

# Proteomics Course

## LECTURE-3 Genomics and Transcriptomics: Why proteomics?



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IIT Bombay



### Lecture outline

- Genomics
- Transcriptomics
- Why proteomics?

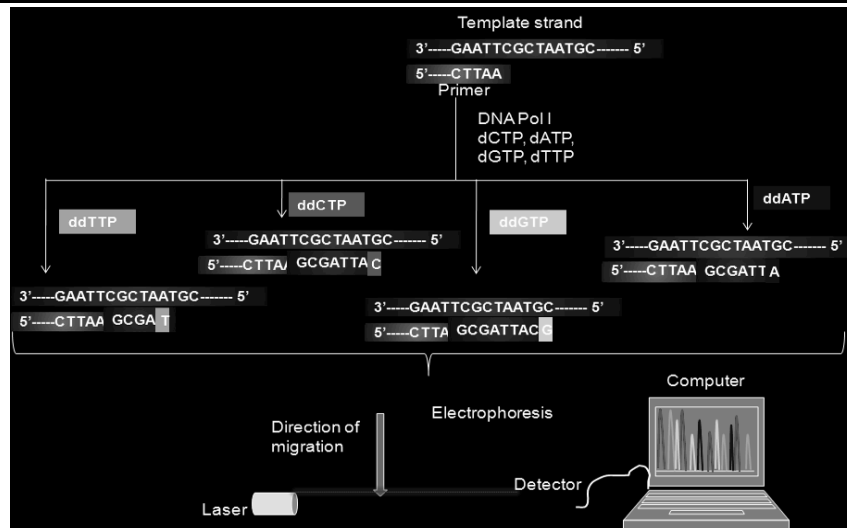
# (I) Genomics

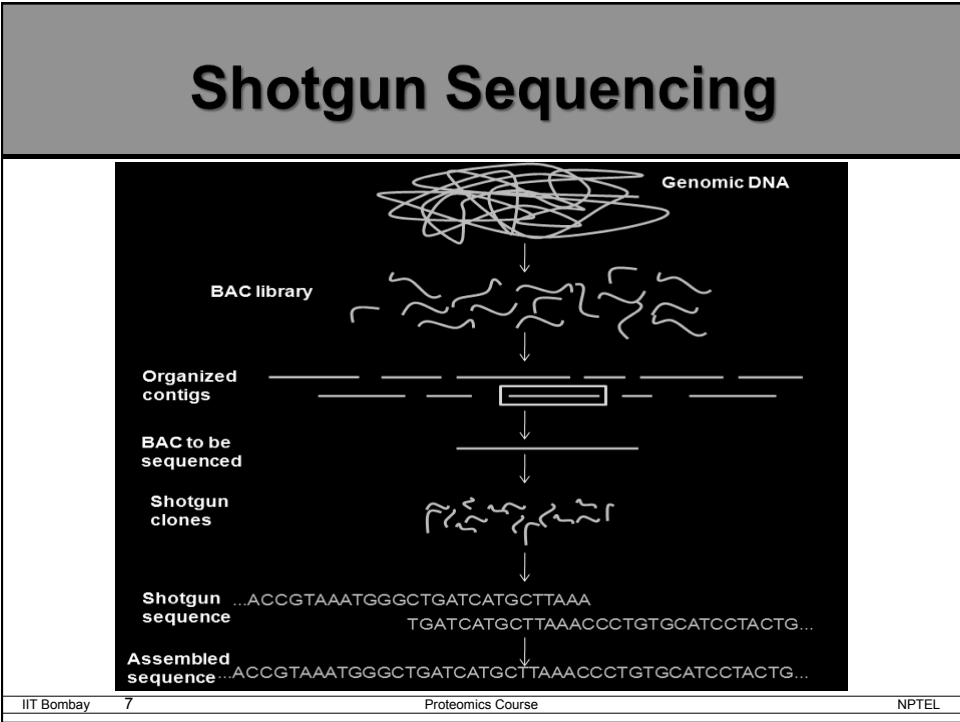
## Genomics

- Genome: The entire sequence of an organism's hereditary information, including both coding and non-coding regions, encoded in DNA is known as "genome".
- Studying genome of an organism by employing sequencing and genome mapping is known as "genomics".

