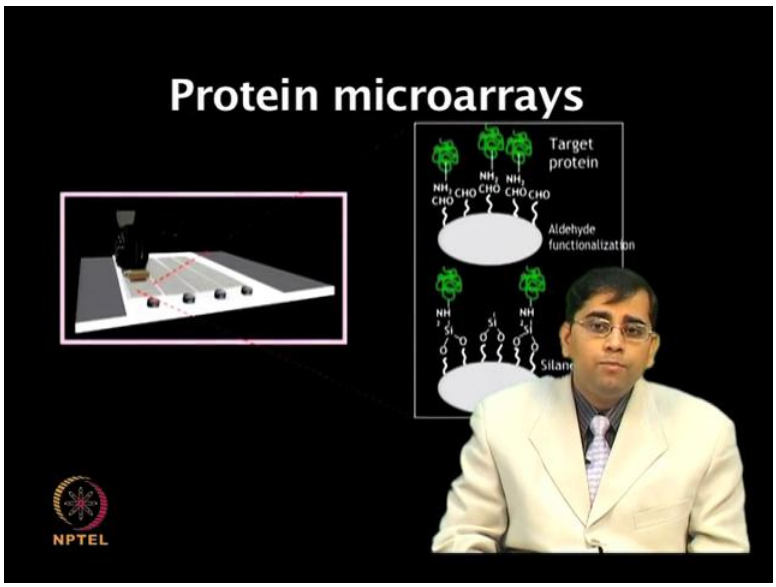

The array surface can be functionalized with a suitable chemical reagent such as aldehyde and celinetervatizations that will react with groups present on protein surface. Protein is printed onto the array surface in extremely small volumes by using robotic printers. There are two types of protein arrays that are commonly used, forward phase arrays, where immobilized antibody is probed by the test lysate. In reverse phase arrays,

cellular lysate is immobilized on the array surface and then, probed using detection antibodies specific to the target of interest.

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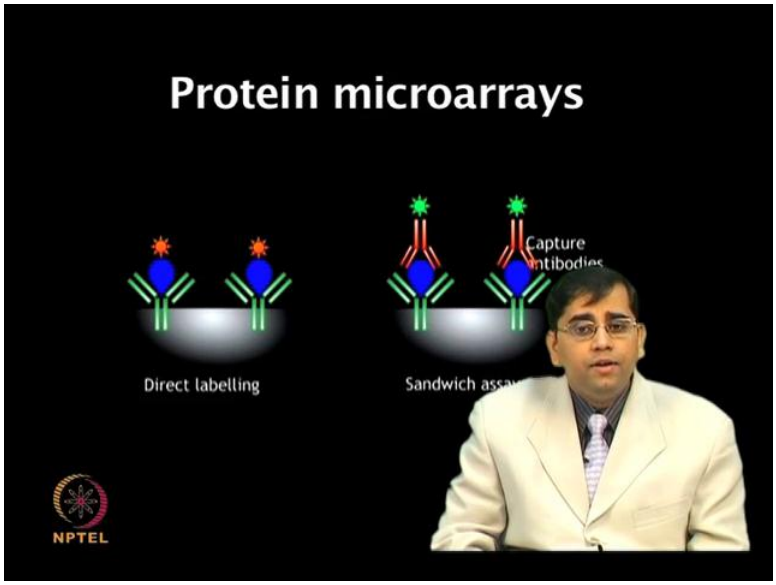

In protein detection using direct labelling at the target, all the target proteins are labeled with a fluorescent tag. In sandwich assay, however a fluorescent tag secondary antibody that recognizes a different epitope on the target antigen binds to it and detected by means of fluorescence.

Protein purification is a laborious process and time consuming, which posses several technical challenges for example, protein purity, protein folding and protein functionality during the purification immobilization and storage (( )). These limitations have motivated the advent of cell-free expression based microarrays, which carry out (( )) transcription and translation and eliminate the drawbacks of traditional cell based methods.

