Proteins and Gel-Based Proteomics
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Mod 02 Lecture Number 6

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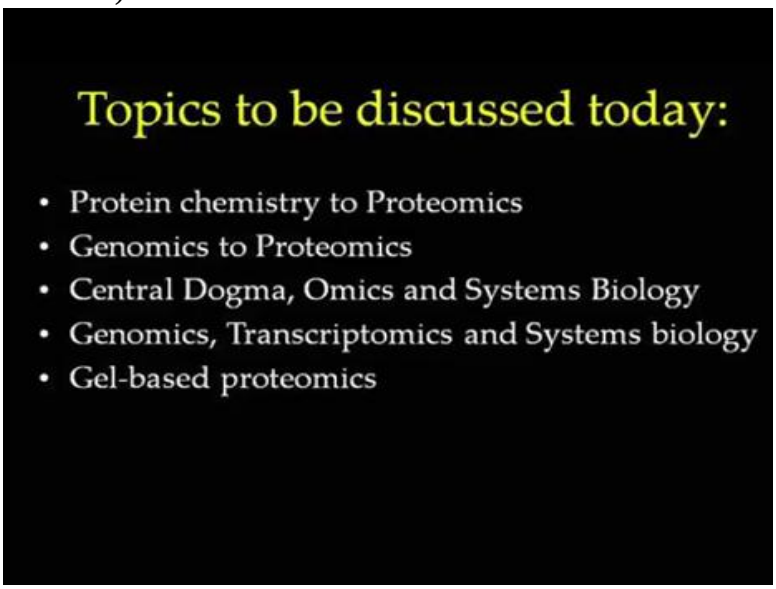

In this lecture, I will discuss about Proteomics.

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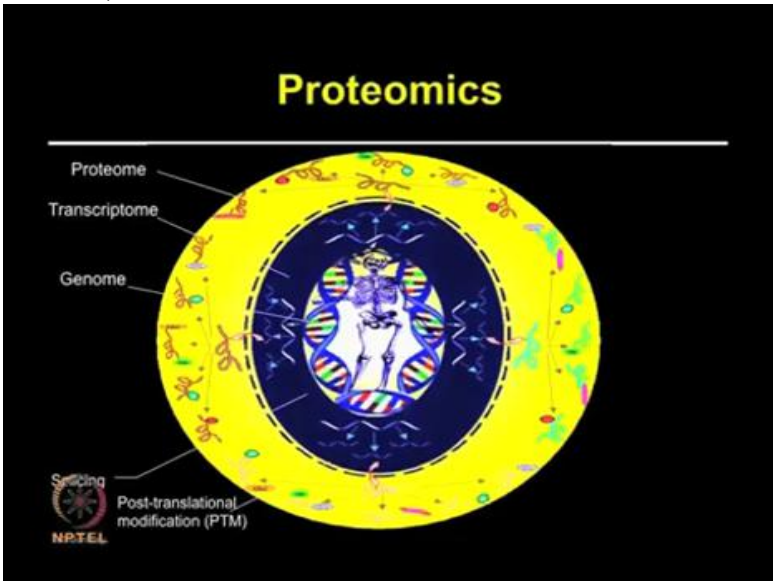

So first of all, what is Proteomics? The proteome describes the protein complement expressed by the genome or more precisely we can say, the protein complement of a given cell at a given time including the set of all the protein isoforms and its modifications. The study of entire compendium of proteins which are encoded 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 alternative splicing and post translational modifications is tremendous. Therefore studying proteins and proteomes are very important.

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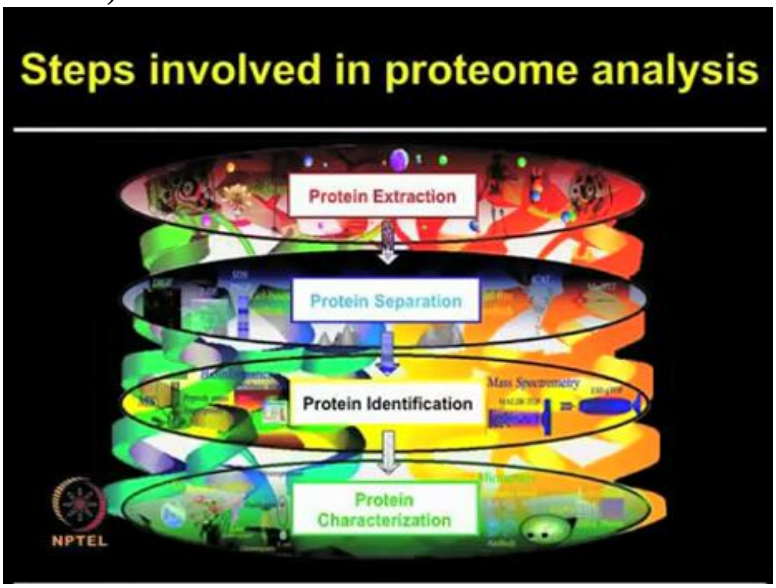

What are different steps involved in the proteome analysis?

As shown here, the protein extraction, protein separation, protein identification and protein characterization, these are the major steps which are involved in proteome analysis. The protein extraction from whole cells, tissues 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 iTRAQ Mass Spectrometry based techniques. The functional characterization of proteins using novel proteomics platforms open new horizon for exploration in biology.

The proteomics discipline can be grouped under two major disciplines, abundance and function based proteomics.

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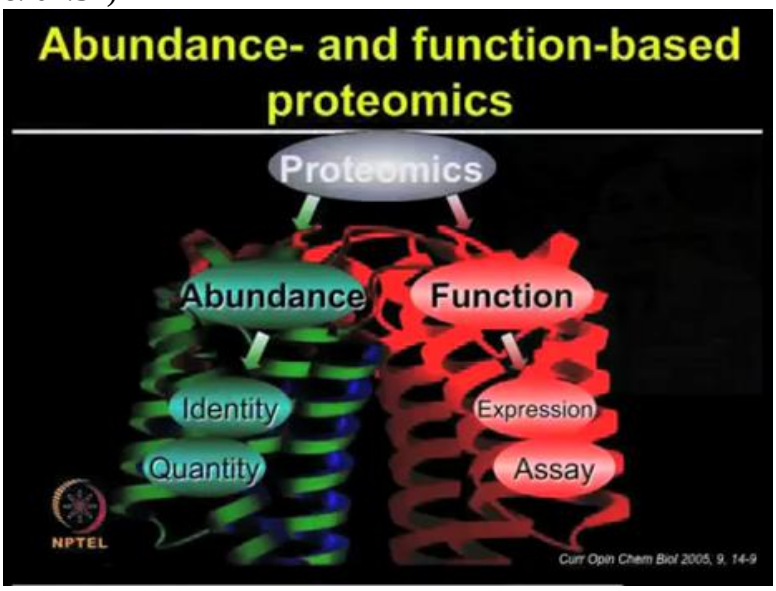

The abundance based proteomics aims to measure the abundance of protein expression where as the functional proteomics aims to determine the role of proteins by addressing protein interactions and their biochemical activities. So how did proteomics field emerge?

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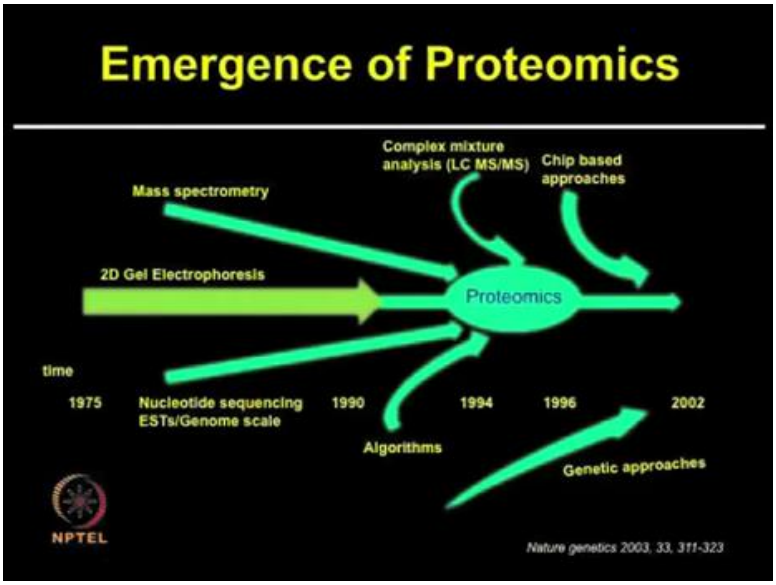

As you can see in the timescale here shown in the slide, advancement of various techniques such as two-dimensional electrophoresis and mass spectrometry, genome sequencing information and computational algorithm together led to the emergence of proteomics field

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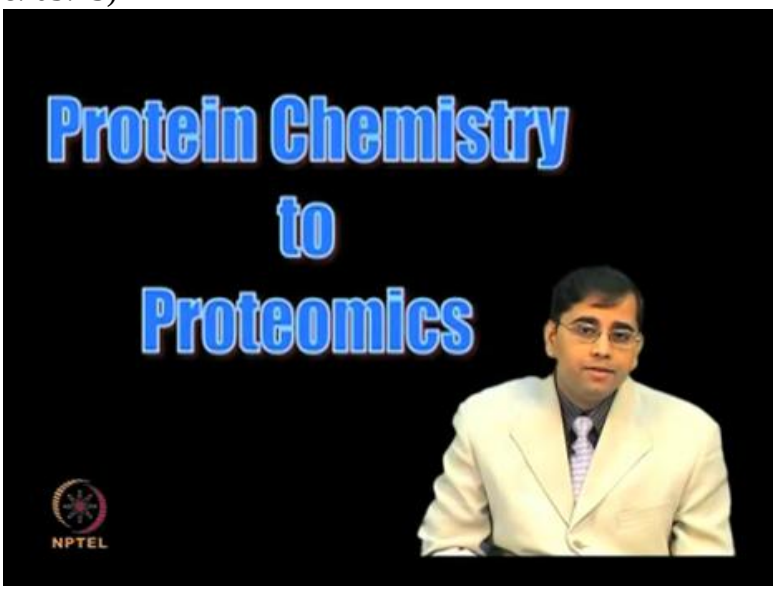

Proteomics research originates from classical protein chemistry and it has embraced new high throughput techniques to analyze complex samples. Many of the techniques used under the modern proteomic umbrella, for example two-dimensional electrophoresis, mass spectrometry have actually originated several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

So what is new? The technological advancements in protein analysis with increased sensitivity, resolution and capability to carry out high throughput studies 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 ionization techniques. This limitation was overcome…

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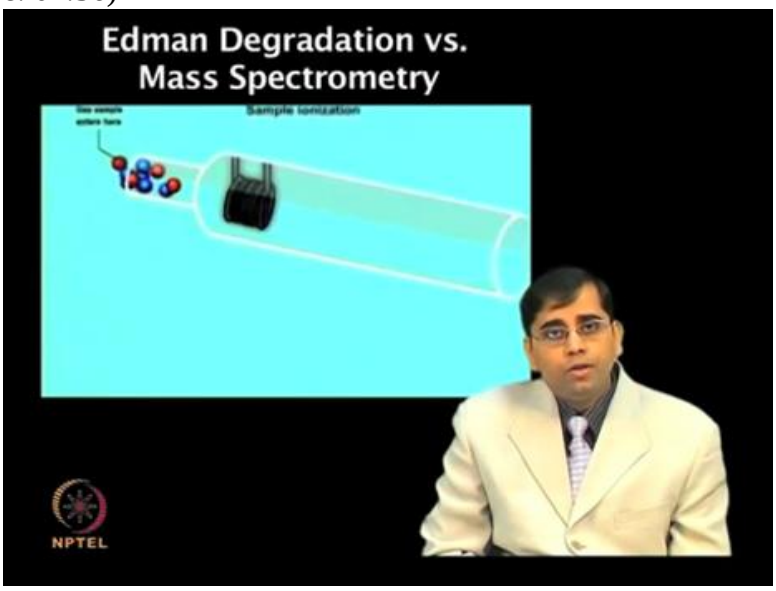

by soft ionization techniques…

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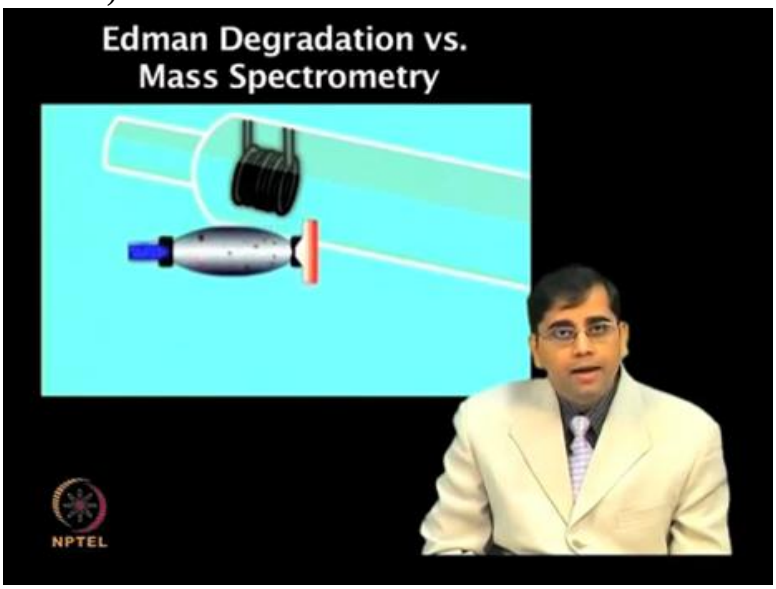

… such as MALDI and electospray ionization.

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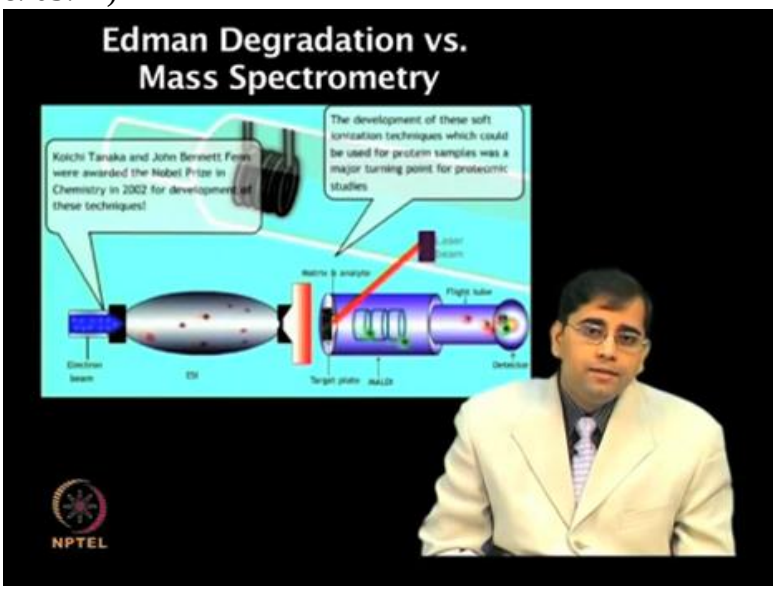

These techniques have greatly improved the proteomic studies as they facilitated mass spectrometry analysis of protein samples.

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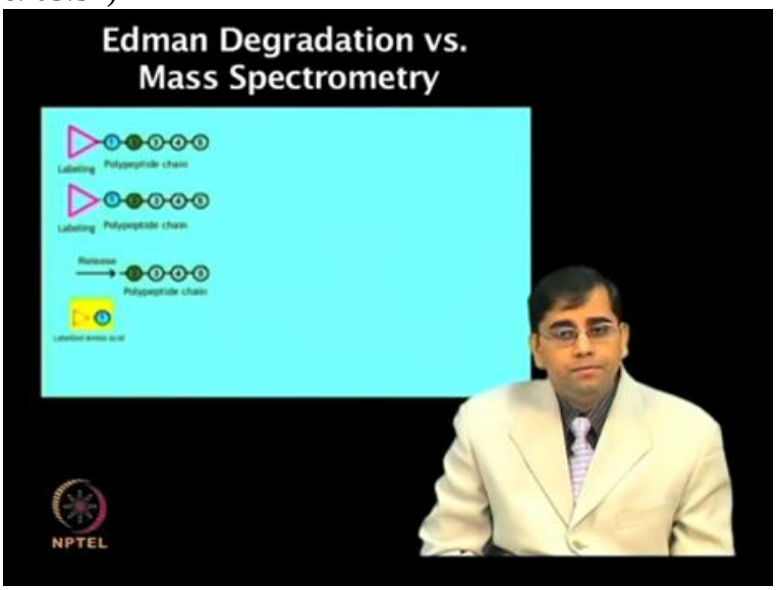

Protein sequencing by Edman degradation is time consuming and cumbersome.

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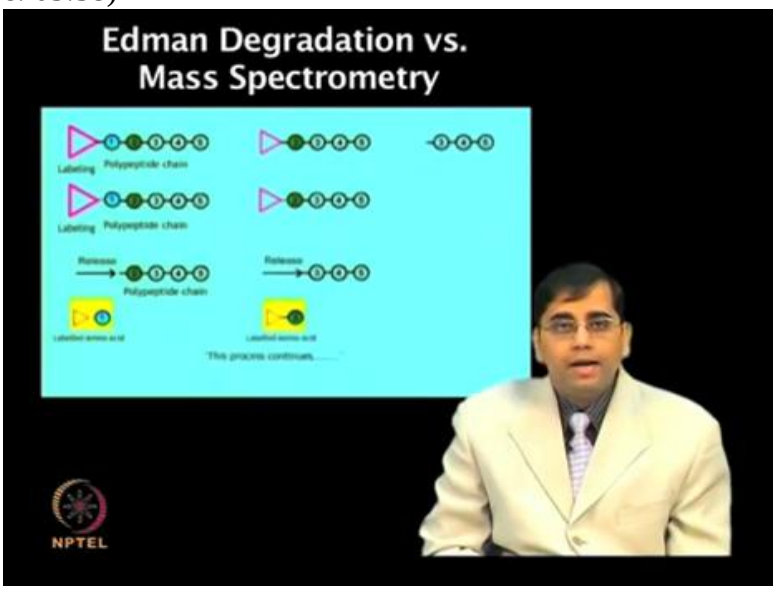

Several rounds of sequencing are required for analysis of polypeptide chains.

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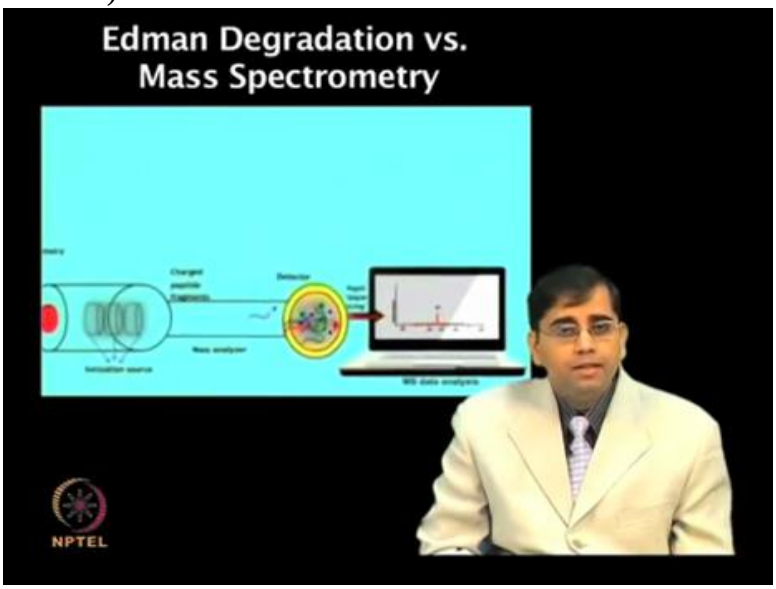

However peptide sequencing by mass spectrometry is much faster and allows large number of samples to be analyzed in a short time.

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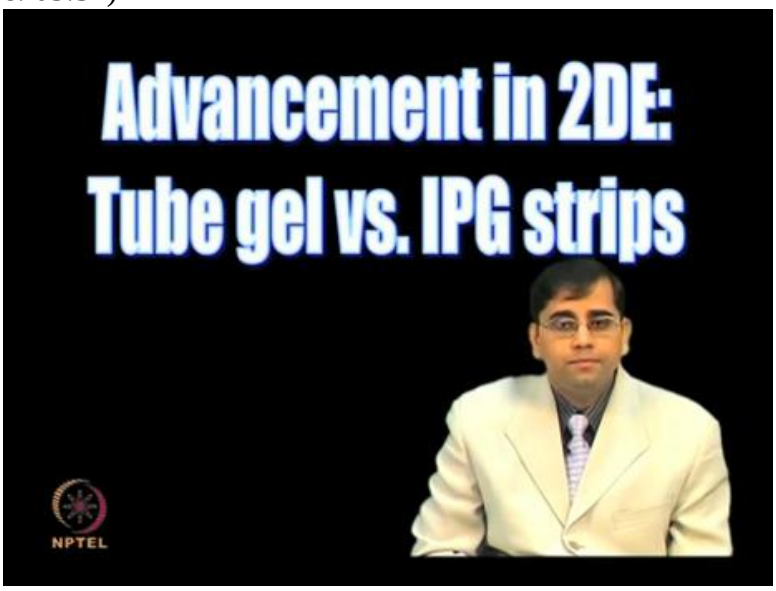

Another aspect, development of immobilized pH gradient strips facilitated proteomic analysis sing two-dimensional electrophoresis. The pH gradient in tube gels are established by ampholyte gradients which are not always very stable …

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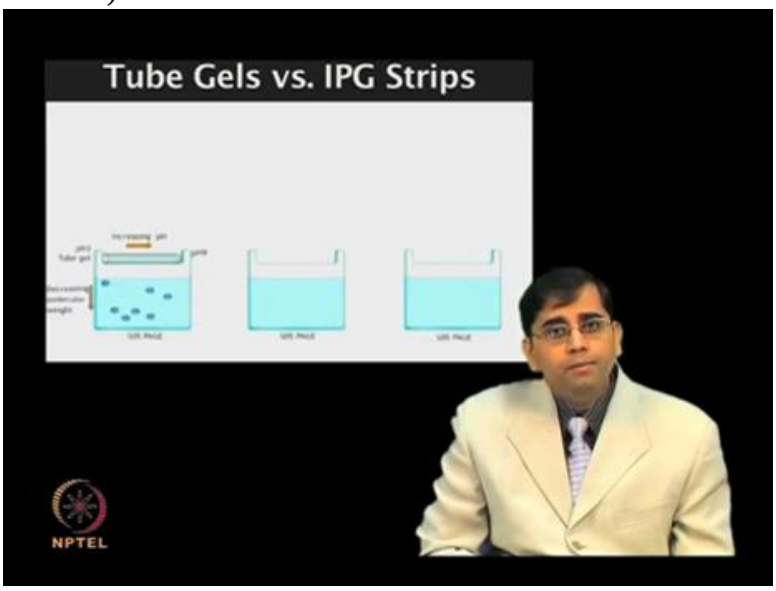

…and tend to break down upon addition of the concentrated samples.