# Genome Sequencing: Traditional methods

## DNA sequencing – Sanger’s method





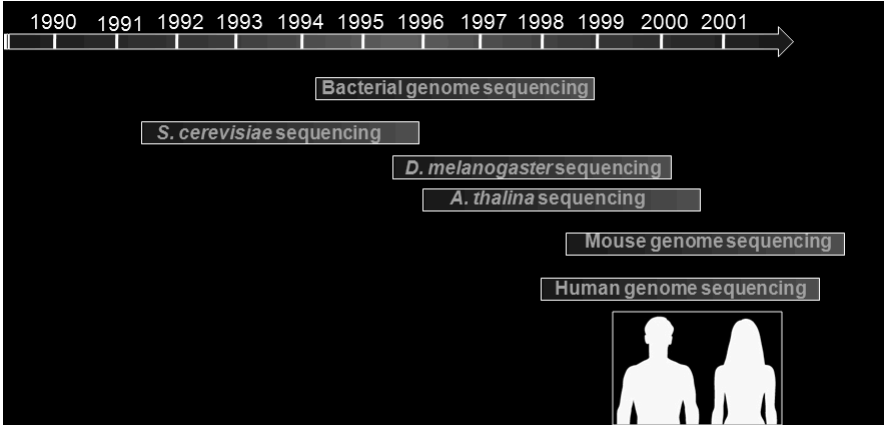
## Traditional DNA Sequencing Methods

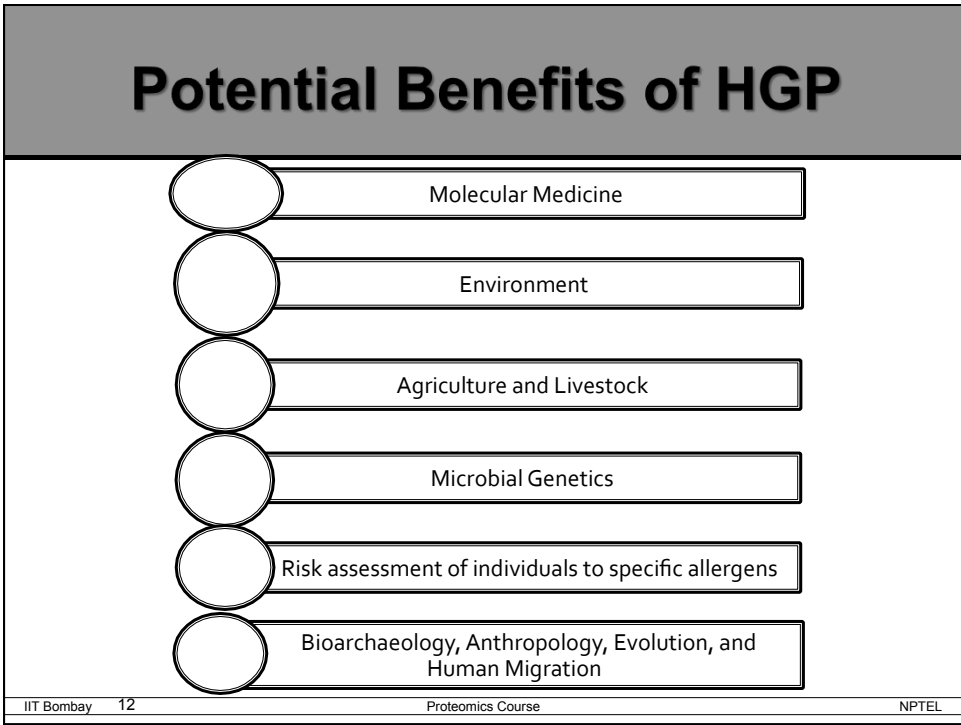
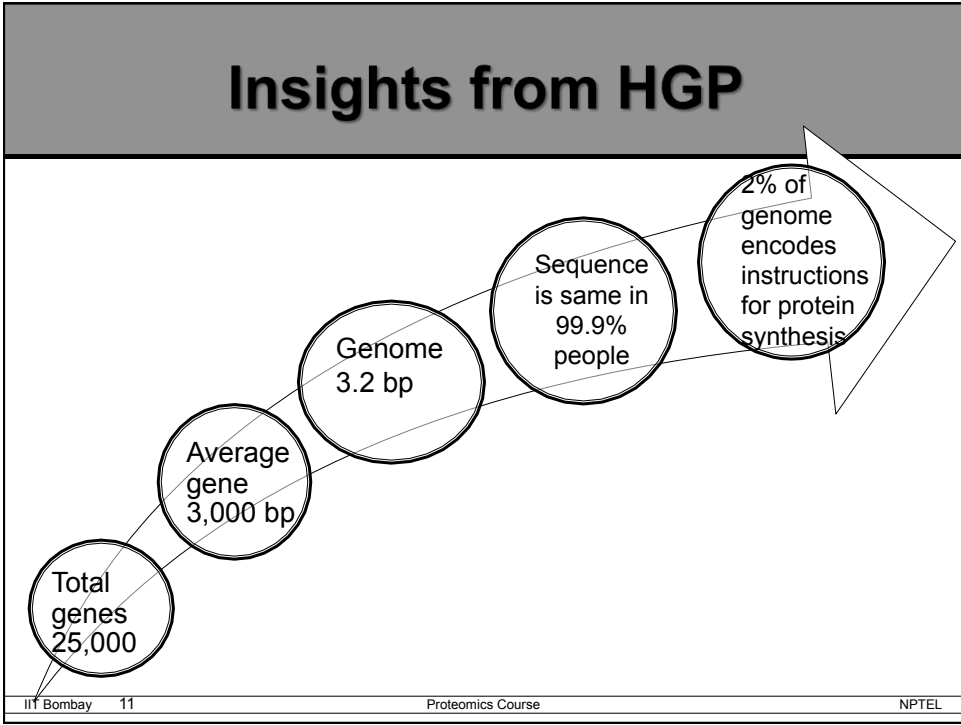
Technique	Description	Sensitivity
Chain termination method (Sanger)	Gold standard but time consuming	High
Pyrosequencing	Based on chemiluminescent detection	Very high
MALDI-TOF	Identifies variant alleles SNPs	Very high