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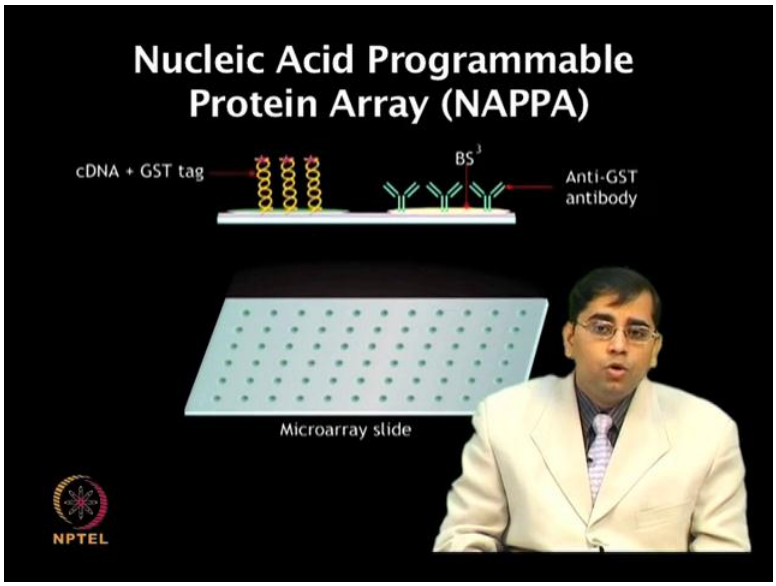

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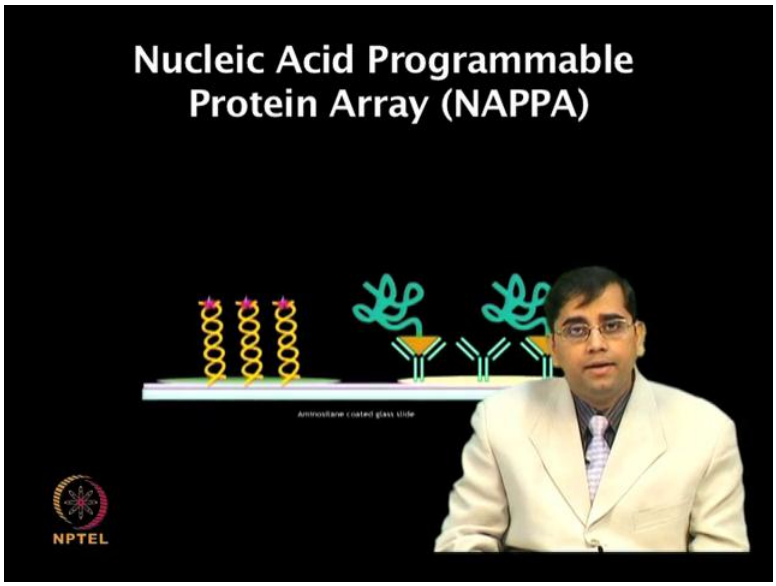

Nucleic Acid Programmable Protein Arrays NAPPA, replaces the complex process of spotting, purified proteins with simple process of spotting plasmid DNA by using recombinational cloning and cell-free expression system, proteins are produced in vitro and captured on array.

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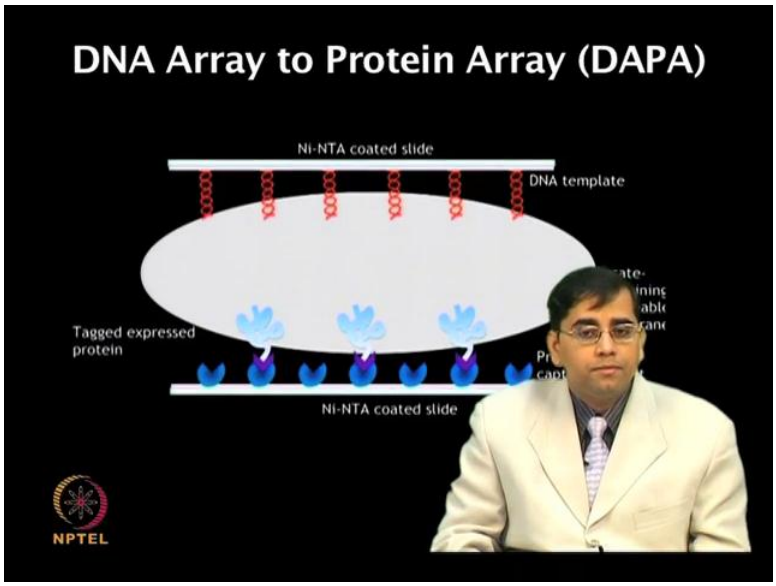