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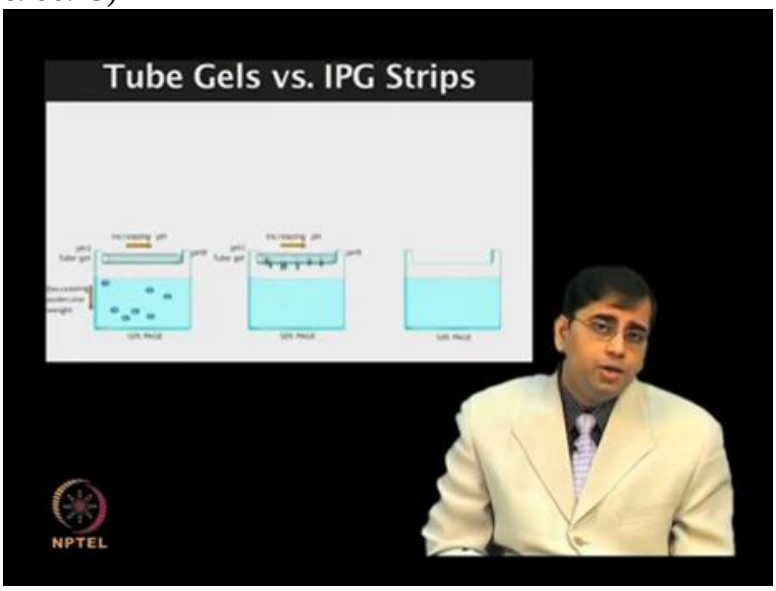

Analysis of protein mixture by two dimensional electrophoresis using tube gels often resulted into…

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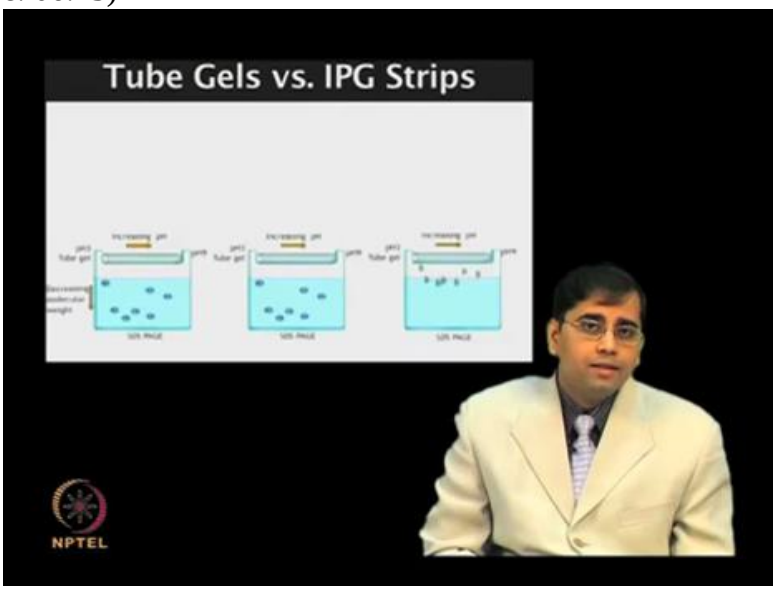

… variations in the gels.

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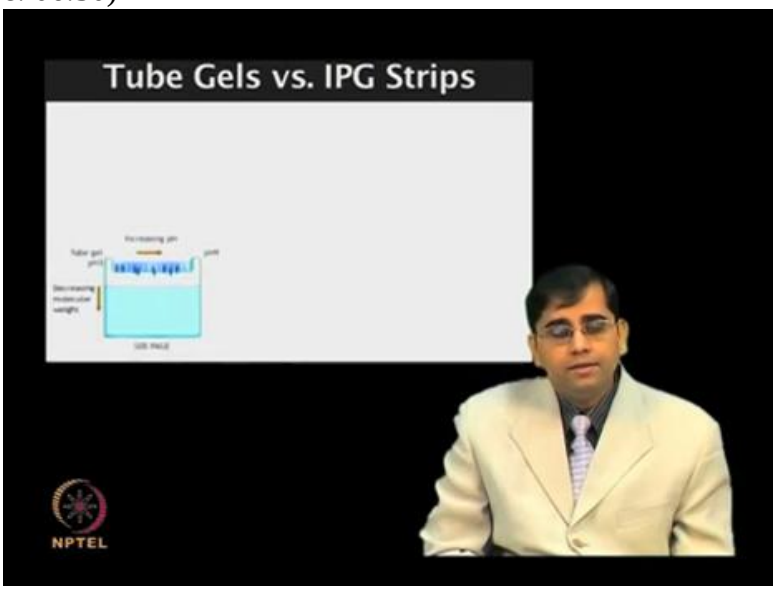

The problem of reproducibility was overcome to a large extent by the development of Immobilized pH Gradient strips or IPG strips.

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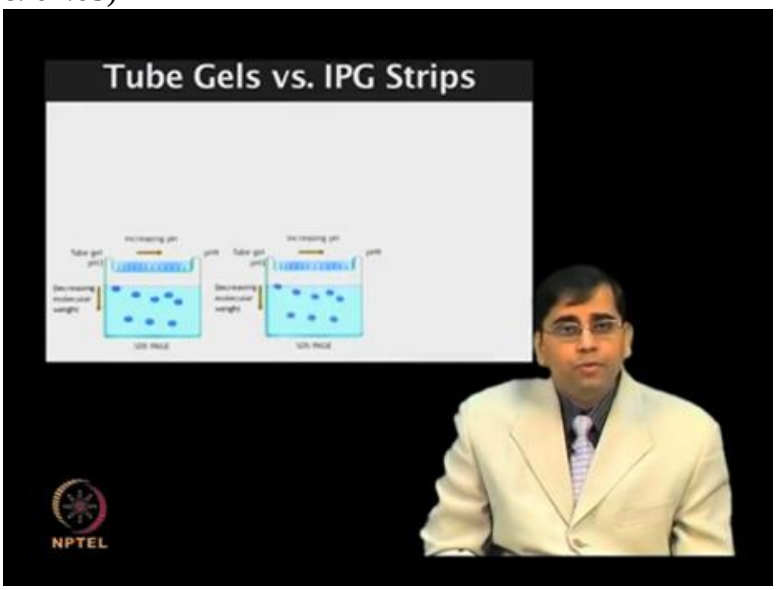

Minimal gel to gel variation was observed when samples were run by…

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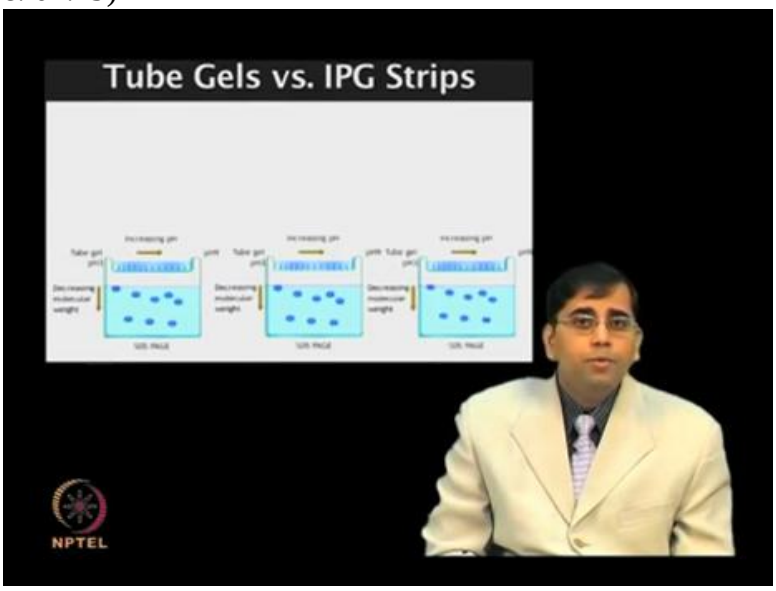

… two dimensional electrophoresis employing IPG strips which made this technique suitable for the large scale proteomic applications.

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Completion of several genome sequencing projects

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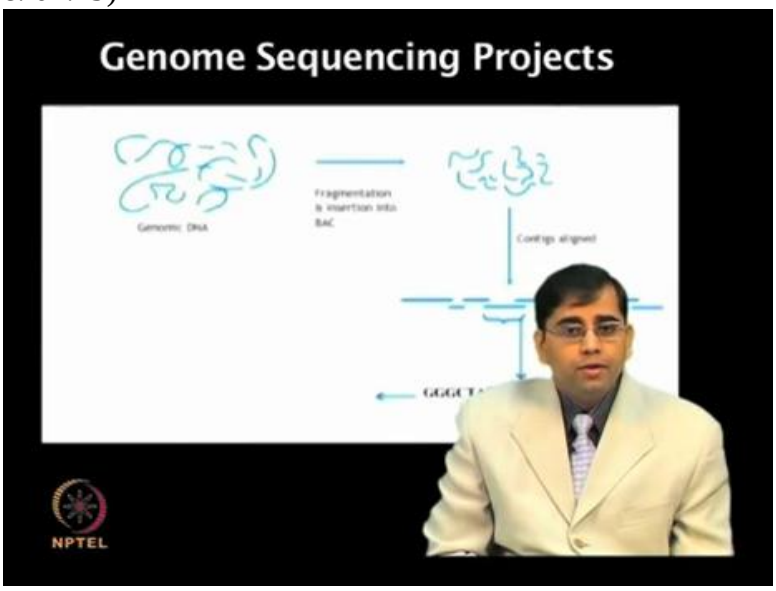

Genome sequencing of several organisms including humans have been successfully completed

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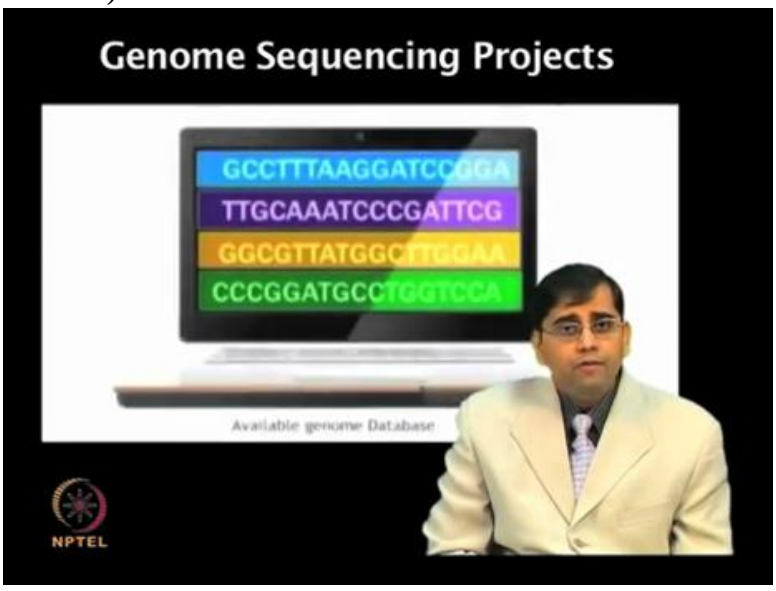

… and these genome databases are extremely useful in correlation of gene and protein sequences.

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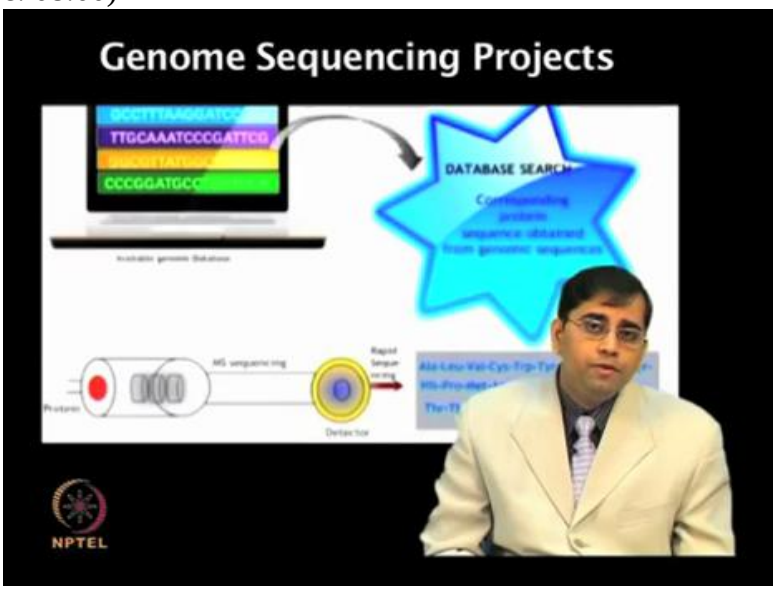

Several databases are now really available which can really help in identification of gene sequence of a protein which has been sequenced by mass spectrometry.

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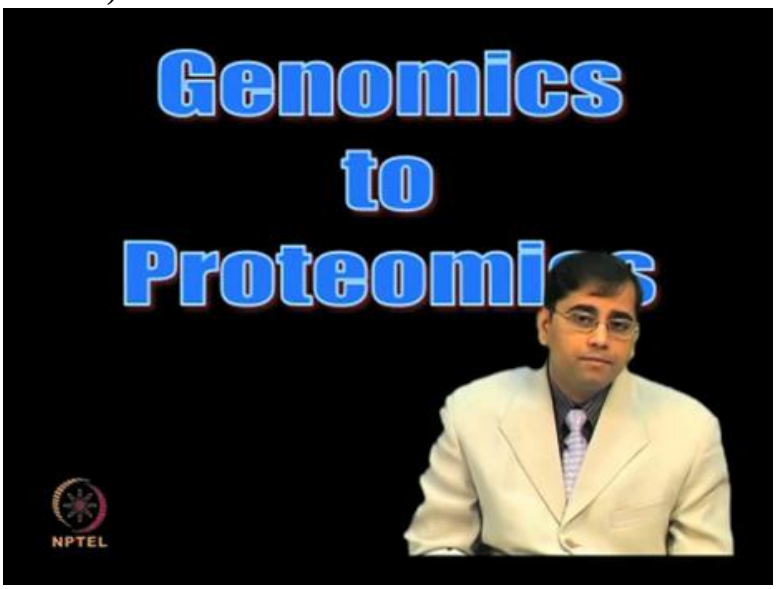

Genome represents an important starting point towards understanding complexity of biological functions. However proteins provide a much more meaningful insight into the mysteries of essential biological processes. To obtain better understanding of cellular processes and regulation there has been an increasing interest in studying proteome. There are several reasons why one needs to study proteomics.

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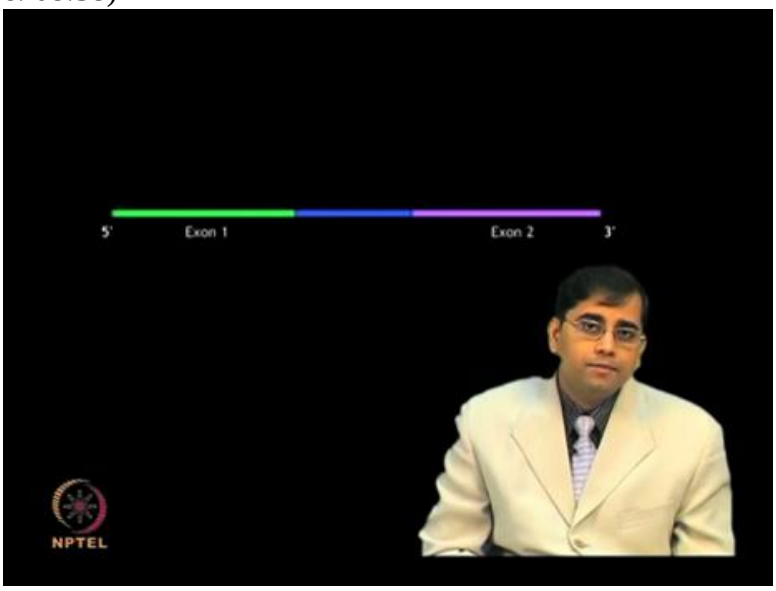

First, the genomic DNA contains large stretches of non-coding regions.

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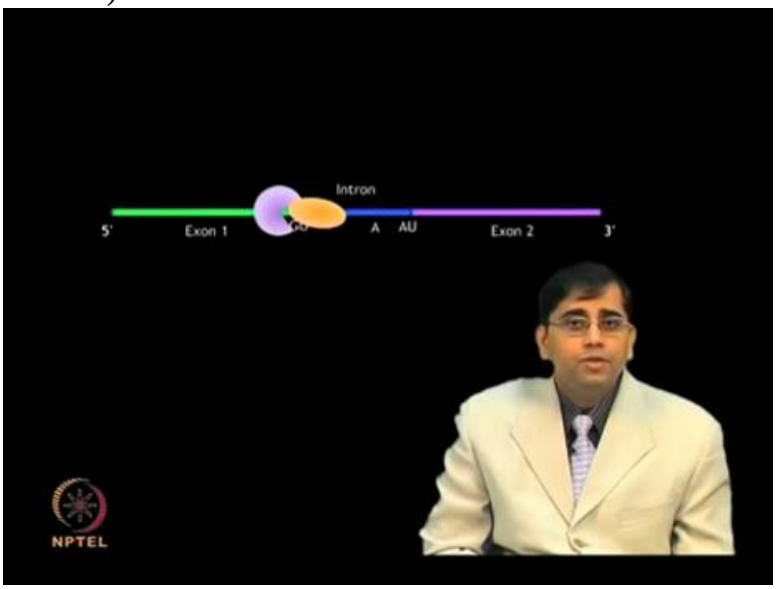

The pre-mRNA is synthesized from the genomic DNA by the process of transcription.

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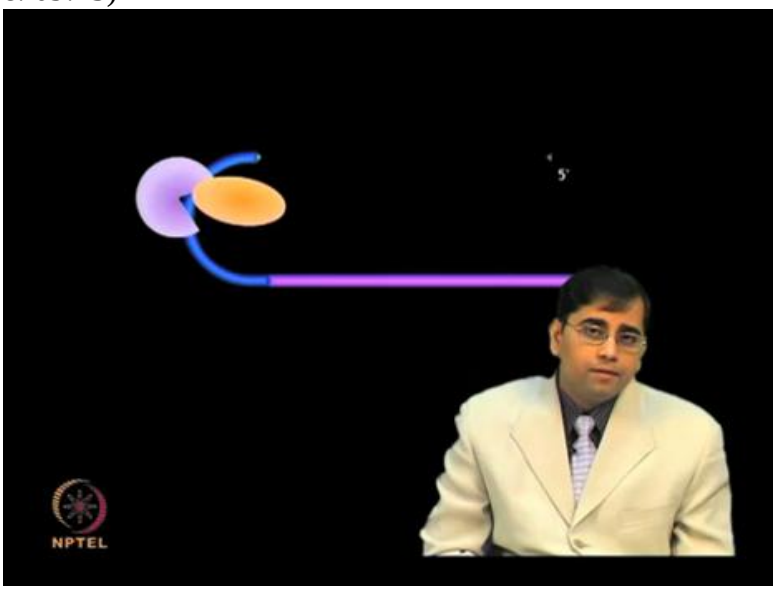

…mRNA contains both exons, the coding sequences

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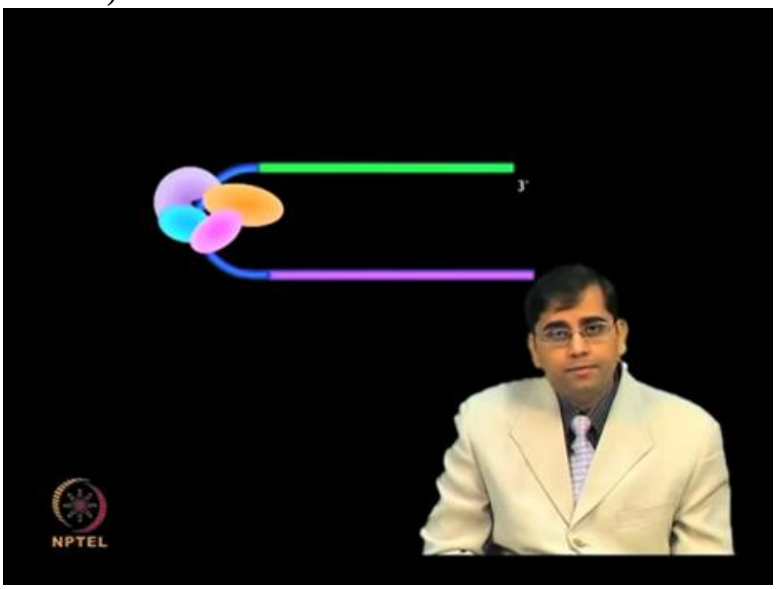

… as well as introns which are intervening non-coding sequences.