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# Genome Sequencing Projects

# Genome Sequencing Projects





## Next Generation Sequencing (NGS)

## Next Generation Sequencing

- Next generation or second generation sequencing technology – sequencing by ligation or by synthesis, including pyrosequencing and reversible chain termination
- Third generation sequencing technology - to improve second-generation sequencing technology and lower the cost, use of scanning tunneling electron microscope, fluorescence resonance energy transfer, single-molecule detection and protein nanopores

## Next Generation Sequencing: Nanopore sequencing

**Nanopore sequencing**

Exonuclease

ssDNA

Nucleoside monophosphates (NMPs)

Cyclodextrin

Nanopore

Event count

Residual pore current

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## NGS Platforms (Commercial)

Illumina

Pyro-sequencing

Helicos

NGS Platforms

SOLiD

Ion Torrent

*Ref: Am J Clin Pathol 2011;136:527-539*

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## NGS vs. Sanger's sequencing

- In NGS preparations are done *in vitro*
  - Transformation of *E. coli* and other limitations are avoided
- NGS methods based on arrays (not capillary)
  - Sequencing time is reduced
- Reduced cost

## DNA Microarrays

# DNA Microarrays

The diagram illustrates the workflow for DNA microarrays. It shows mRNA being converted to cDNA, which is then labeled with fluorescent dyes Cy3 and Cy5. The labeled cDNA is hybridized to a microarray chip. A schematic shows a cDNA strand hybridizing to a complementary sequence on the chip. To the right, there is an image of a microarray scanner and a corresponding fluorescence image showing a grid of spots.