In DNA Array to Protein array DAPA, slides bearing, the DNA template and the protein tag capturing agents are assembled phase to phase with lysate containing permeable membrane placed in between. The expressed protein slowly penetrates the membrane and gets immobilized on the slide through its capture agents.

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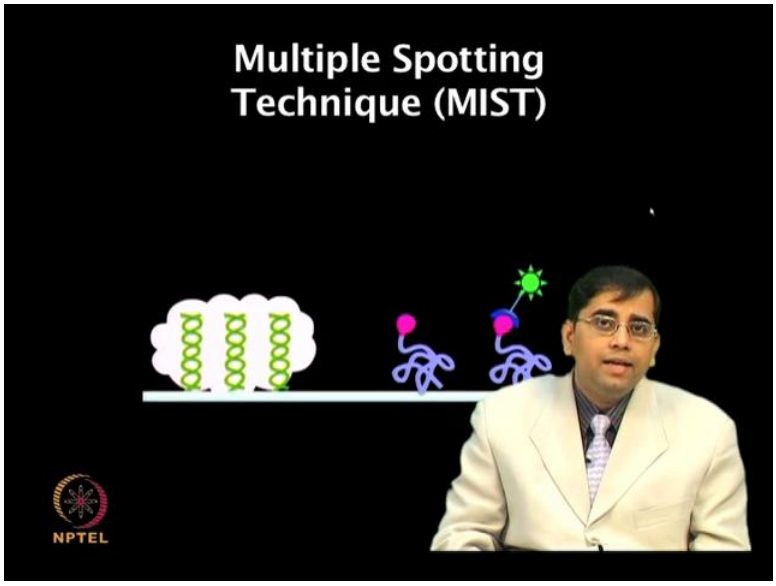

Multiple Spotting Techniques MIST, it involves addition of template DNA onto the solid array surface and second spotting step involves addition of cell free lysate.

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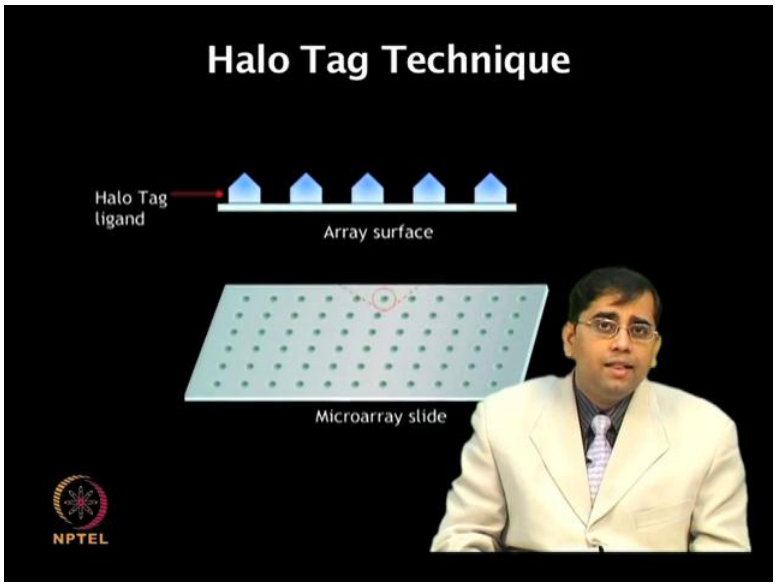

In halo tag method, slide is activated with a halo tag (( )), which captures the expressed protein through fun covalent interaction thereby preventing any material loss and assuring oriented capture of proteins.