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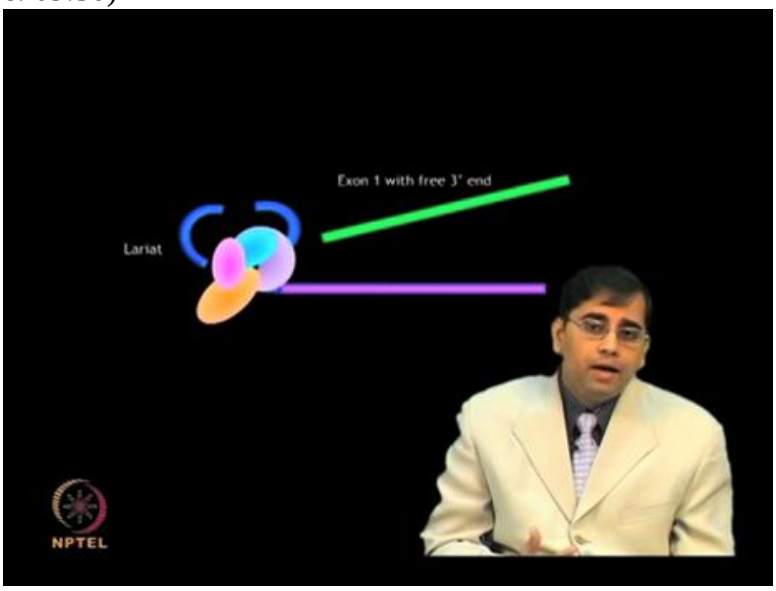

By involving series of steps

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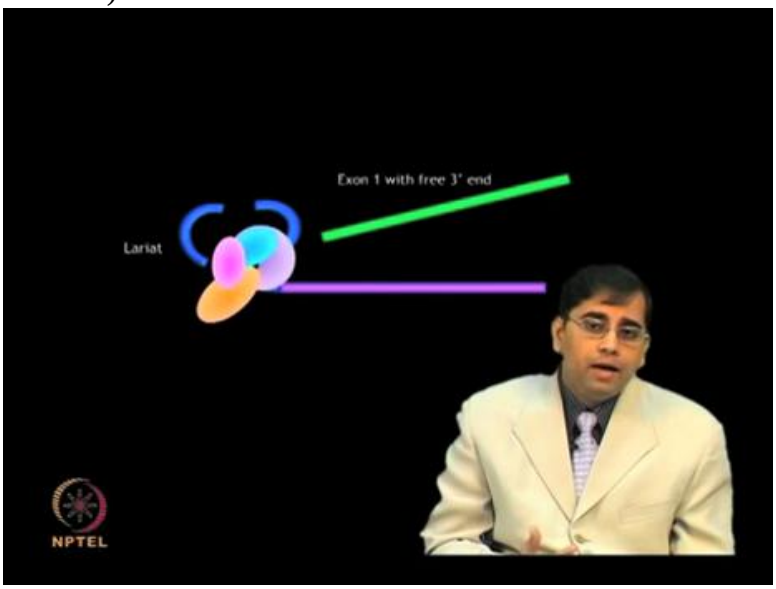

… finally the three prime hydroxyl group of the first exon attacks

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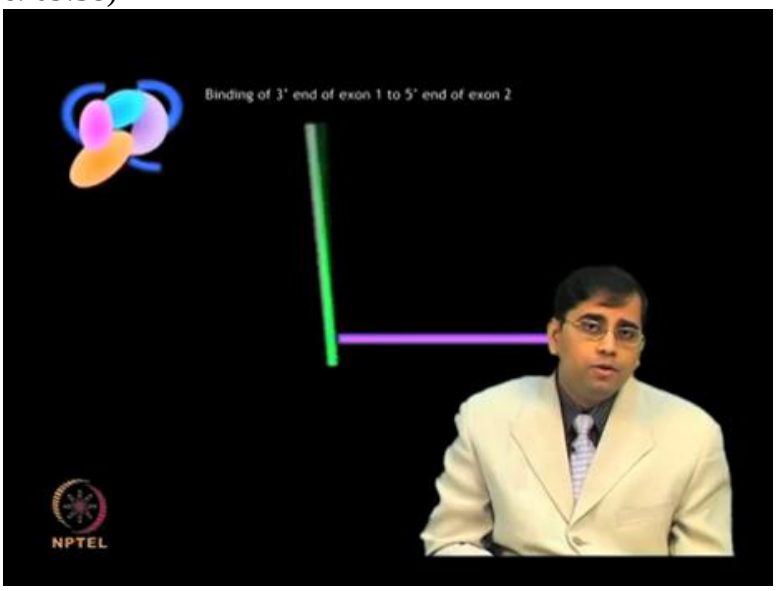

… the 5 prime end of the second exon such that

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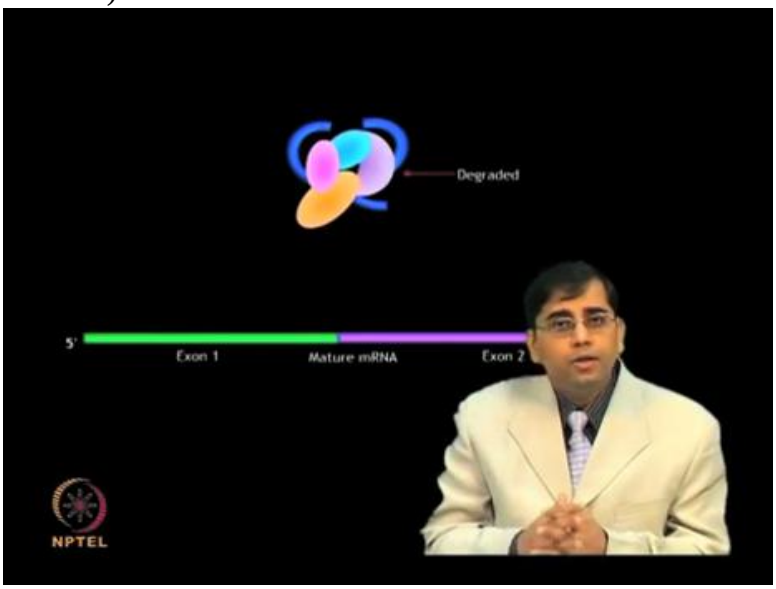

…they are joined together…

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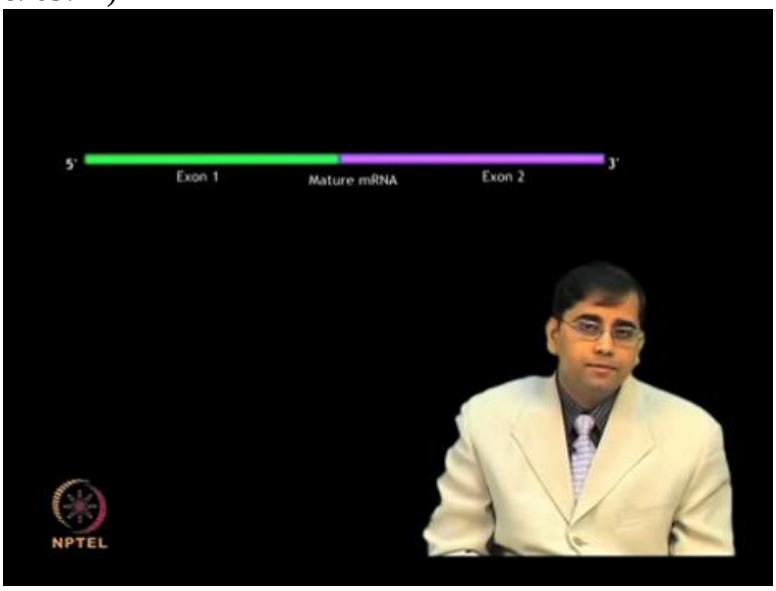

… to give the mature mRNA.

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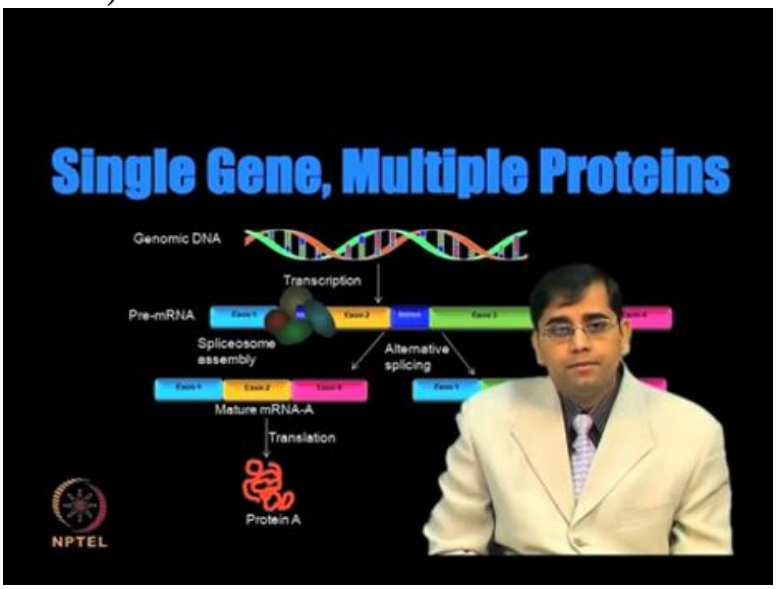

Second important factor is… single gene can give rise to multiple proteins.

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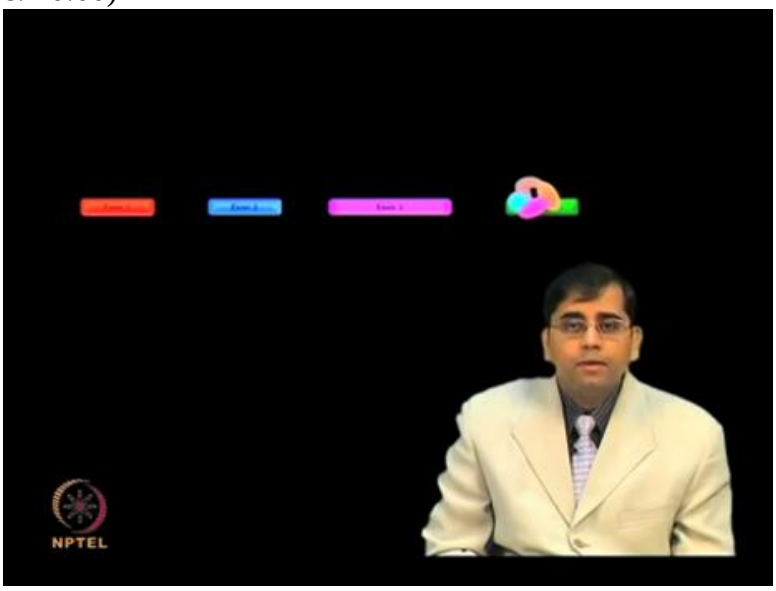

The alternative splicing is a process by which, exons or coding sequences of pre mRNA

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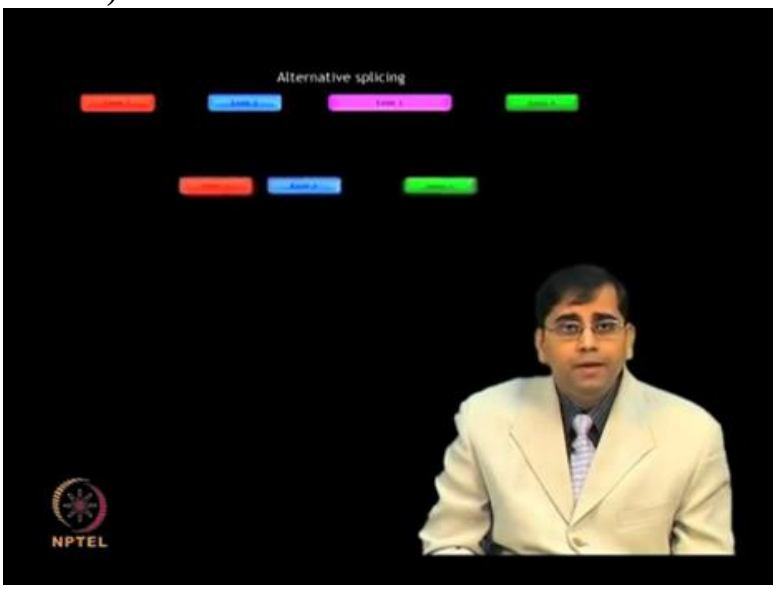

produced by transcription of a gene

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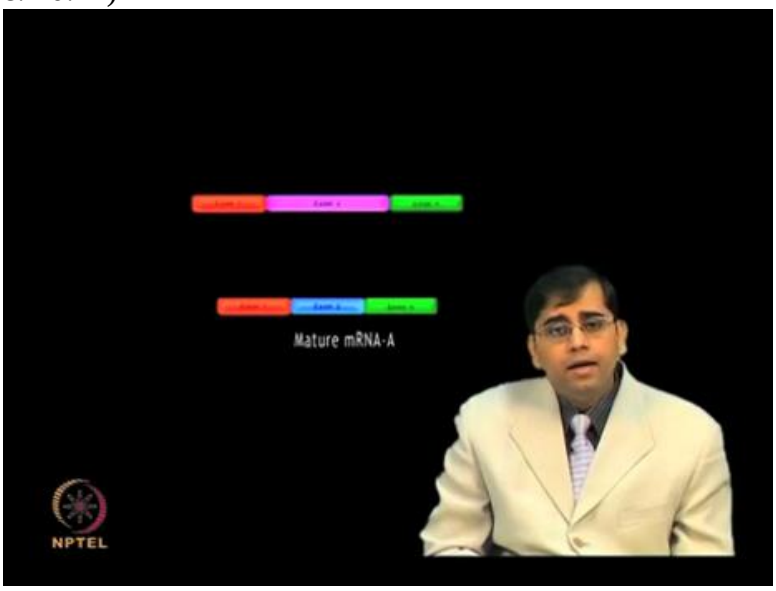

…are combined in different ways…

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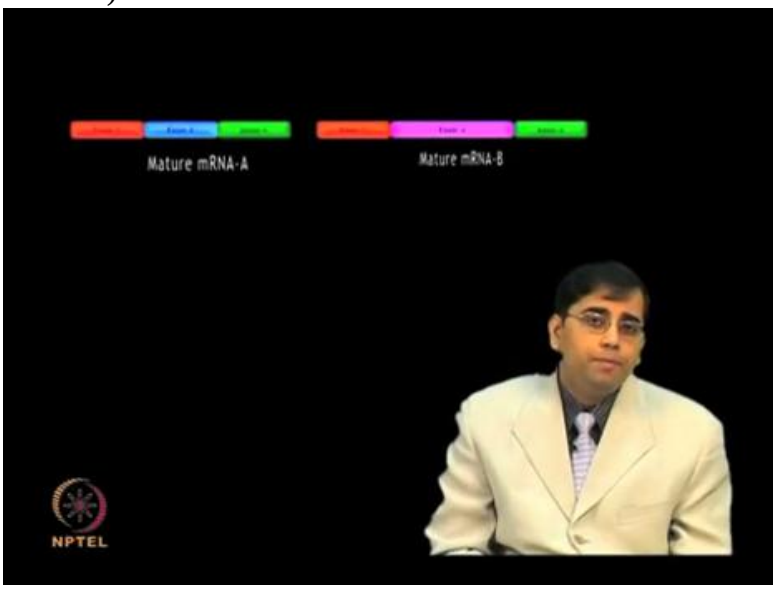

…during RNA splicing

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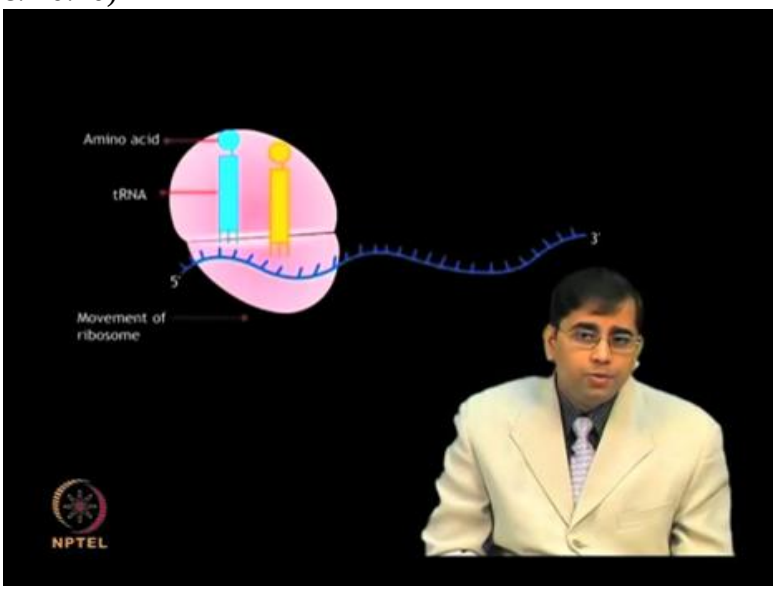

Resulting mature mRNA gives rise to

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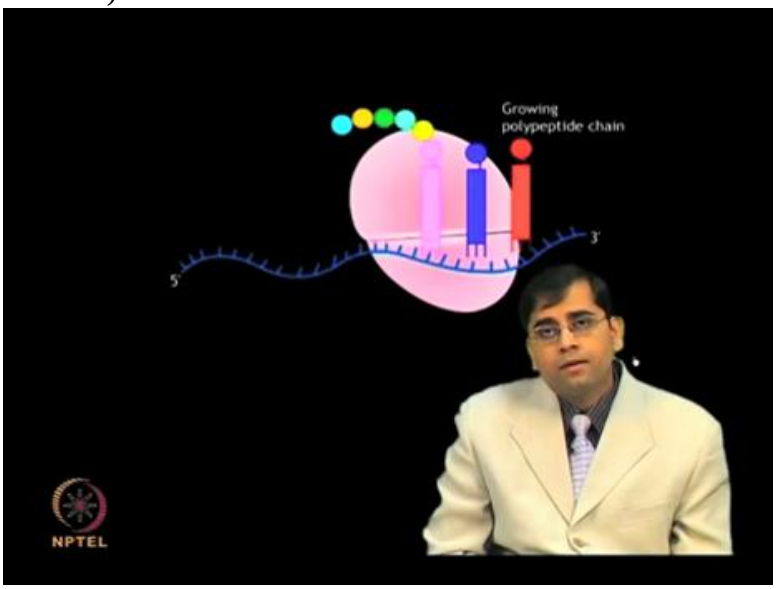

different products by translation, most of which are isoforms of one another.

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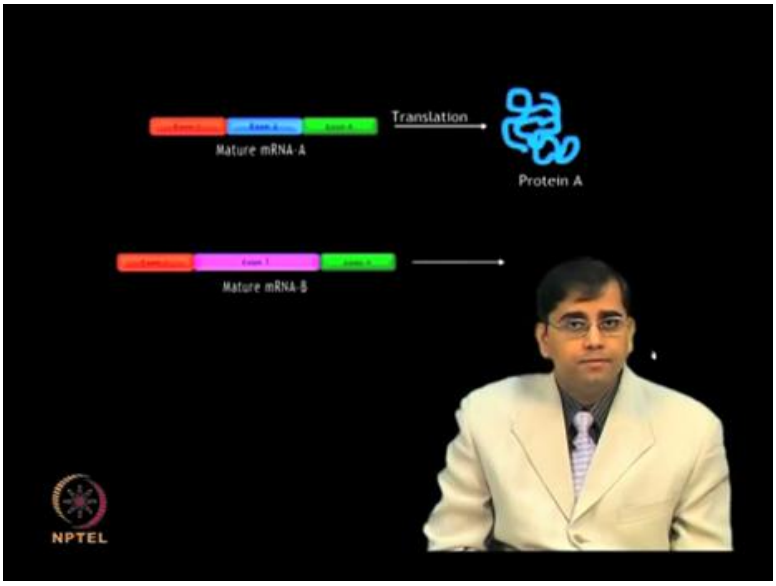

The diversity of proteins encoded by a genome is greatly increased due to alternative splicing.

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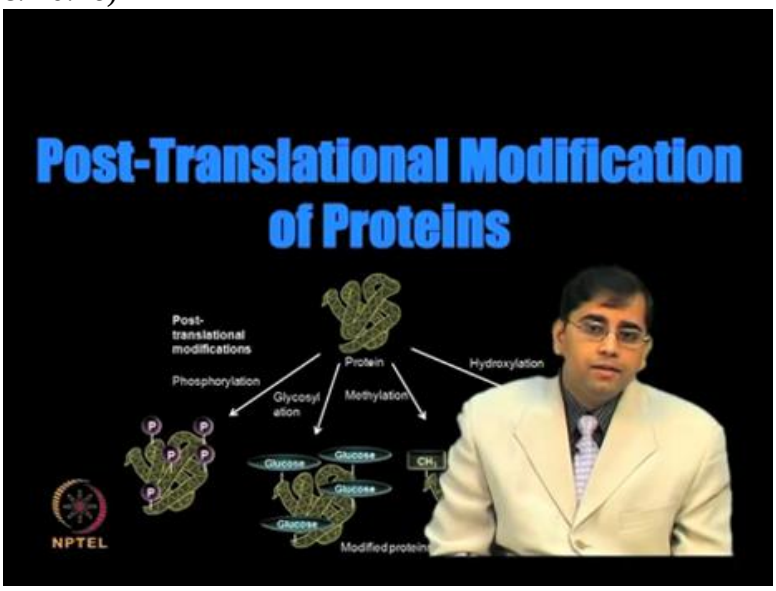

Third important factor is post translational modification of proteins.

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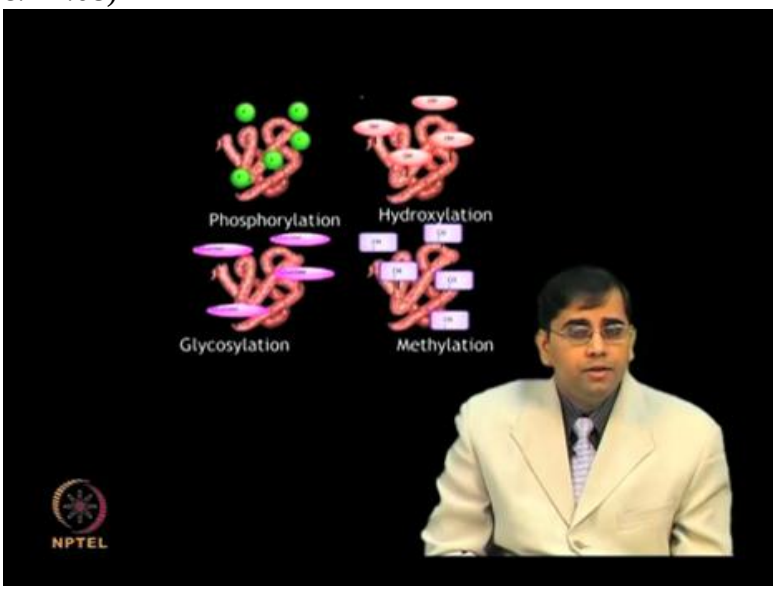

The proteins obtained by translation undergoes folding and various post-translational modifications such as phosphorylation, glycosylation, alkylation, hydroxylation etc. to give the final functional proteins.

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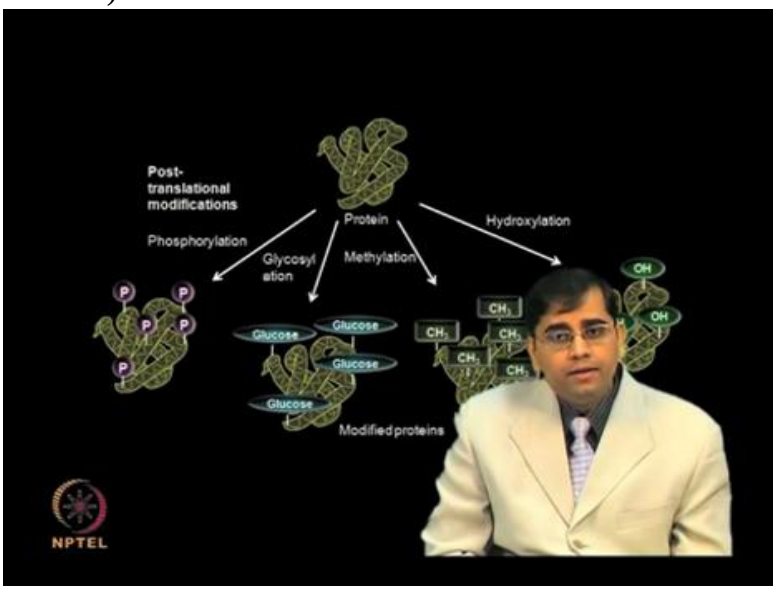

PTMs generate diversity, complexity and heterogeneity of gene products and its functional consequences can be modulation in protein dynamics and alteration of its functional activity.