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# (II) Transcriptomics

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## Transcriptomics

- Transcriptome - analysis of all species of transcript, including mRNAs, non-coding RNAs and small RNAs
- Transcript analysis is used to quantify the expression level changes of each transcript during development and under different conditions

## Techniques for evaluating gene expression

- Northern blotting
- Quantitative real-time polymerase chain reaction
- Differential display
- Serial analysis of gene expression
- Microarray

## Reverse transcription PCR

The diagram illustrates the Reverse transcription PCR (RT-PCR) process in three stages:

- mRNA:** A single-stranded messenger RNA molecule is shown at the top.
- Reverse transcriptase:** An arrow points down to the next stage, where the enzyme reverse transcriptase has converted the mRNA into a double-stranded cDNA (complementary DNA) molecule.
- First cycle:** A primer (a short DNA sequence) binds to one end of the cDNA. Taq polymerase (a DNA polymerase) then extends the primer to synthesize a new DNA strand, creating a double-stranded intermediate.
- Second cycle:** The process repeats. Primers bind to both ends of the double-stranded intermediate, and Taq polymerase synthesizes two new DNA strands, resulting in four double-stranded DNA molecules.

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## Real-time PCR

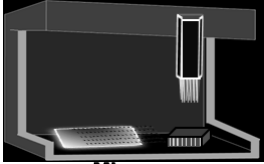
The diagram illustrates the Real-time PCR process:

- Target ssDNA:** A single-stranded DNA target is shown with a primer and a probe. The probe is a short DNA sequence with a **Quencher** (a small molecule that blocks fluorescence) and a **Reporter** (a fluorescent molecule) attached to its ends.
- Annealing:** The primer and probe bind to the target DNA.
- Taq polymerase:** The enzyme Taq polymerase extends the primer, synthesizing a new DNA strand.
- Fluorescence quenched:** As the probe is extended, the Taq polymerase's 5' to 3' exonuclease activity degrades the probe, separating the quencher from the reporter.
- Polymerization & probe degradation:** The process continues, resulting in the release of the reporter molecule.
- Reporter fluorescence:** The released reporter molecule emits fluorescence, which is detected in real-time.

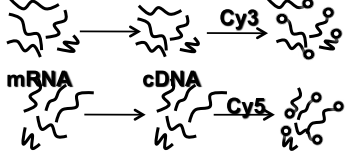
A graph on the left shows **Relative fluorescence** on the y-axis and **# of cycles** on the x-axis. The curve shows a sigmoidal increase in fluorescence over cycles, with the inflection point representing the end of the exponential amplification phase.

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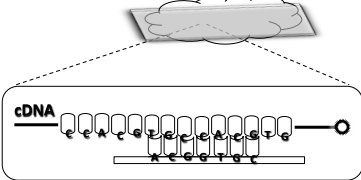
## cDNA Microarrays