Microarrays have become integral part of clinical and drug discovery process.. The protein microarray experiment involves blocking the slides with milk or super block, application of primary antibody and washing with milk followed by incubation of secondary antibody and signal detection, although microarray experiments are simple, but data analysis is very challenging. Biological research has witnessed (( )) time shift from focused reductionist approaches to a greater dependence on data provided by large omic techniques to provide insight into the biological systems and organization of physiological networks.

The microarray scanning and data analysis will be discussed with application expert of a Spinco for molecular devices, single or multiple slides can be scanned by using a scanner. Using defined scanning parameters robotic arms can select slides and position it for the scanning, the laser power wheel can adjust the laser strength, fluoresce signal is collected from the photo multiplier tubes, each channel (( )) (( )) and TIFF images are saved.

Data analysis becomes crucial to make cells out of massive amount of data, it is very important have good understanding of both the biology involved and the analytical

techniques, rather than relying only on the software. Challenges of microarray data analysis will be discussed with professor Sudesh from Tulane University, USA.

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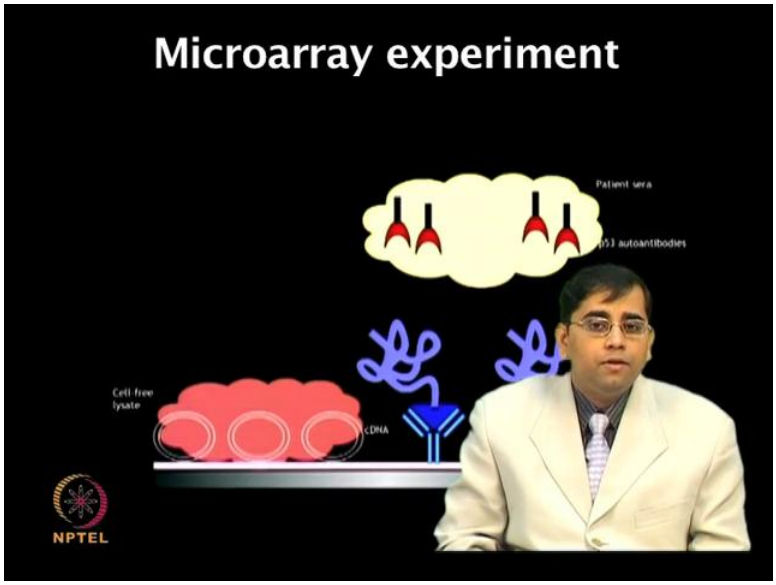

Protein microarrays have found wide applications for discovery and functional proteomic studies. Microarrays are used for analyzing both antigens and antibodies in blood samples, and other biological fluids for biomarker discovery. Some of the representative applications will be discussed in this module. Several conventional Label based detection approaches such as fluorescence chemiluminescence and radio isotopes are in practice, but researches are exploring methods for label-free analysis to get rid of interference due to the tagging molecules; and reduce the complexity (( )).

The label-free techniques rely on measurement of inherent properties of the query molecules such as mass dielectric properties and allow direct rapid real time measurement of biomolecules in high throughput manner, eliminating the requirement of secondary reactants.

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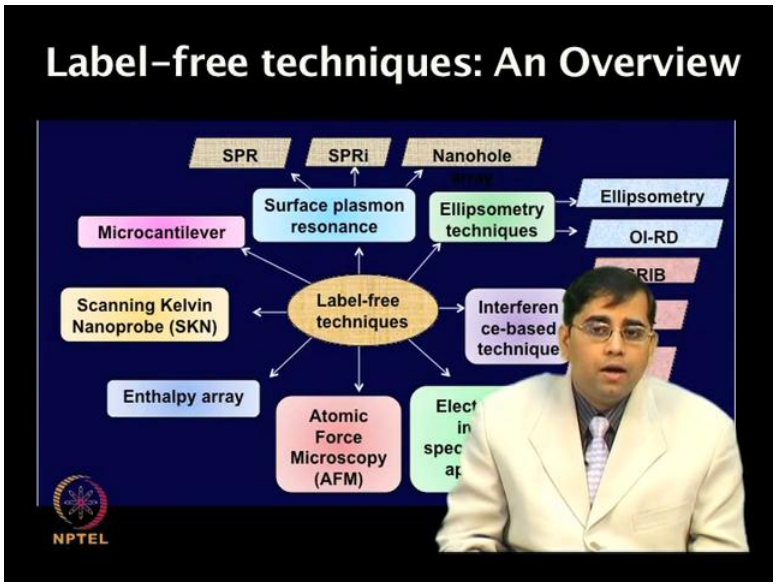