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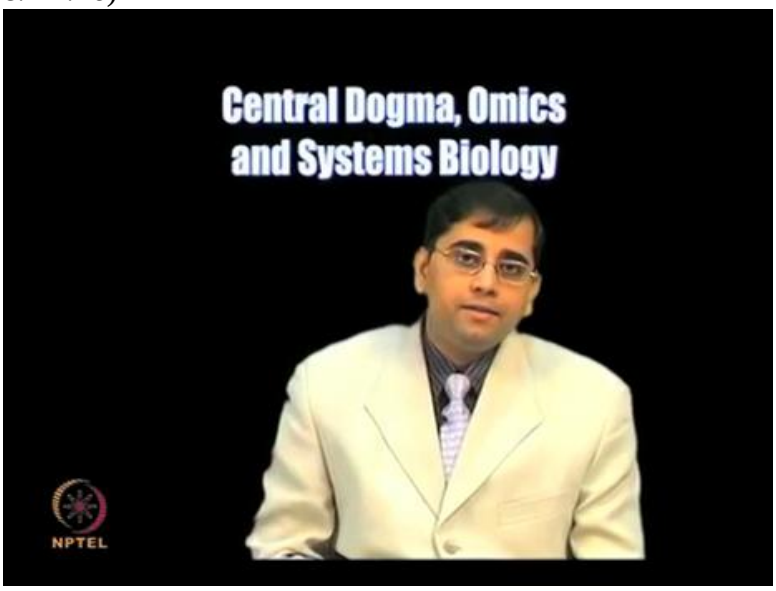

During the last decade, we have witnessed the revolution in biology as this discipline has fully embraced Omics tools. The emergence of genome-wide analysis to understand cellular DNA, RNA and protein content by employing genomics, transcriptomics and proteomics at Systems level has revolutionized our understanding of control networks that mediate the cellular processes. These concepts will be discussed in first module.

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Genes are the blueprint for life and proteins are the effector molecules.

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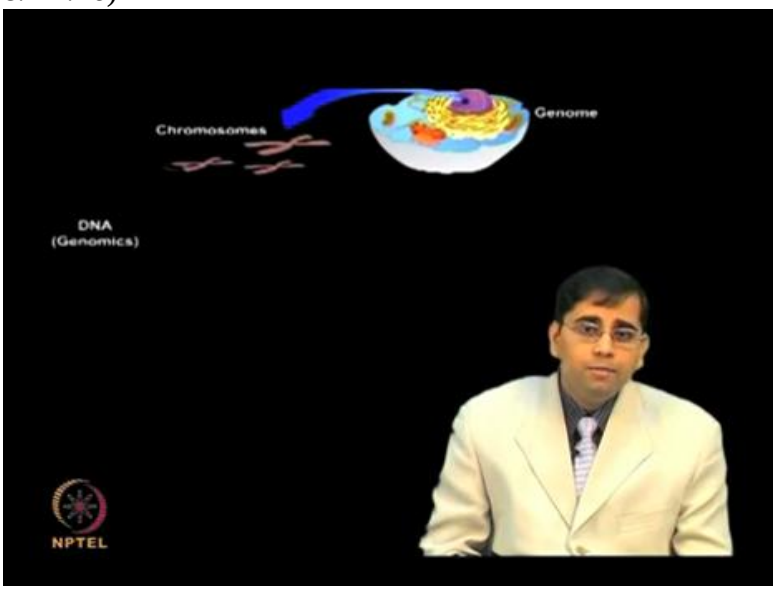

Due to this fact, the central dogma has guided research at the Systems level.

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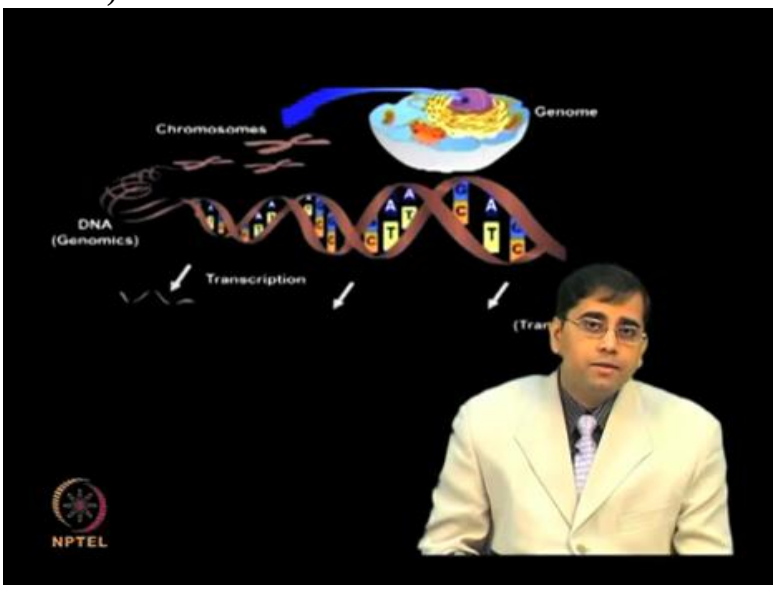

After completion of human genome sequence, numbers of genes around 25000s are surpassed by an estimated number of proteins in millions.

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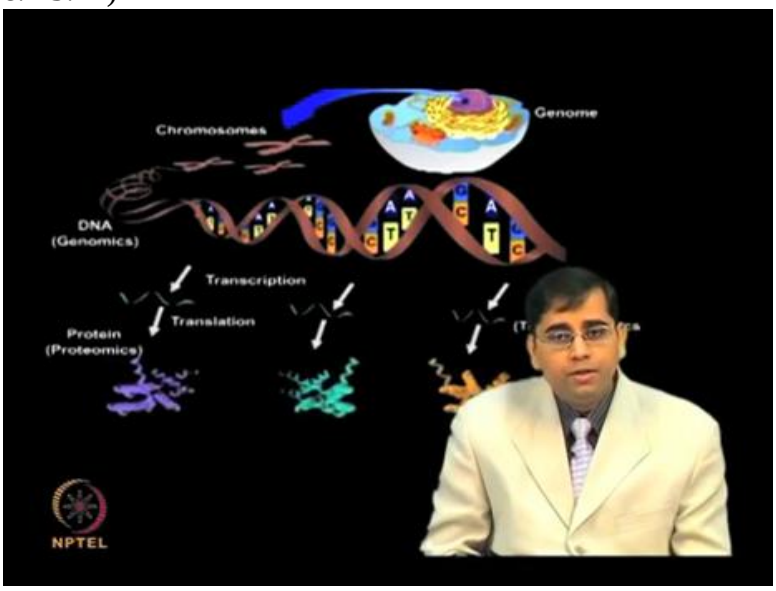

Studying large scale study of protein structure and function requires a thorough understanding of protein composition and their various structural levels

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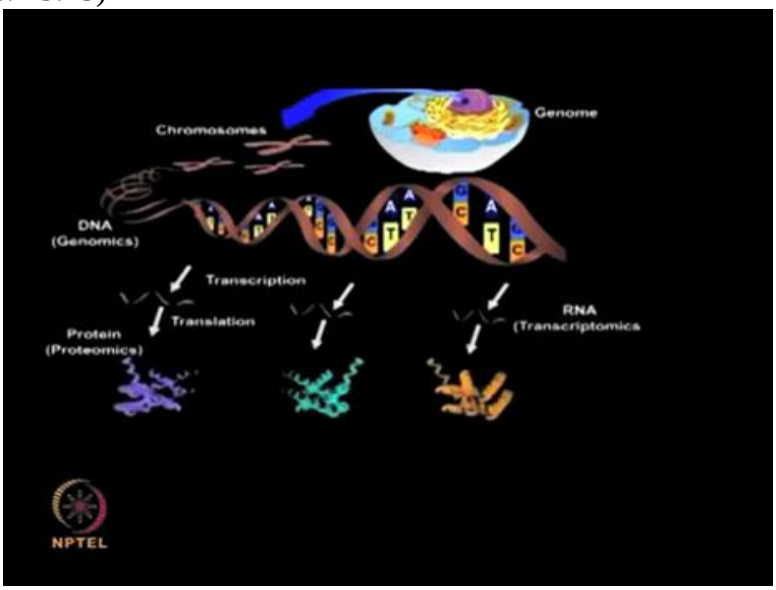

by employing high throughput tools.

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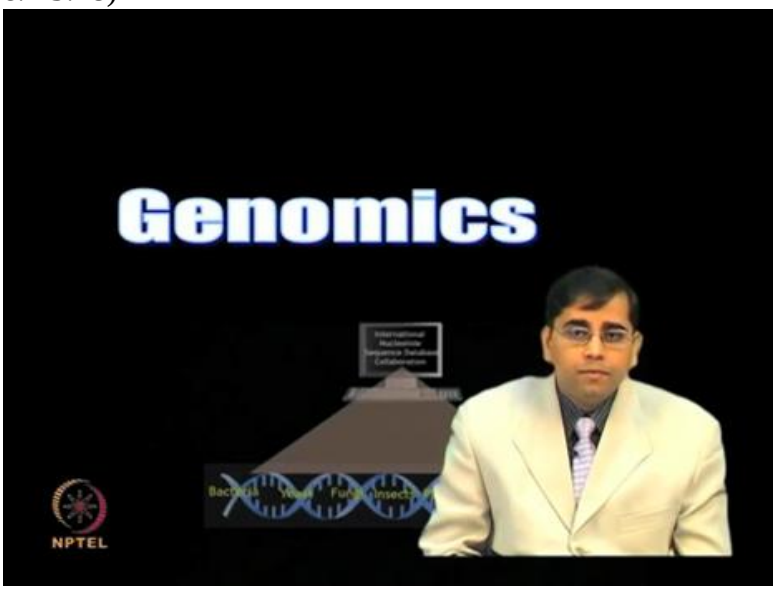

Studying genome of an organism by employing sequencing and genome mapping is known as genomics.

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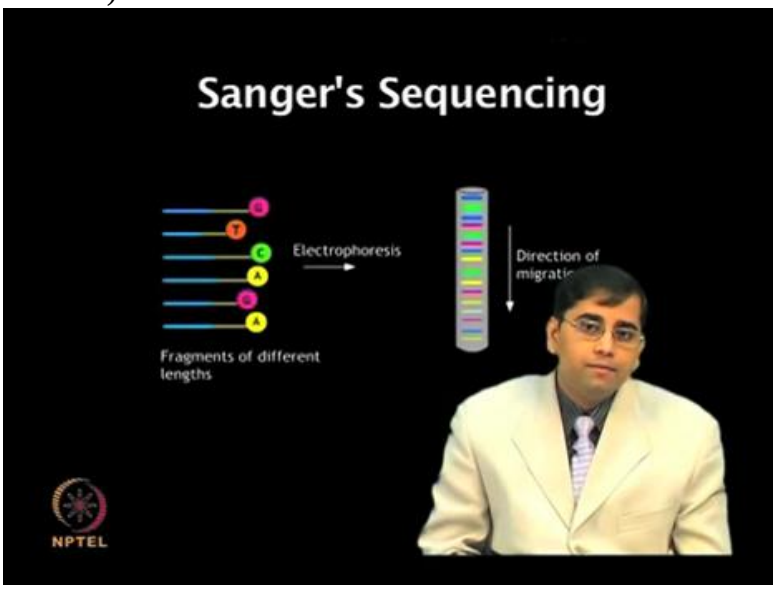

Several genome sequencing projects that aim to elucidate…

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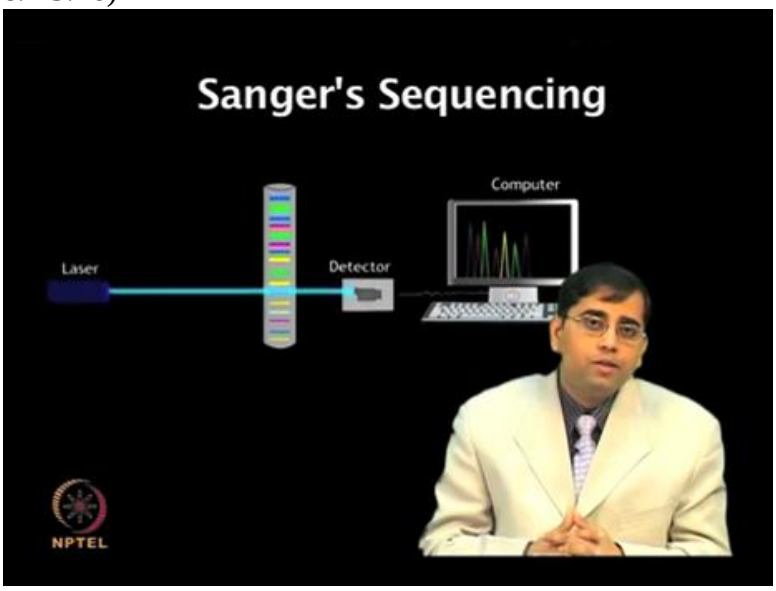

…the complete genome sequence of organisms have been undertaken by several research groups all over the world.

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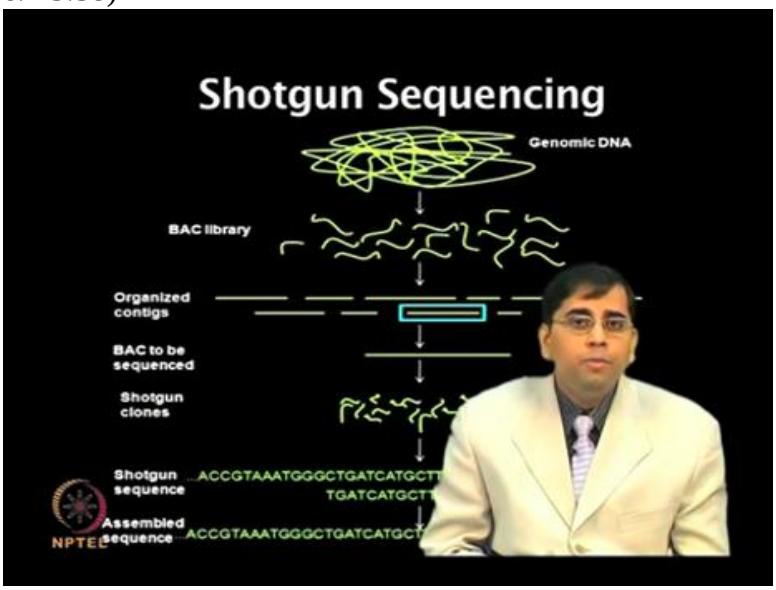

From a genomic library, clones were isolated and ordered into a detailed physical map.

Further individual clones were sequenced by Shot Gun sequencing to provide the complete genome sequence.

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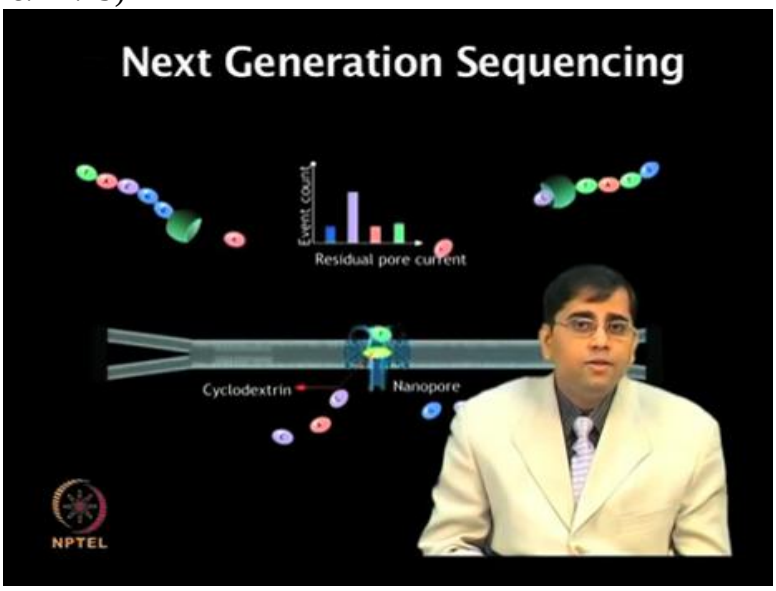

Recently Next Generation Sequencing NGS strategies have dramatically increased the pace of sequencing by several order of magnitudes. Next Generation Sequencing based on nano-pore structures is known as nano-pore sequencing.

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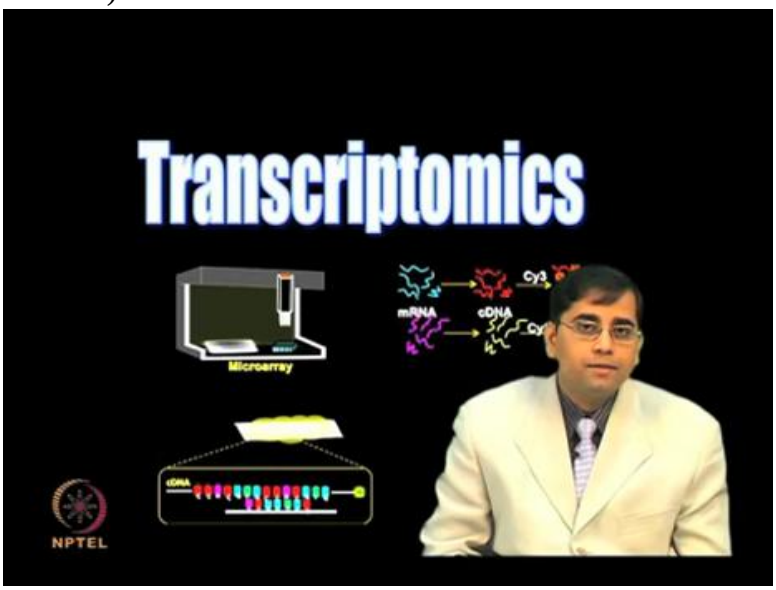

Transcriptomics: Study of all the mRNA molecules expressed by a particular cell type of an organism is known as transcriptomics. The transcriptomic analysis measures the genes that are being actively expressed at any given time and varies significantly with external environmental conditions. Various techniques such as microarrays, QRT PCR etc, have been widely used for transcriptional analysis.

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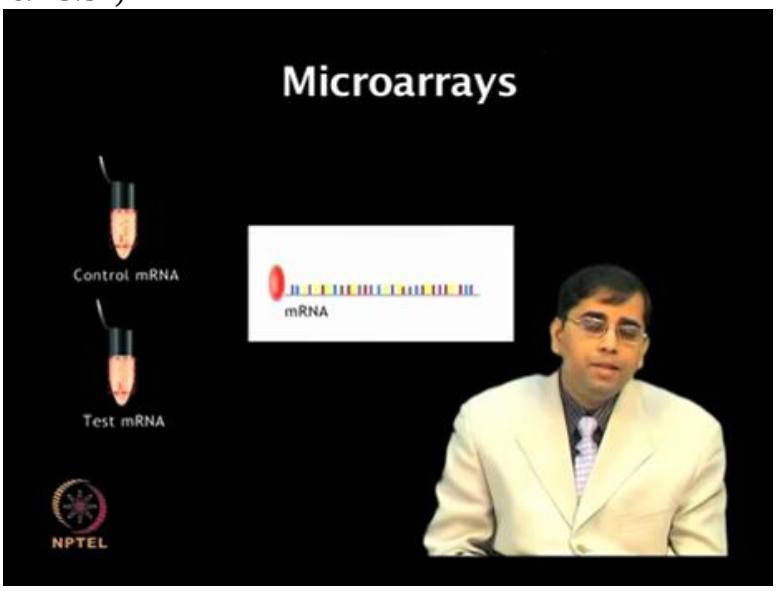

In a microarray experiment the mRNA from control and test samples are extracted….

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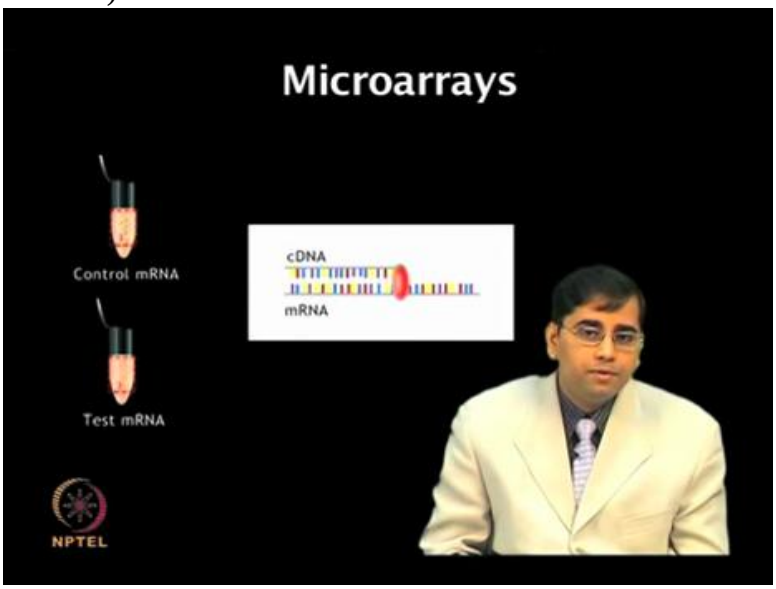

…and reverse transcribed …

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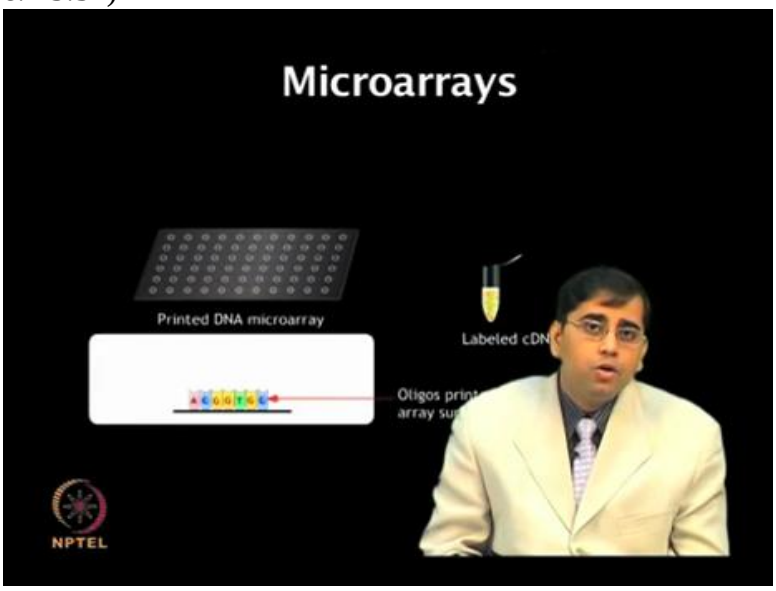

…into its corresponding cDNA.