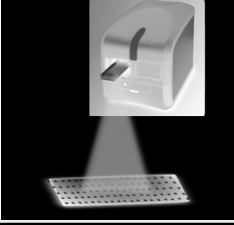
**1. DNA Array printing**



**2. Reverse transcription & labeling**



**3. cDNA hybridization**

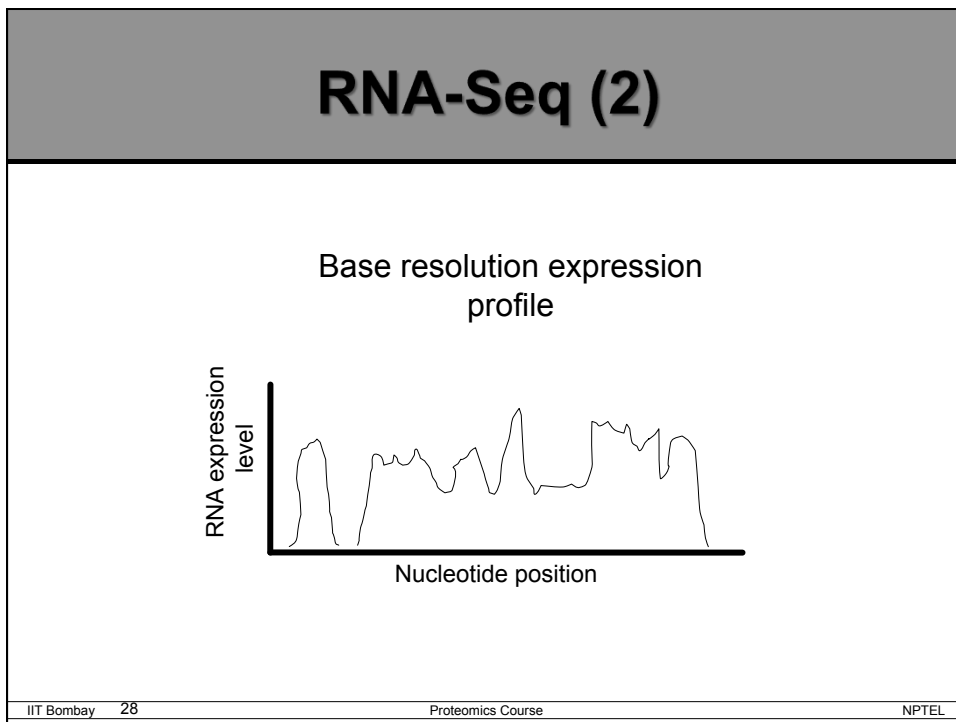
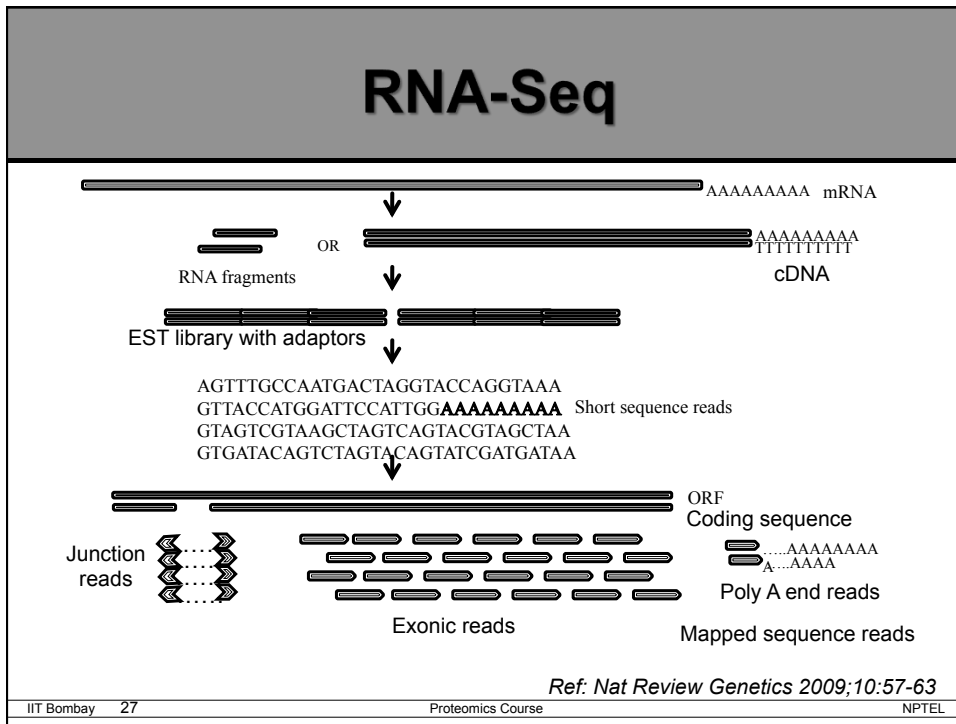


**4. Array scanning**

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## RNA Sequencing

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## (III) Why proteomics?

## Proteomics

- “Proteome” entire complement of proteins expressed by genome of an organism under defined conditions
- The study of entire compendium of proteins encoded by a genome is known as “proteomics”