Many label-free techniques such as S P R,S P R imaging, ellipsometry and interference based and nano technique based techniques are emerging, rapidly as a potential compliment to the labelling methods and it will be discussed in module label-free detection techniques. S P R based biosensors are in great demand as they provide label free, real time detection of bimolecular interactions, SP R sensograms descries the changes in S PR signal versus time.

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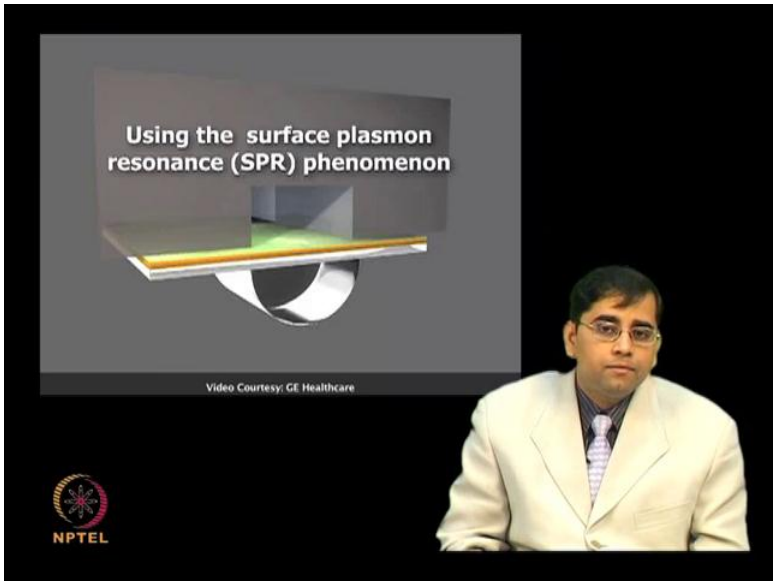

Since introduction of biacore S P R instrument, the S P R spectroscopy has been widely used to characterize biological surfaces and monitor bio molecular binding events. The biacore technology will be discussed with research product expert of GEHealthcare; this optical biosensor can be used to monitor macromolecular interactions in real time, without labelling requirements.

The S P R biosensors have played an important role in research into biomolecules, and their interactions. Performing good S P R experiment and accurate interpretation of binding reactions from biosensors are always very challenging. Performing good S P R experiments data collection and processing can eliminate artifacts and provide good quality data.

The success of S P R experiments depends on the kinetic measurements in real time, monitoring adsorption of unlabelled (( )) molecules to the surface and ability to monitor weakly bound interactions due to high surface sensitivity. Detailed guidelines for the S P R data analysis has been provided by the researches are need to be discussed in this module.

Several nano techniques such as carbon nanotubes, nanowires, quantum dots, gold nano particles are increasingly being used for proteomic applications. In the field of nano proteomes as demonstrated here, the binding of target protein to the functionalized carbon nanowires lead to detectible changes in electrical conductance.

The phenomenon of diffraction and interference and use of diffraction based biosensors will be discussed with Professor Cynthia Goh from University of Toronto. This technique hold great promise to become a technically robust and user friendly platform for clinical and diagnostic study. This century is considered as century of biology in which life science research is undergoing a profound transformation by employing various omic technologies.

Unraveling a structural and functional details of proteins at the proteome scale is very daunting task; however, proteomics has come to mean virtually everything in protein research and it has quickly evolved to become integral aspect of human biology and medicine.

Today, I gave you an overview of modules and lectures, which will be discussed in this course. It was not possible to discuss all the modules and all the details, but during our subsequent lectures I will take you to a journey of protein and proteomics research by providing basic concepts and details of proteomic techniques. I hope it will enthuse you to learn about proteomic techniques and proteomic concepts, thank you for your attention.