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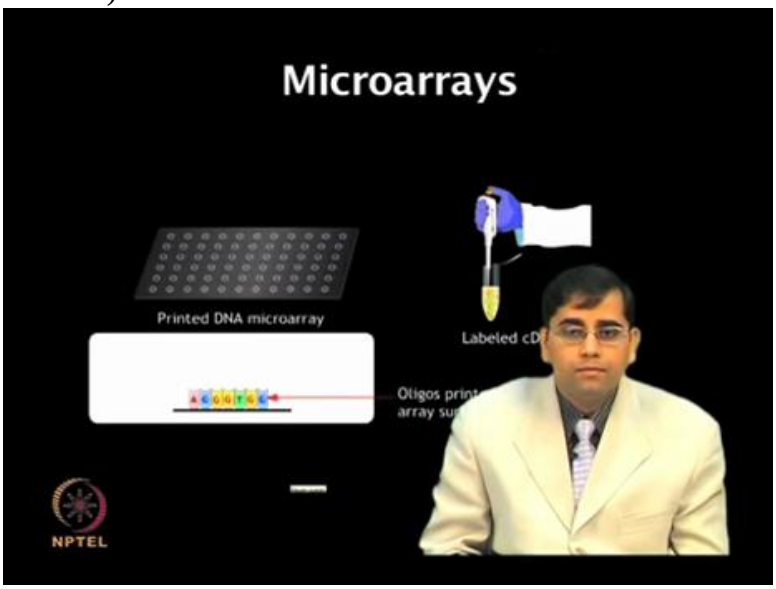

The cDNA samples are labeled with Cy5 and Cy3 dyes and

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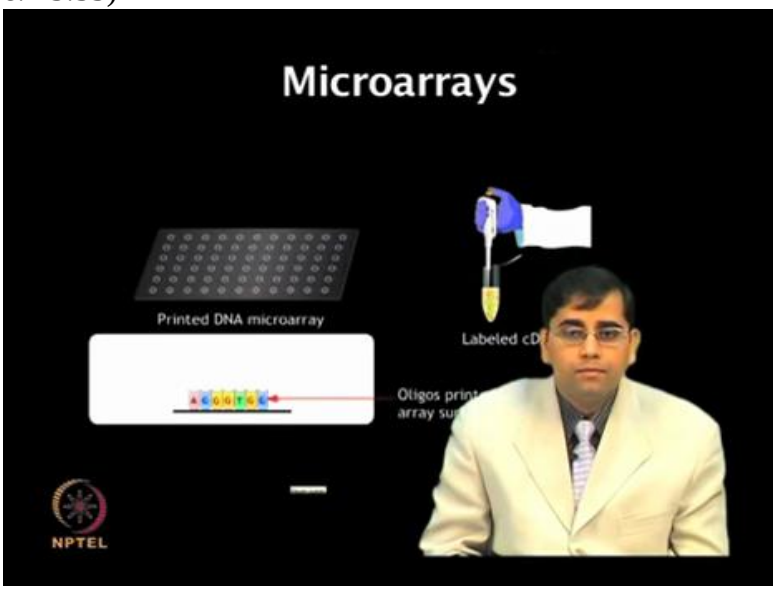

mixed cDNA sample is incubated on printed DNA microarray.

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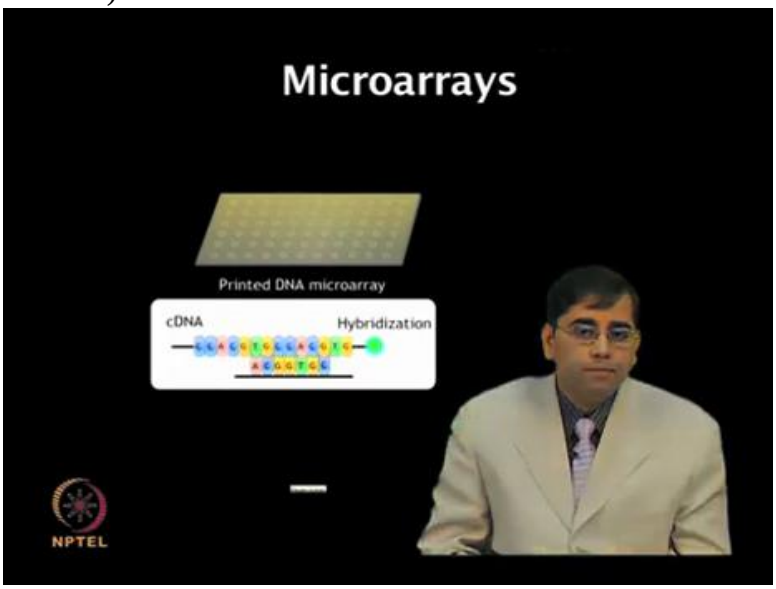

This allows hybridization to occur between the probed polynucleotide on array surface and the labeled cDNA sample of interest. In this manner, expression level of thousands of genes can be measured and analyzed simultaneously.

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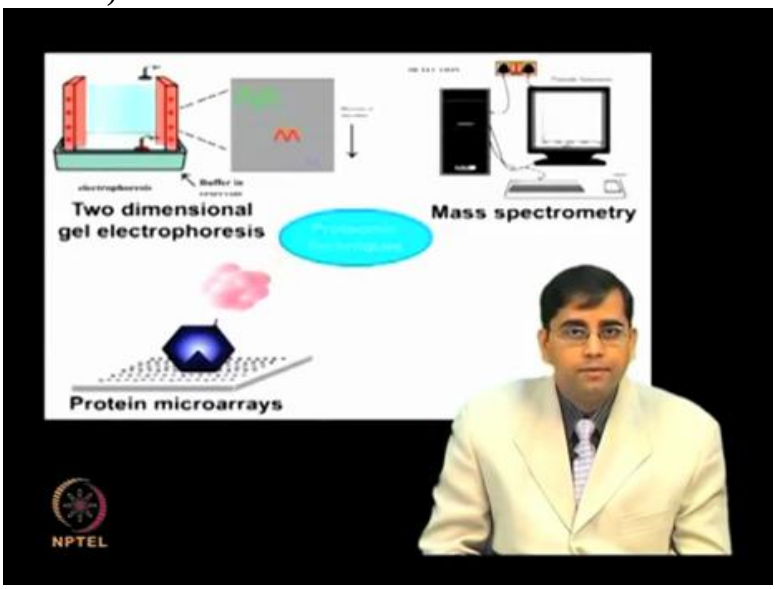
Different types of proteomics technologies such as…

(Refer Slide Time: 16:30)



Two-dimensional electrophoresis, mass spectrometry, Microarrays and label-free techniques will be discussed in more detail later.

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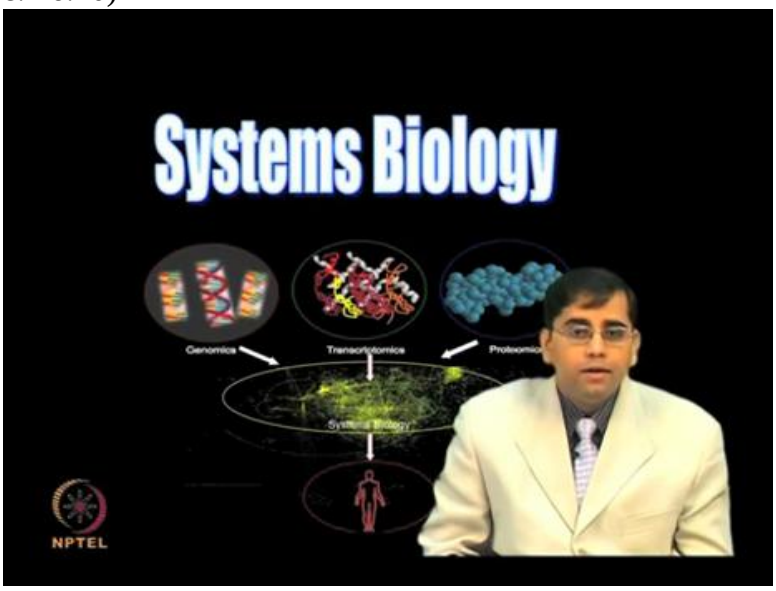

In Omics era, technological advancement in genomics, proteomics and metabolomics have generated large scale datasets in all the aspects of biology. These large datasets have motivated the computational biology and Systems approaches with objective of understanding the biological system as a whole.

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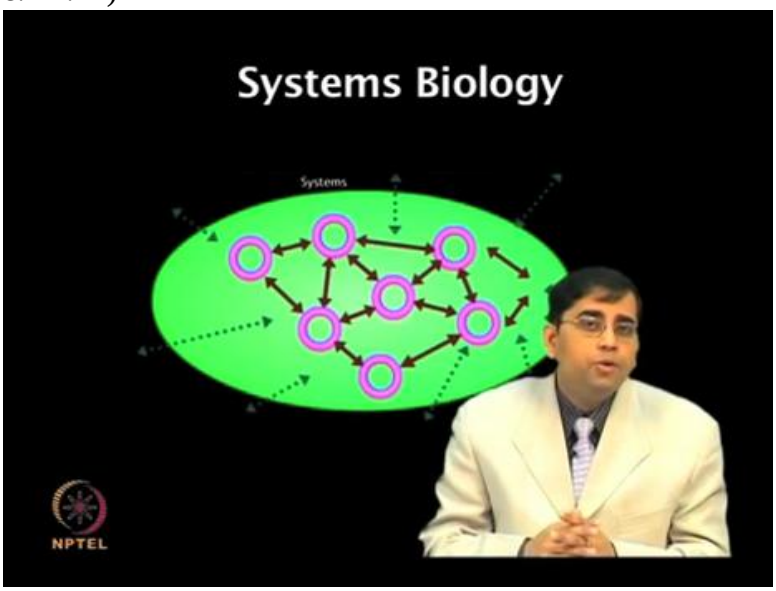

The system biology and biological network modeling aims to understand the biological processes…

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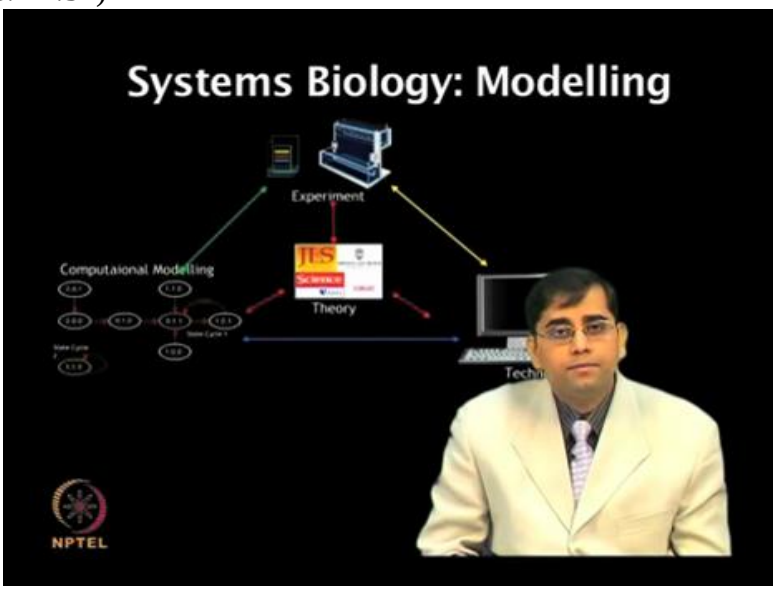

… as whole system rather than the isolated parts while synergistic application of experiment, theory, technology and modeling.

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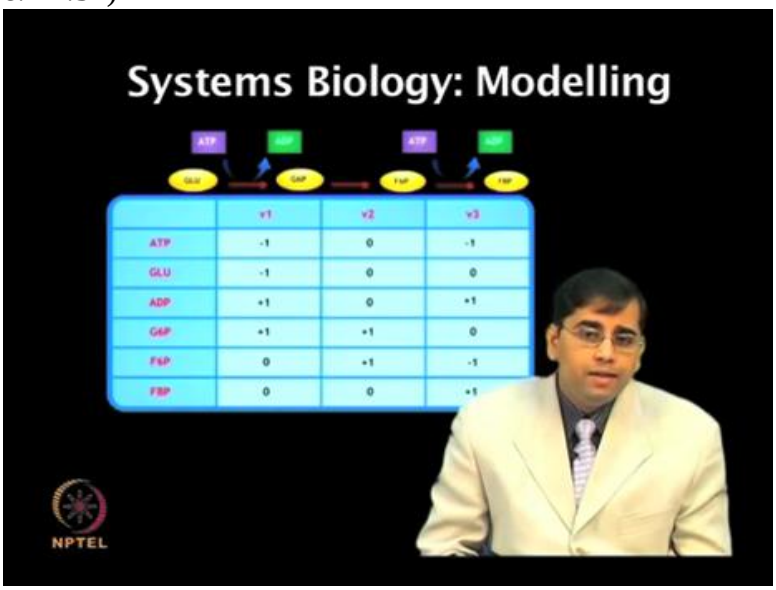

The systems level studies aim to develop computationally efficient and reliable models of underlying gene regulatory networks.

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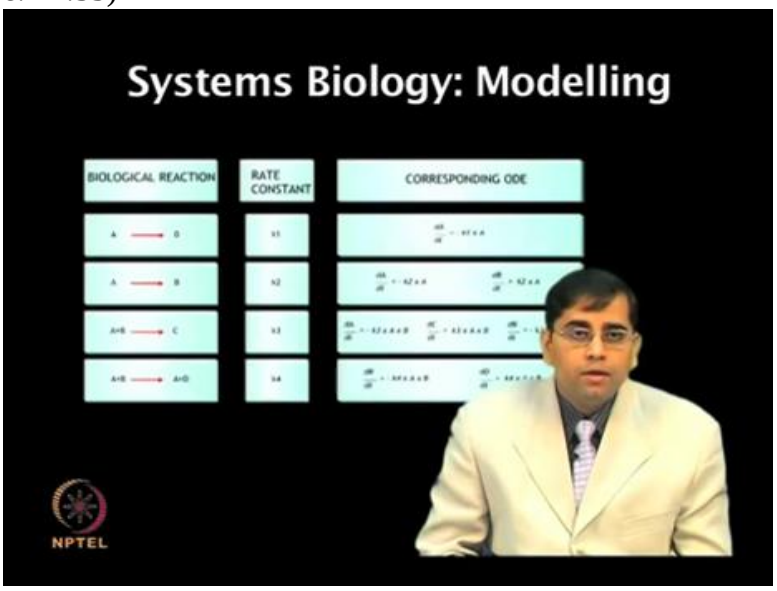

The quantitative analysis measures and aims to make models for precise kinetic parameters of system's network component. It also uses properties of network connectivity.

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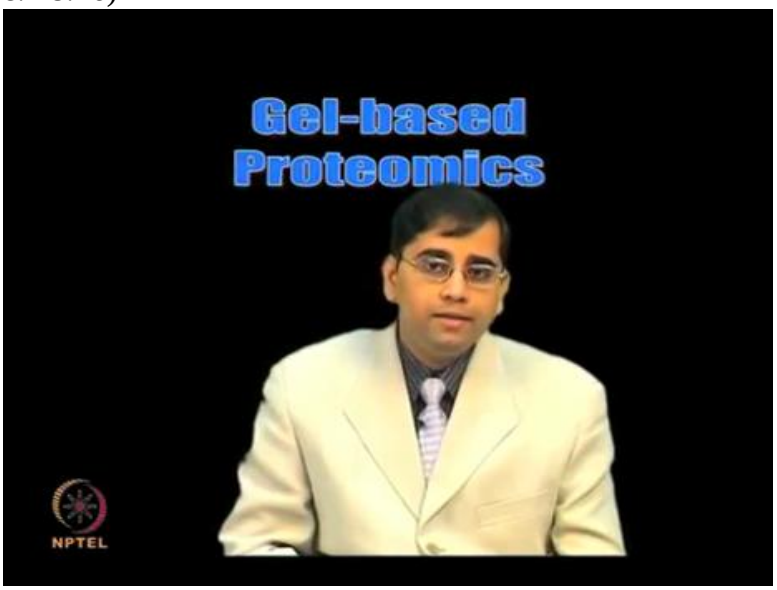

Several techniques used in proteomics typically aim to elucidate the expression, localization, interaction and cellular function of proteins.SDS PAGE, two-dimensional electrophoresis, difference gel electrophoresis are various commonly used gel-based proteomics. Protein extraction is the first step for the proteomic analysis.

The protein extraction methods aim that most if not all the proteins in a cell or its organelle are extracted by the procedure and the presence of interfering components are reduced or minimized.

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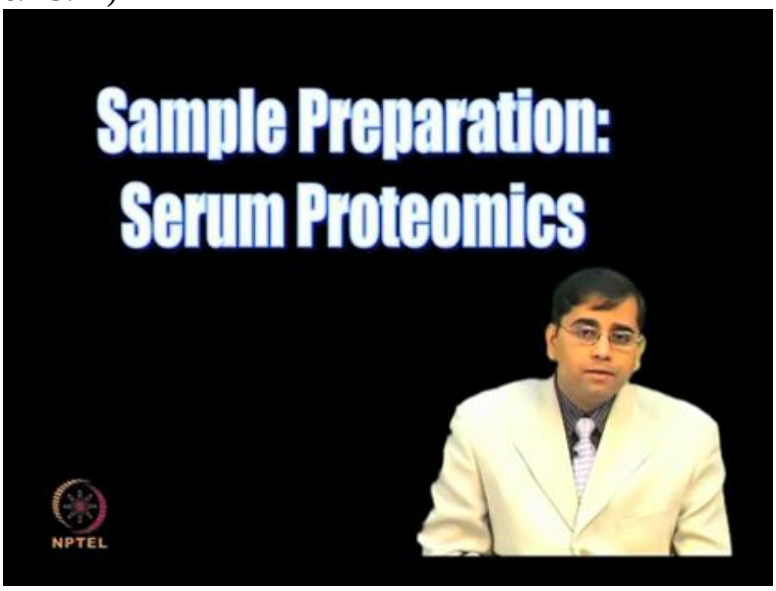

Different biological systems, different biological samples pose different types of challenges.

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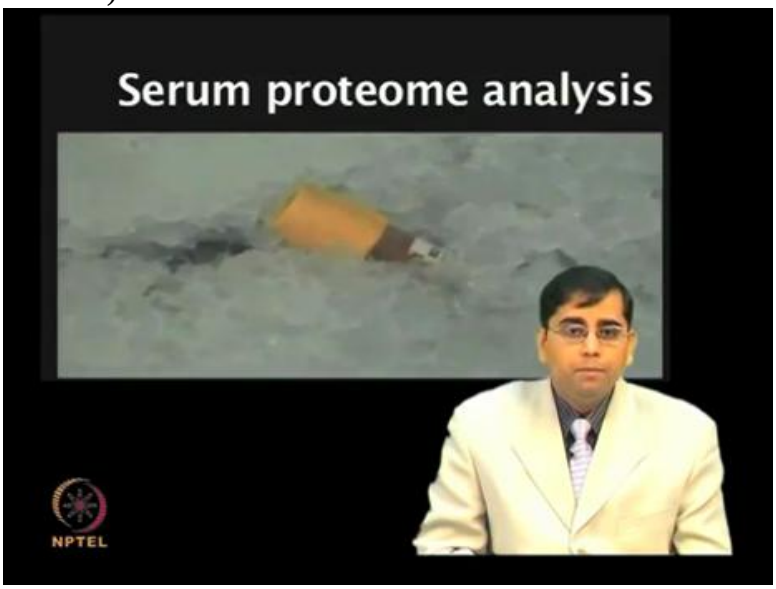

For example serum proteome analysis shown here illustrates that proteins in biological systems such as serum may have difference

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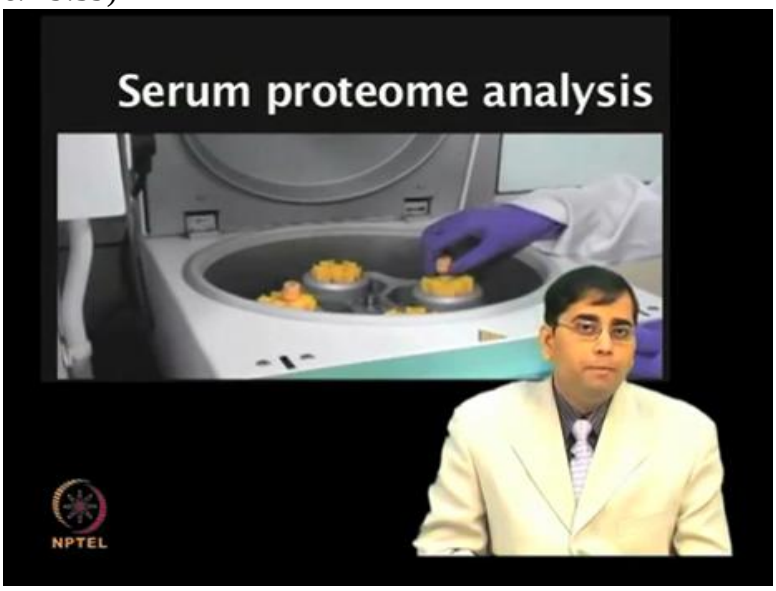

of several order of magnitude.

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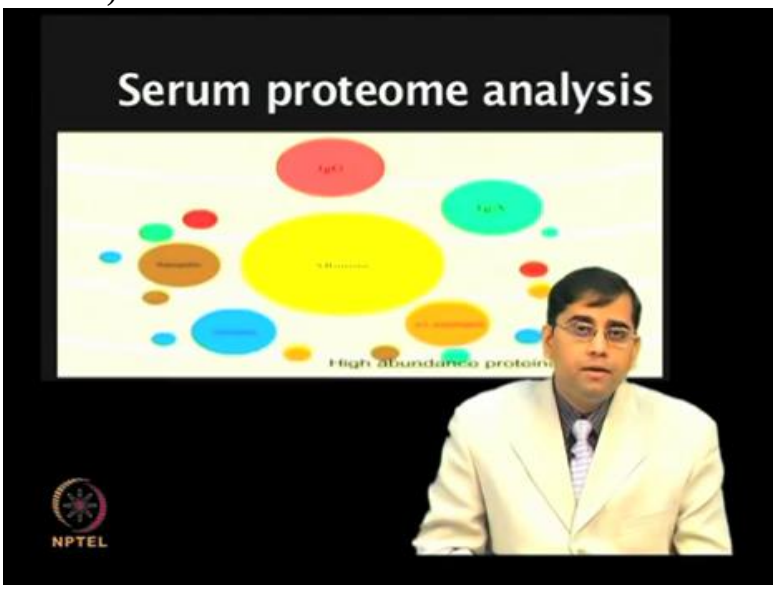

Albumin and immunoglobulin are the most abundant proteins in serum…

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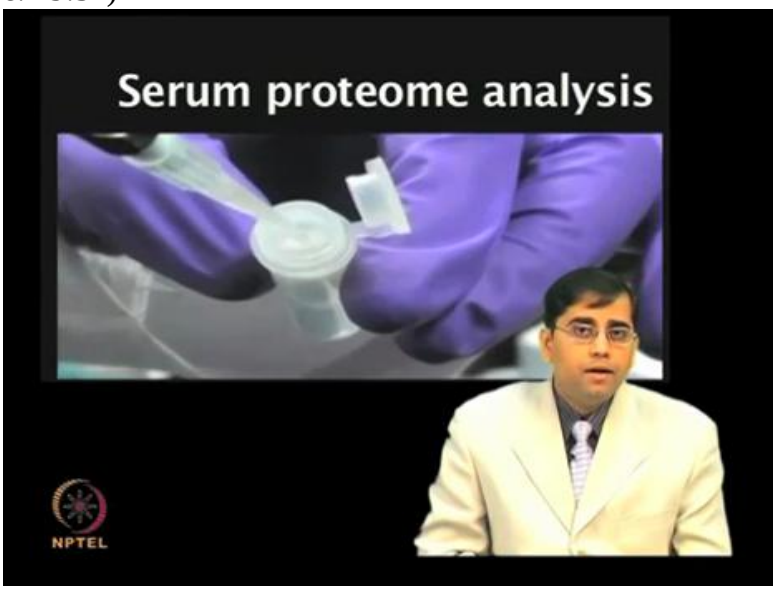

… which mask other low abundant proteins which are present in the lower concentration

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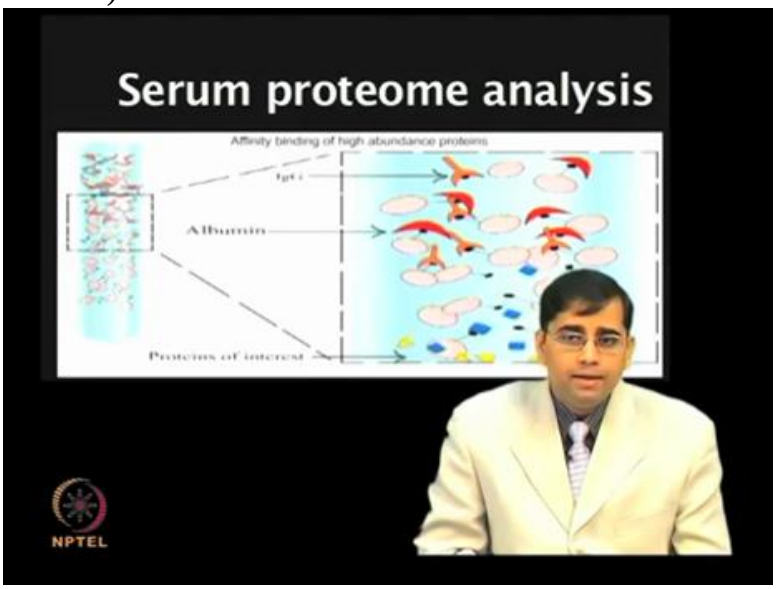

It is therefore preferred to remove these high abundant proteins by using affinity chromatography methods.

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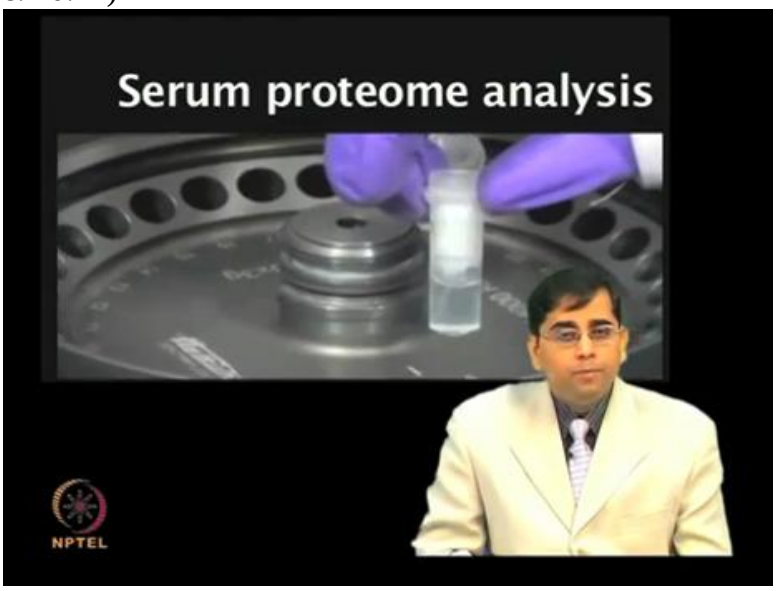

Once the serum has been processed using these chromatography methods…

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these proteins can be extracted.

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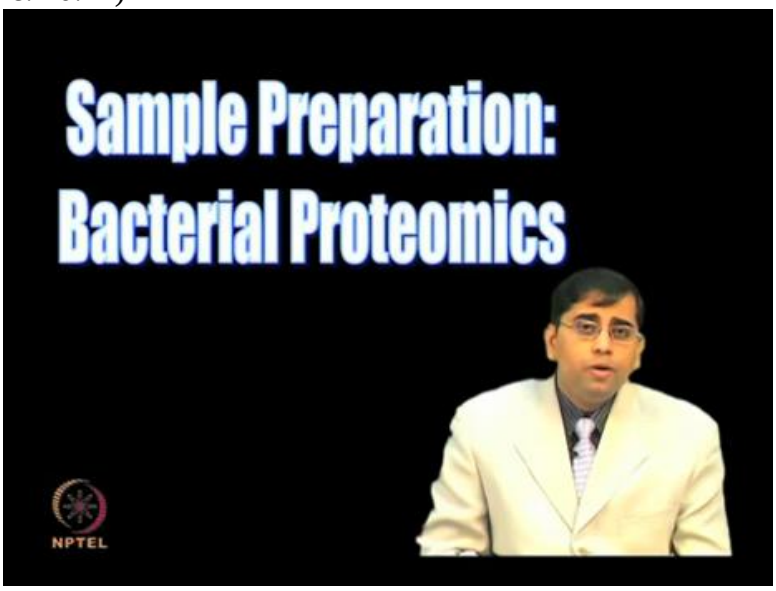

In bacterial protein sample preparation, sonication is an important step

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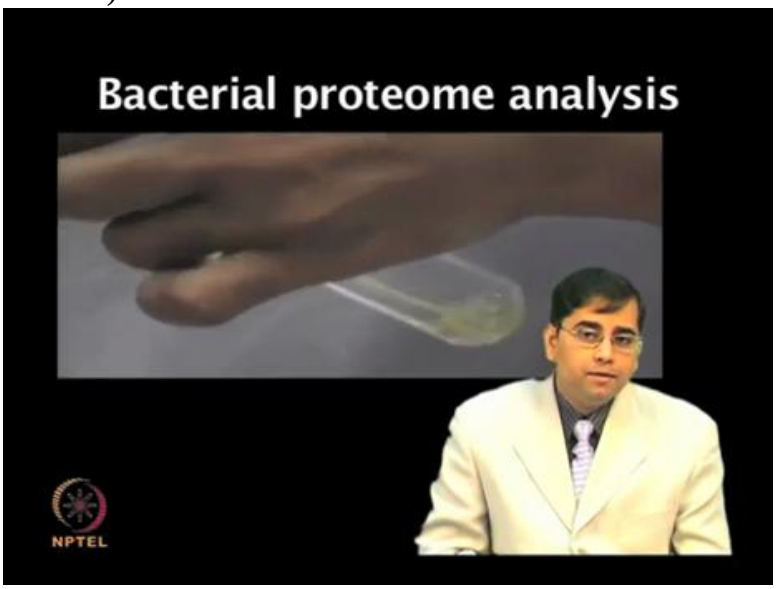

…to disrupt the bacterial membrane.

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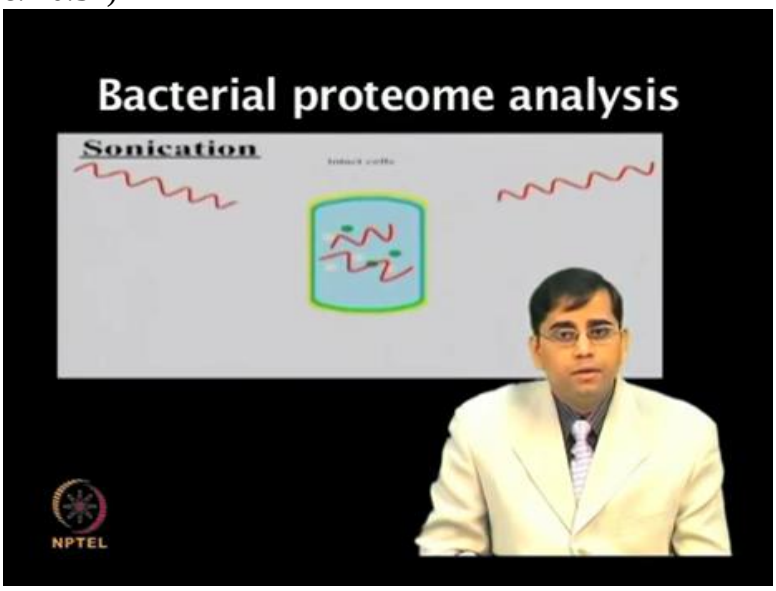

Sonciation breaks open the cellular contents…

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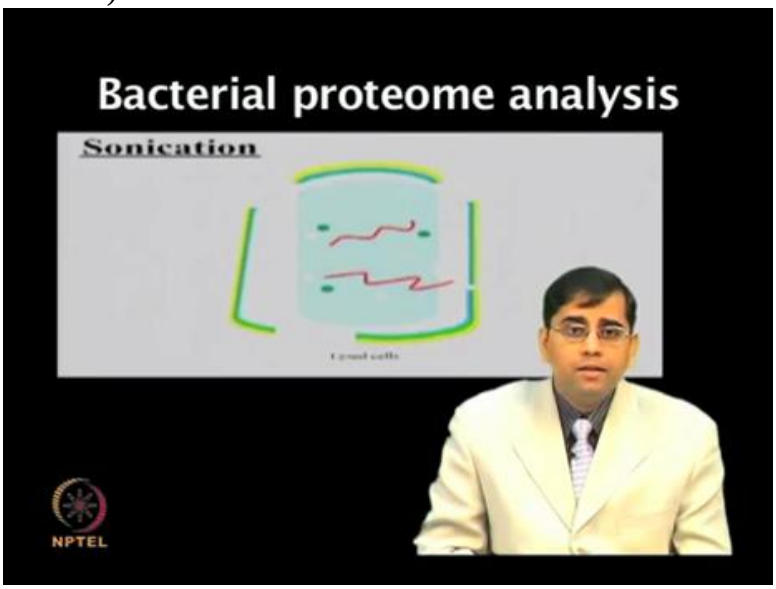

the cellular membrane to release the intracellular contents.

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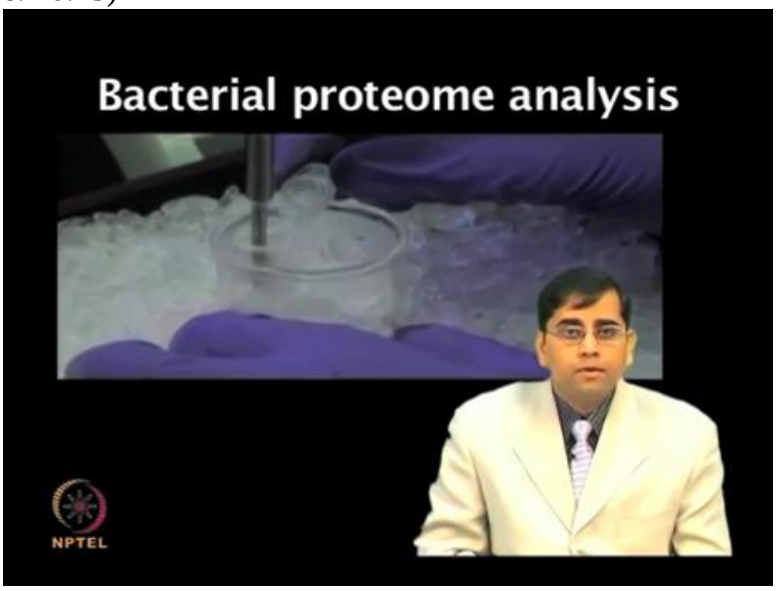

Protein extraction can be performed by using different methods…

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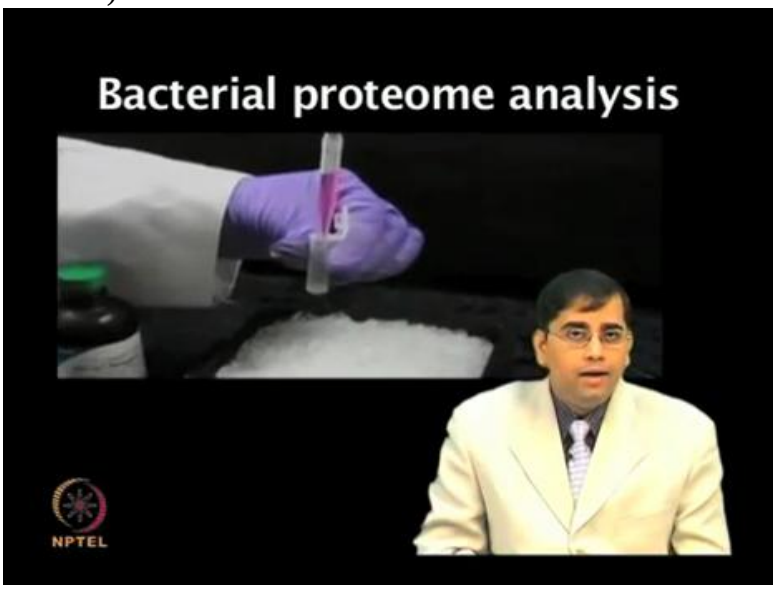

…and protein palettes are reconstituted in lysis buffer

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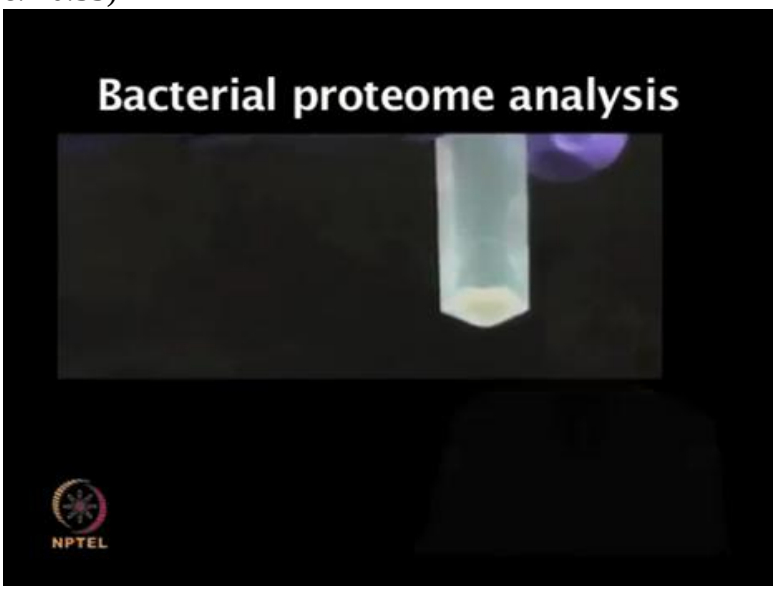

…for proteomic analysis.

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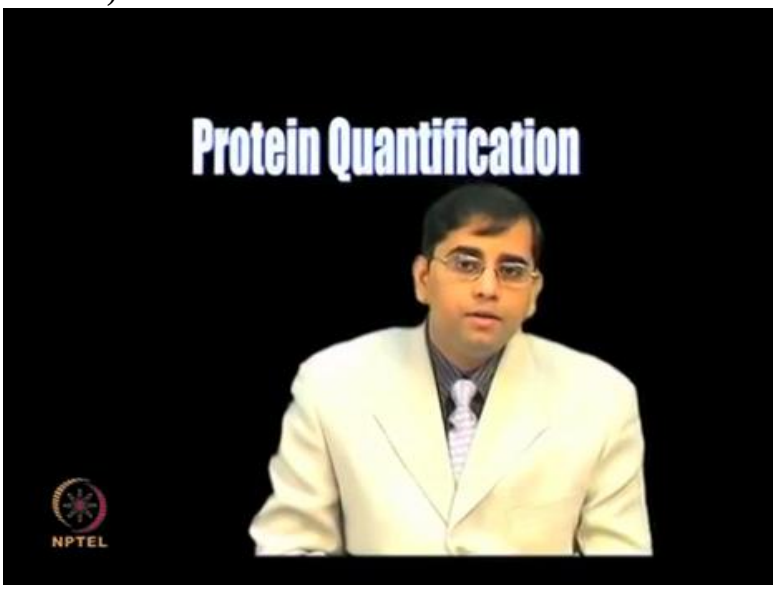

Protein quantification is sensitive to detergents or certain ions. Therefore it is crucial to select the correct quantification method.

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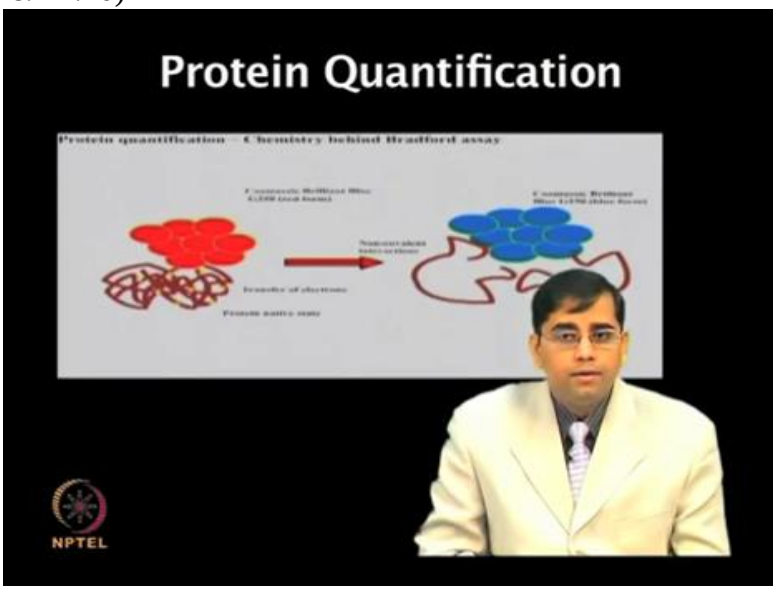

In Bradford color reagent, transfer of electrons converts the dye to its blue form thereby giving the solution blue color.

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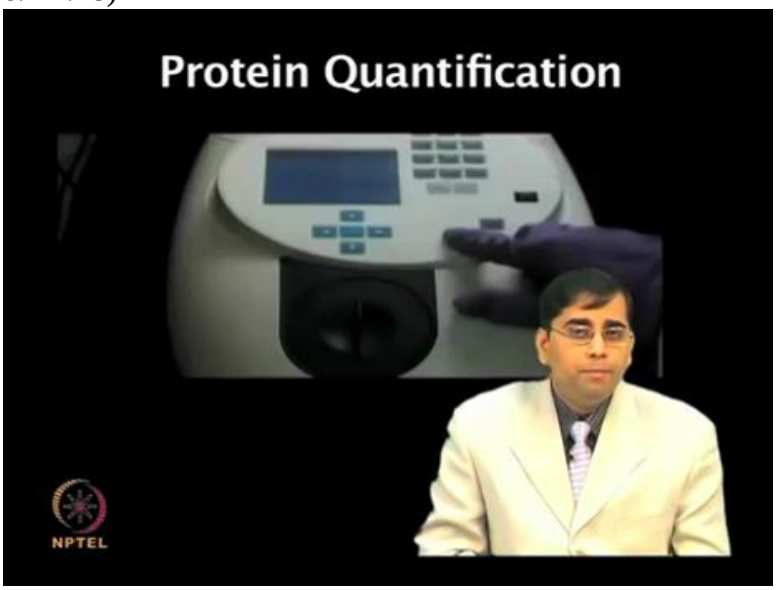

Absorbance of standard and unknown protein samples can be measured at 595 nanometers…

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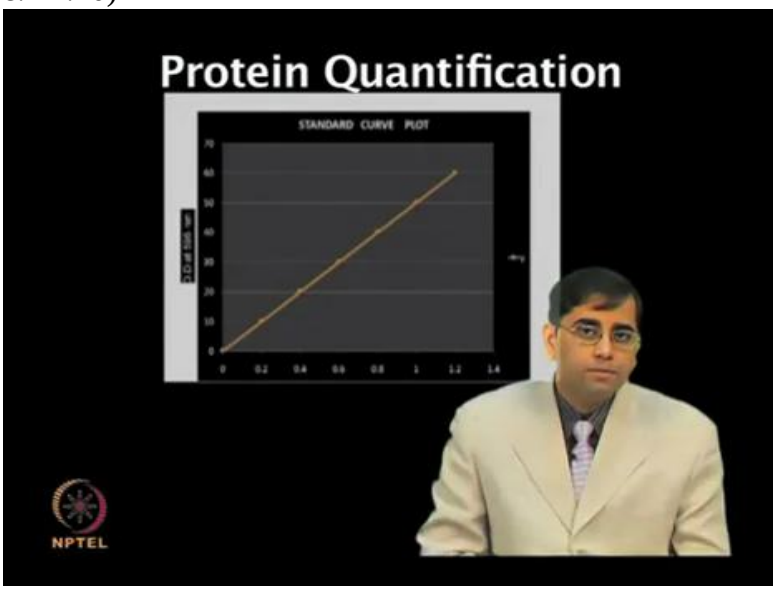

…and protein concentration can be determined from the standard plot of the absorbance values.

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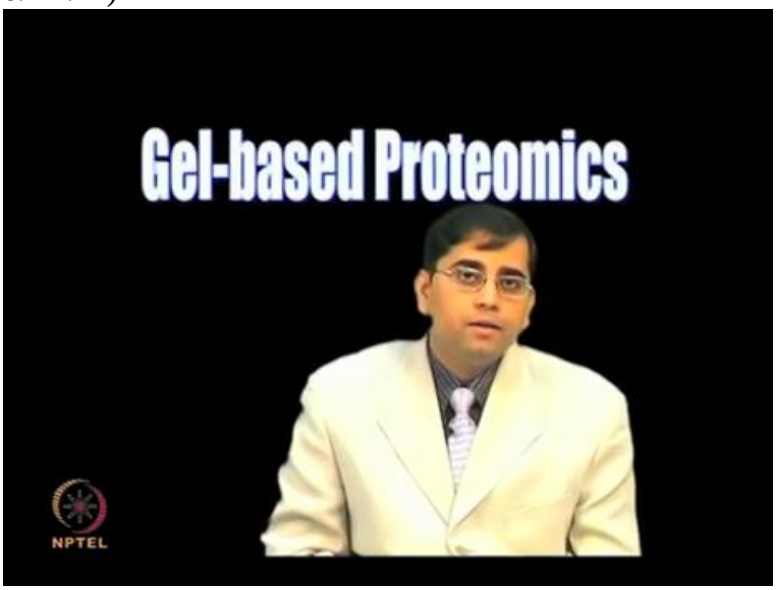

In gel-based proteomics, proteins are commonly analyzed using SDS PAGE and two-dimensional gel electrophoresis.

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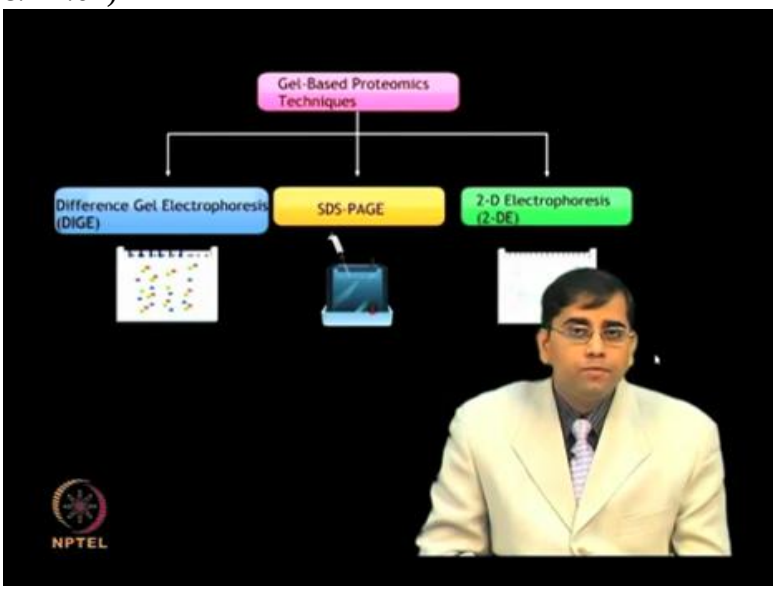

Separation in SDS PAGE occurs almost excessively on the basis of molecular weight whereas in 2DE, the first dimension separation is based on isoelectric point and second dimension separation based on molecular weight. Some of the limitations of two dimensional electrophoresis can be overcome by difference gel electrophoresis or DIGE technique.

2DE or DIGE in combination with mass spectrometry has been the standard technique for proteome analysis.

The two-dimensional electrophoresis involves protein separation on a pH gradient based on their isoelectric point using isoelectric focusing followed by separation in second dimension using SDS PAGE

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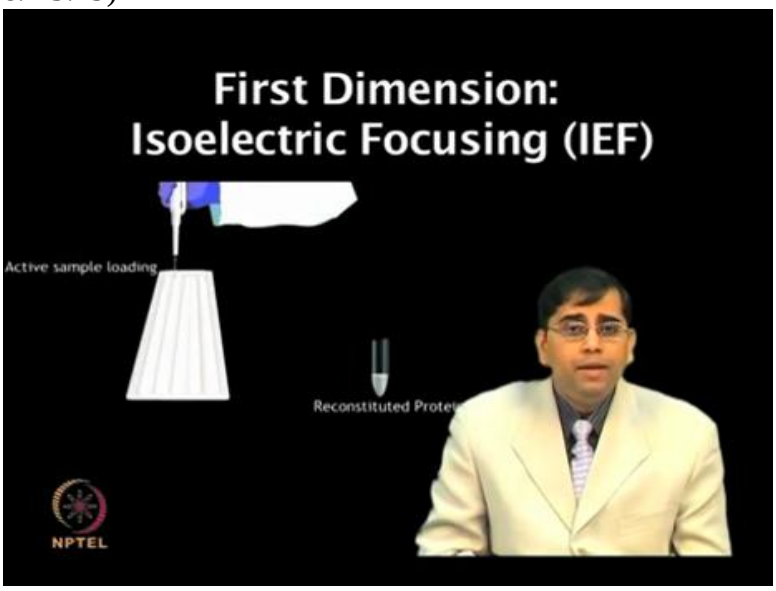

To perform 2DE add the reconstituted protein sample to the rehydration tray and place IPG strip for rehydration.

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Isoelectric focusing involves the application of electric field which causes the proteins to migrate to the position on the pH gradient strips…

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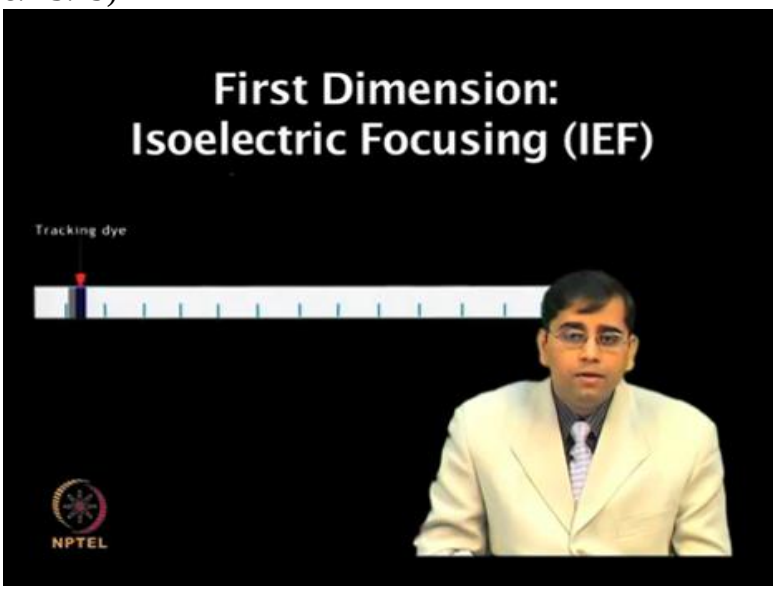

that matches the pI of the specific protein after which it does not move in the electric field owing to the lack of charge.

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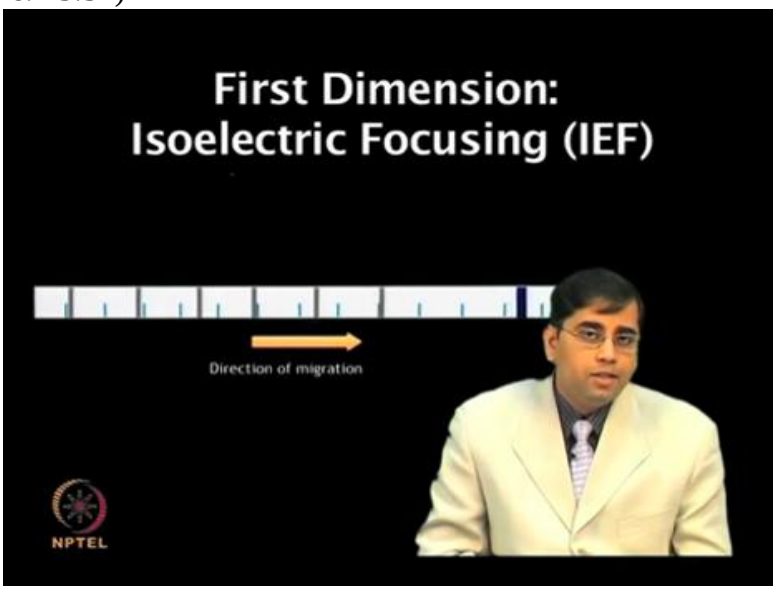

The proteins migrate along the strip and come to rest at a point where their net charge becomes zero known as isoelectric point.

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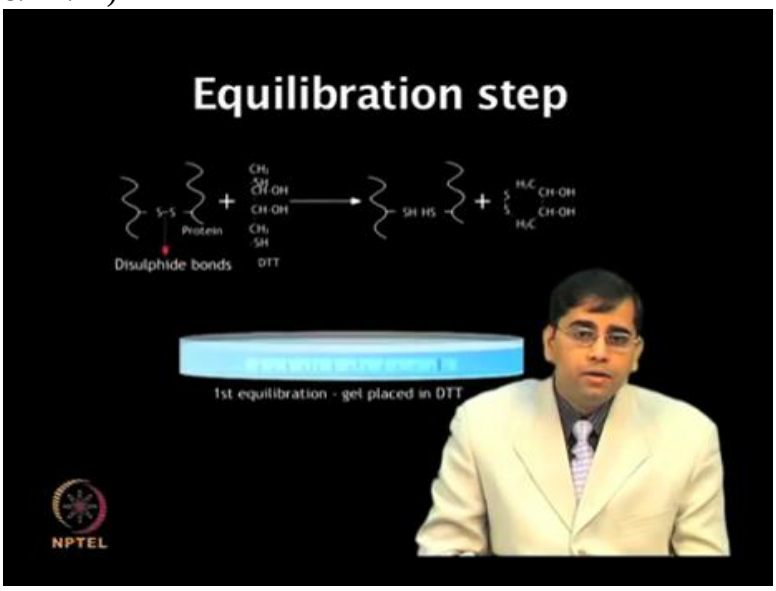

Prior to the second dimension separation, an equilibration step is required. In equilibration, dithiothreitol brings about cleavage of the protein disulfide bonds…

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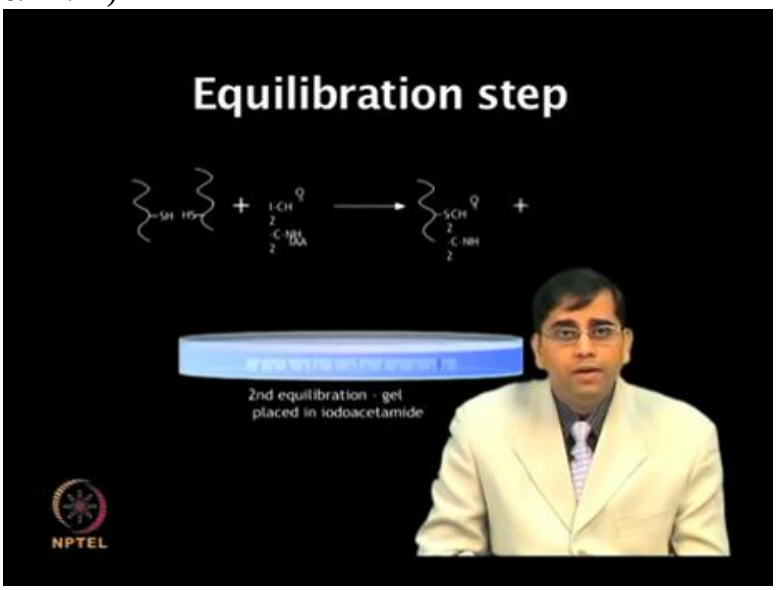

…while iodoacetamide prevents reformation of these bonds by binding to the sulfhydryl groups

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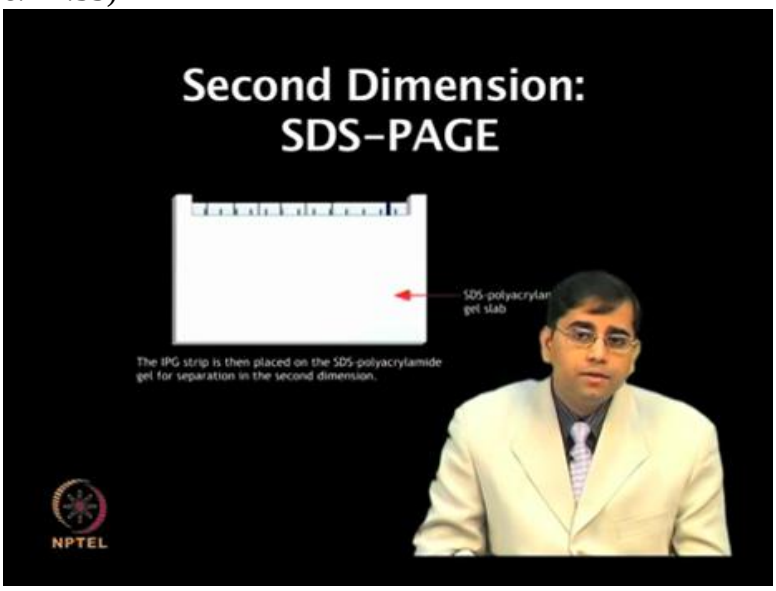

On SDS PAGE gel, proteins get separated.

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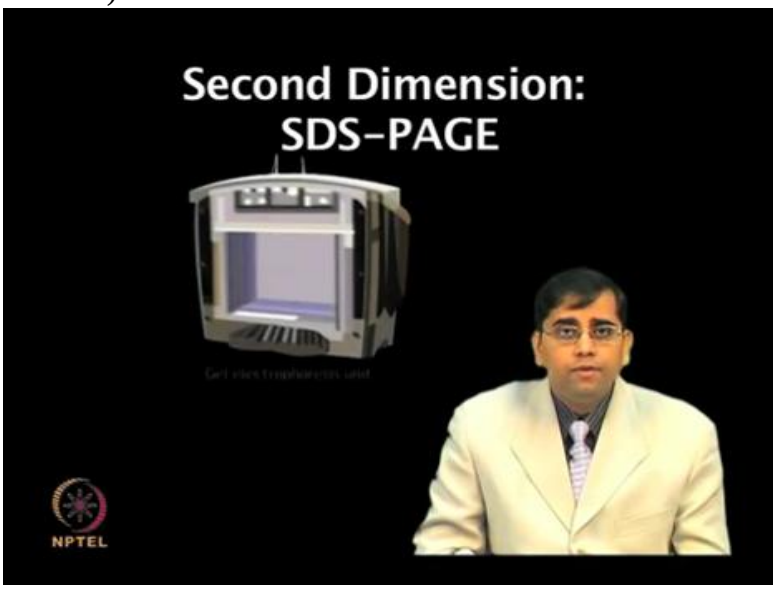

On the basis of their molecular weight, with the low molecular weight proteins having high mobility …

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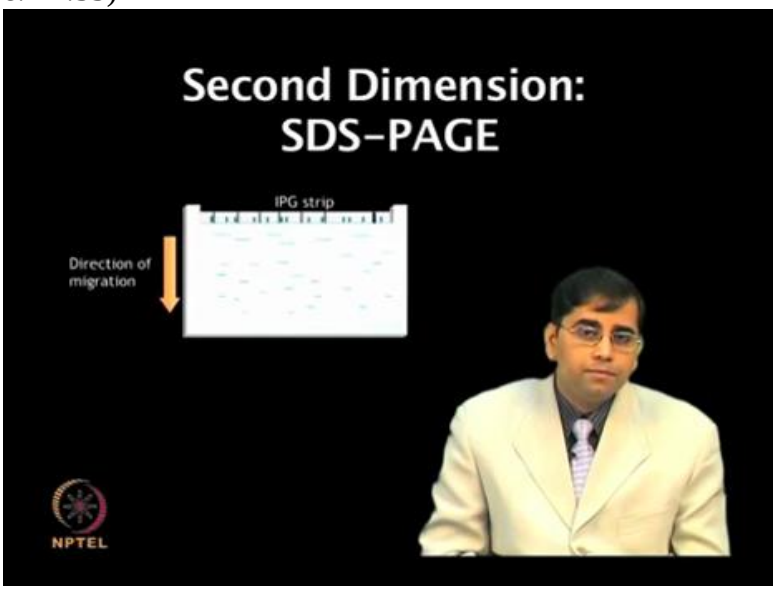

…are migrated further through the gel and the high molecular weight proteins remain close to the point of sample application.

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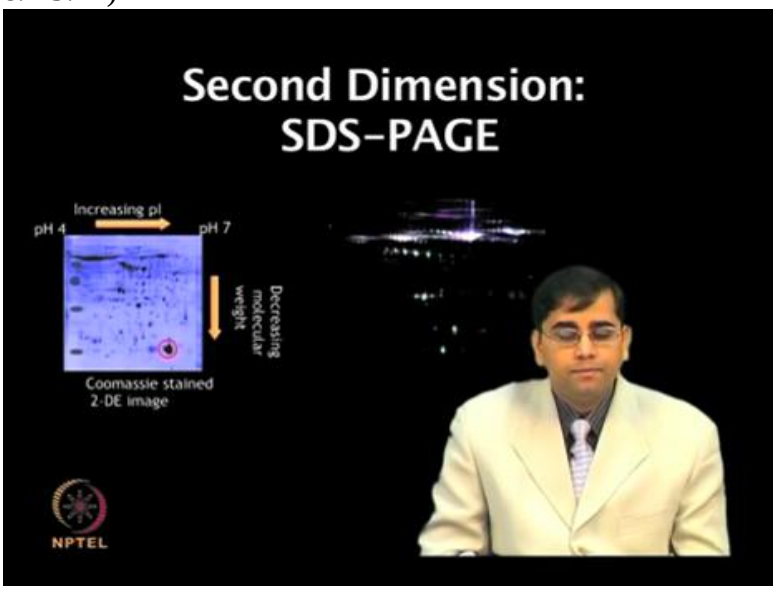

Gel can be visualized by different staining methods such as Coomassie staining, silver staining and Cyanine dyes.

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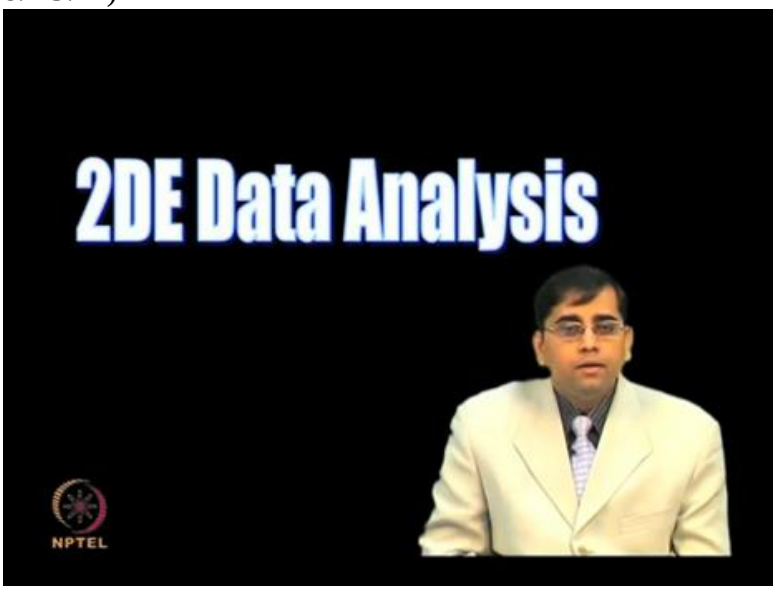

The gel data analysis will be discussed…

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…with an application expert of GE Healthcare

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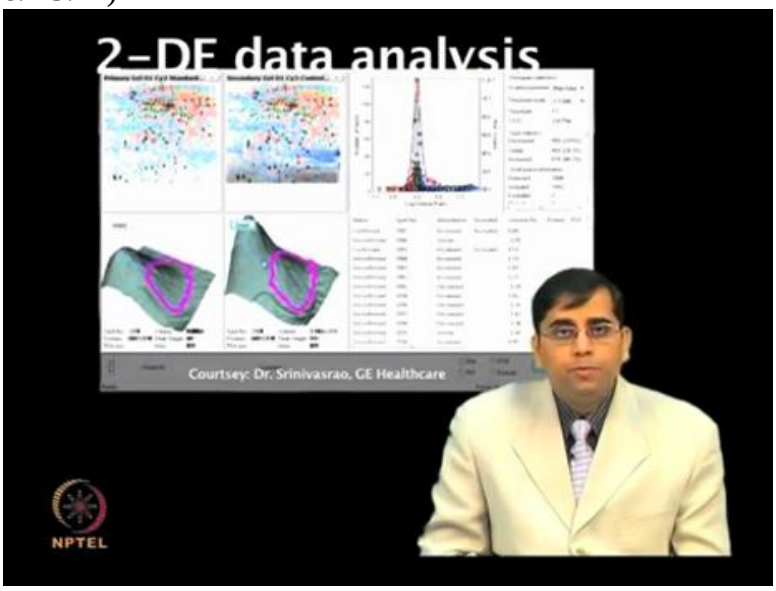

The gel analysis involves image processing, detection of spots, making matched sets, landmarking, viewing histograms etc. Various information regarding the spots such as their area, volume, intensity; and statistical parameters such as standard deviations can also be calculated.

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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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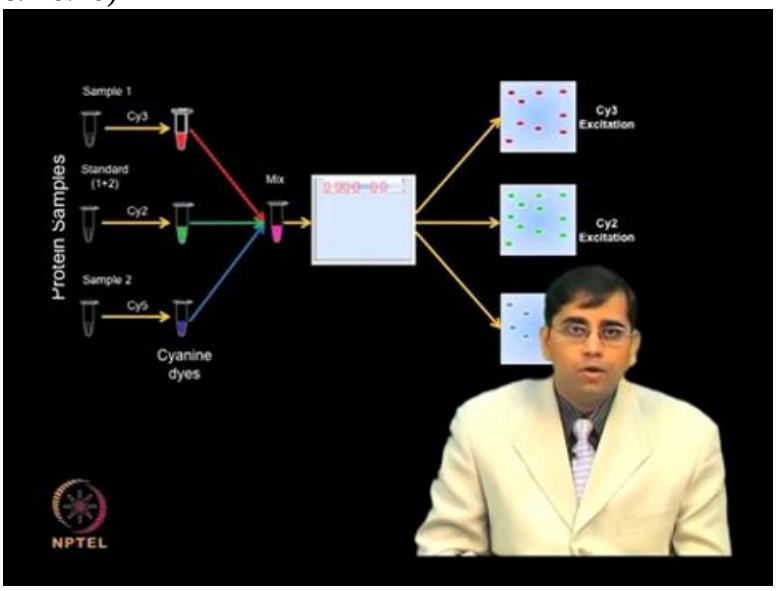

Fluorescence two-dimensional difference in gel electrophoresis or 2D DIGE is an advanced 2D technique that allows for…

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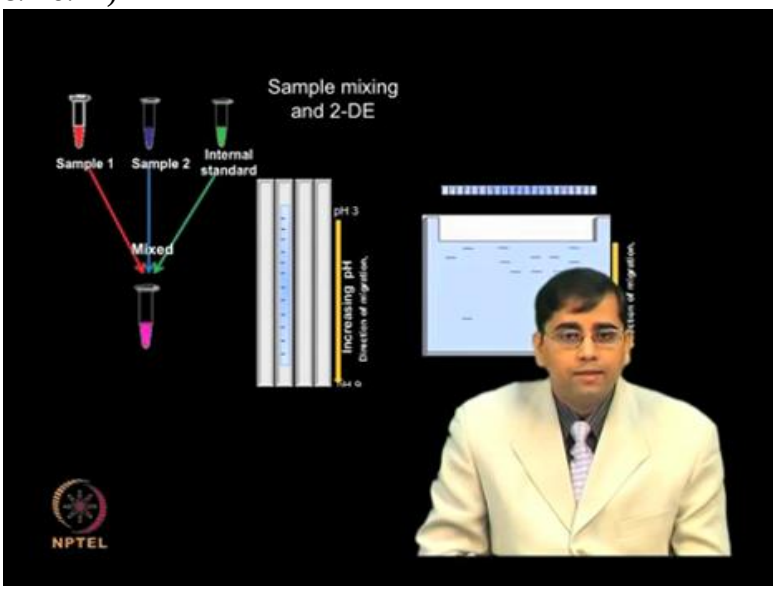

…accurate quantitation with statistical confidence while controlling non-biological variations

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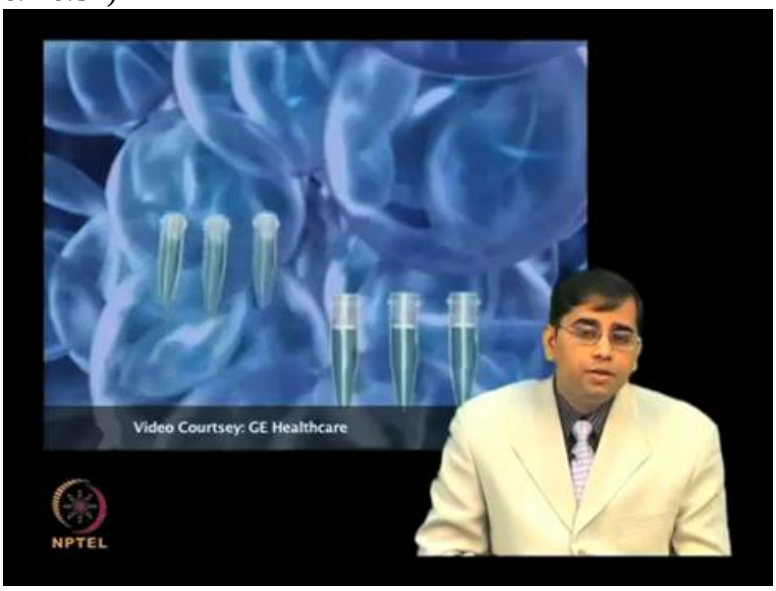

In DIGE, proteins extracted from different types of cells or tissue samples are labeled with different fluorescent reagents…

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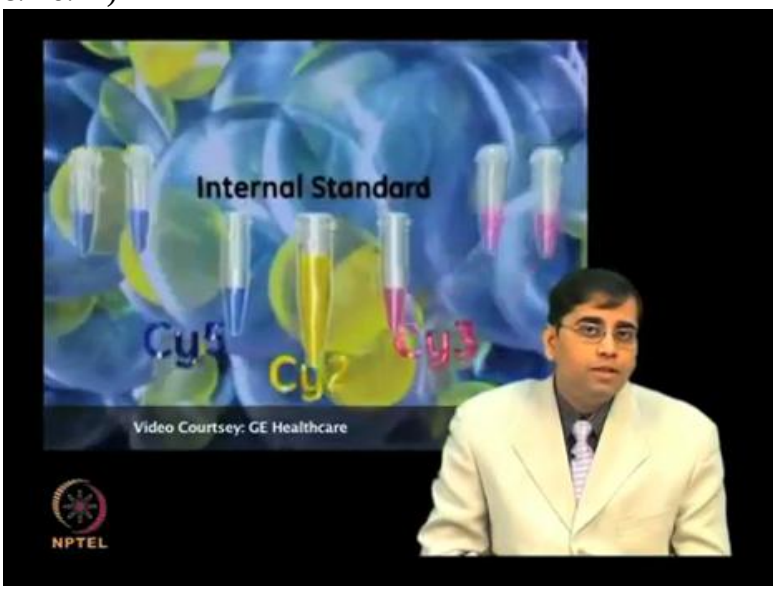

… such as Cy2, Cy3 and Cy5 …

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…mixed and then…

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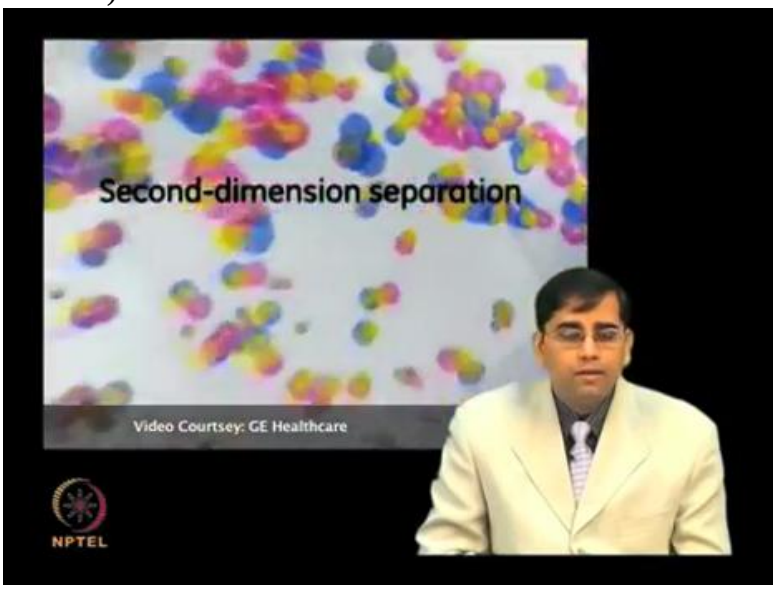

separated by two dimensional electrophoresis on a single gel.

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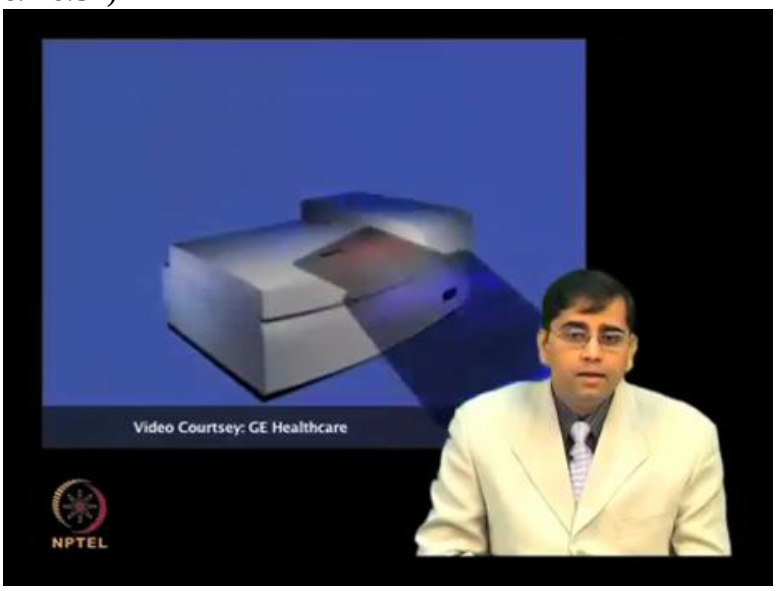

The proteins are detected separately using the excitation wavelength…

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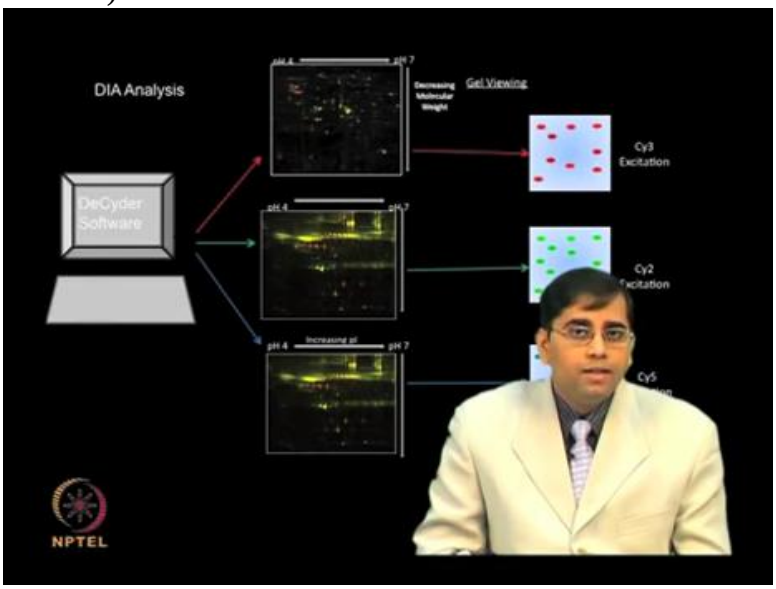

… specific to the different fluorescent reagents Cy2, Cy3 and Cy5.

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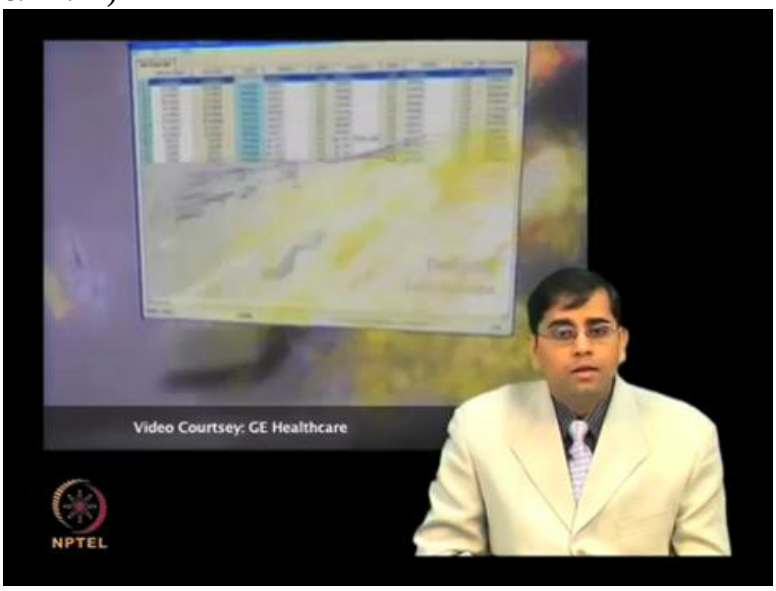

The commercial software such as DeCyder facilitate the automated analysis of DIGE gels and provide Differential Expression Analysis, Principal Component Analysis, Pattern and Discriminant Analysis.

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Two-dimensional electrophoresis, DIGE followed by mass spectrometry technique has been applied for…

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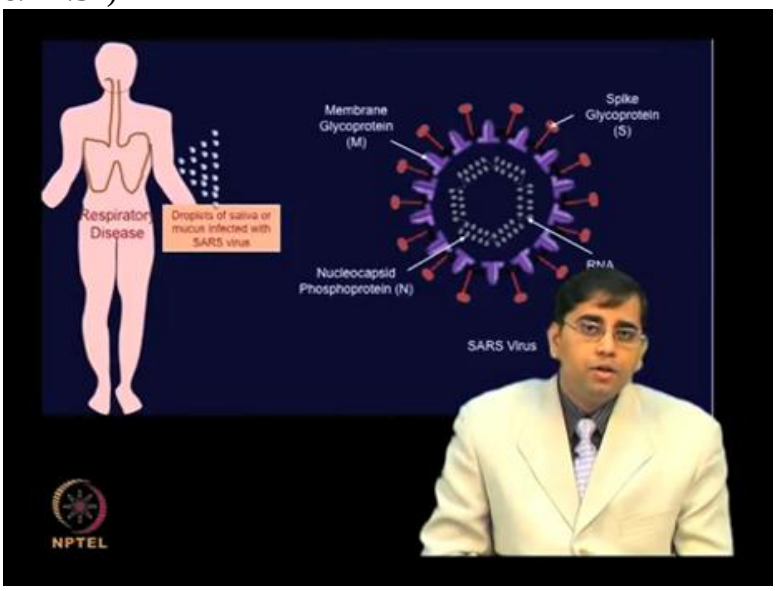

many…

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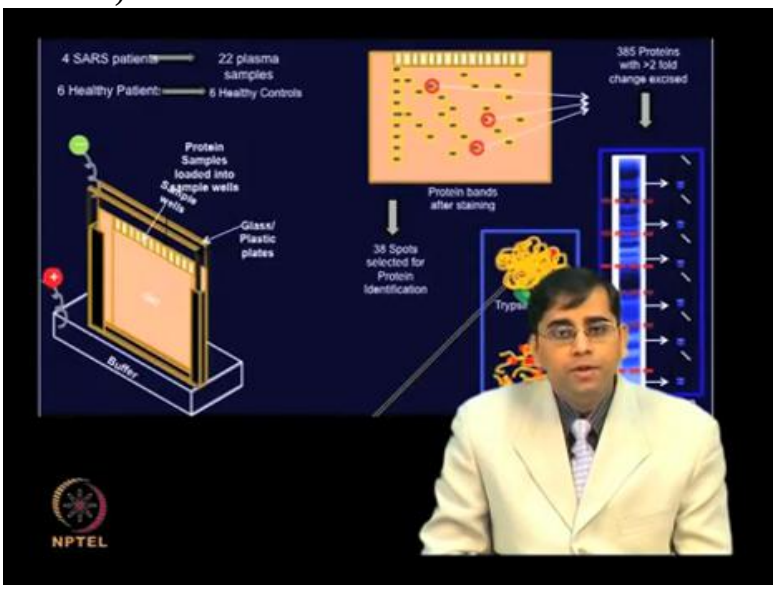

…applications. Some of these applications…

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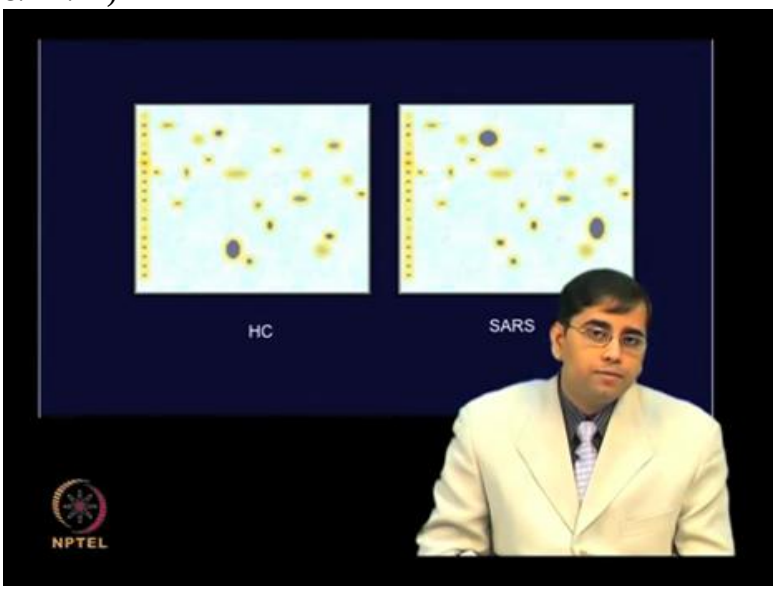

will be discussed…

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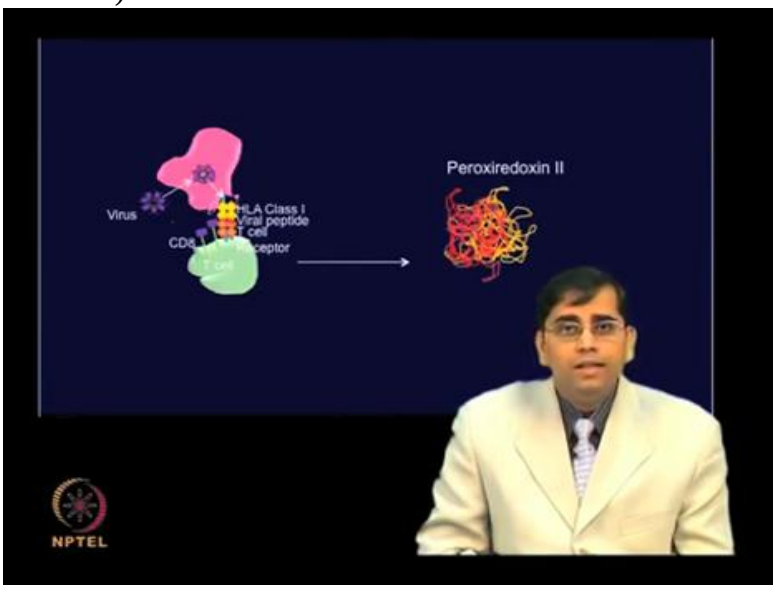

in this module…

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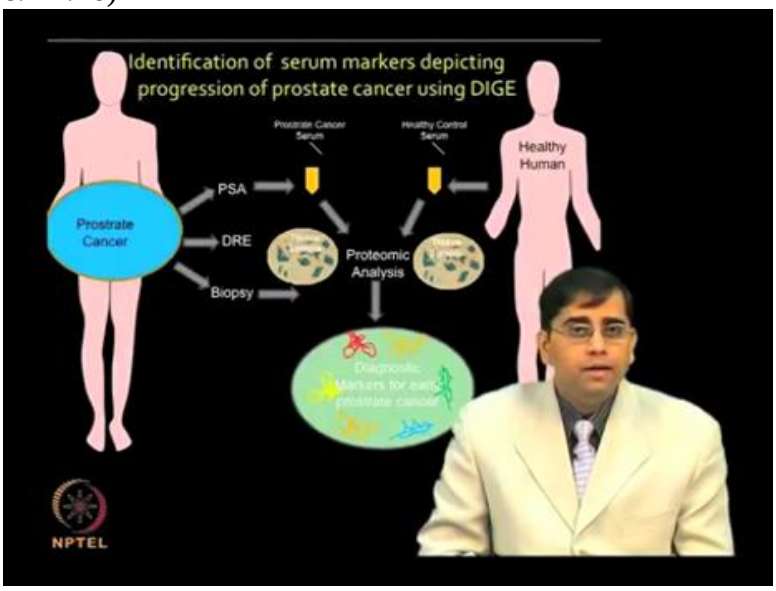

of…

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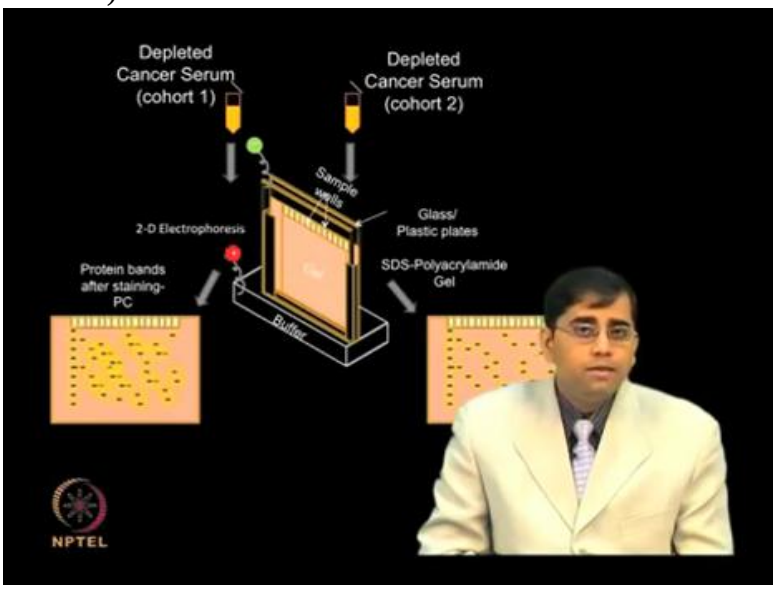

gel-based proteomics.

This century is considered as century of biology in which life science research is undergoing a profound transformation by employing various Omics technologies.

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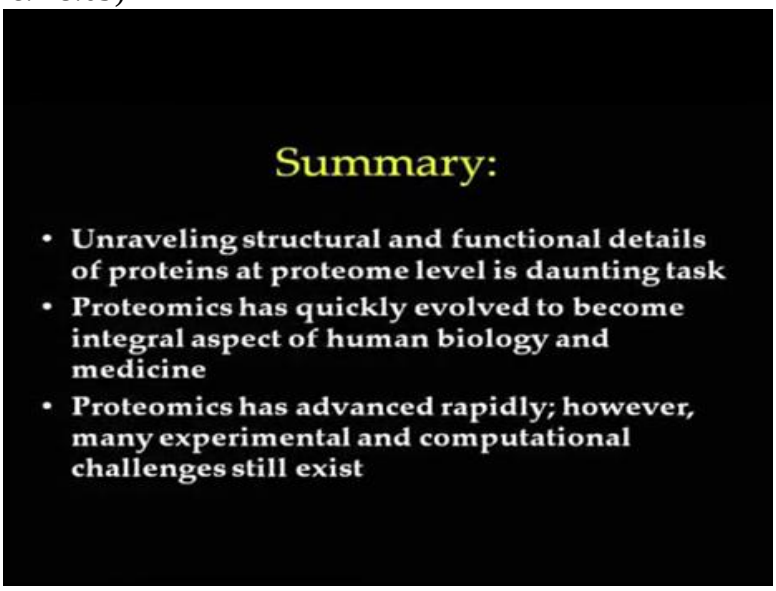

In summary unraveling structural and functional details of proteins at the proteomic scale is very daunting task. However proteomics has come to mean virtually everything in protein research and it has quickly evolved to become an integral aspect of human biology and medicine.

During the 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.