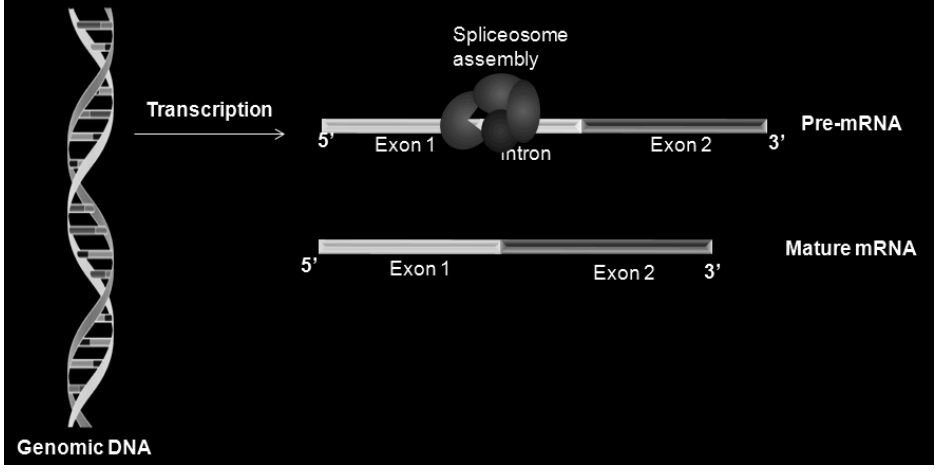
## Genomics Vs. Proteomics

## Genomics vs. Proteomics

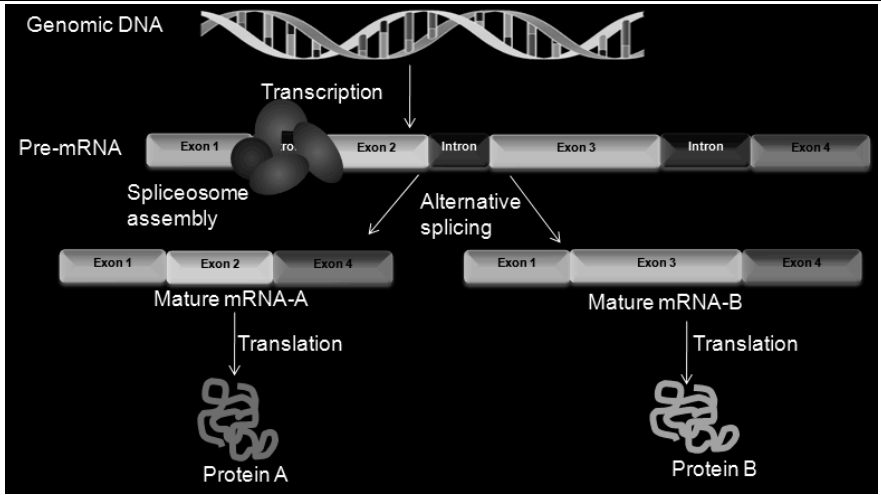
- Availability of completed genome sequences of several species has shifted the focus towards identification and characterization of all gene products of organism
- Genome represents only the starting point but products of gene expression, proteins, provide a much more meaningful insight into essential biological processes.



# Genomics vs. Proteomics

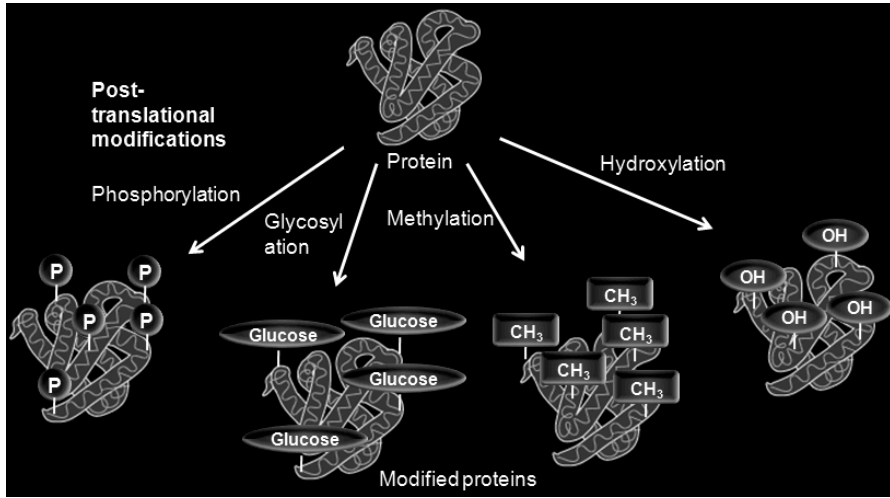


# Genomics vs. Proteomics (2)



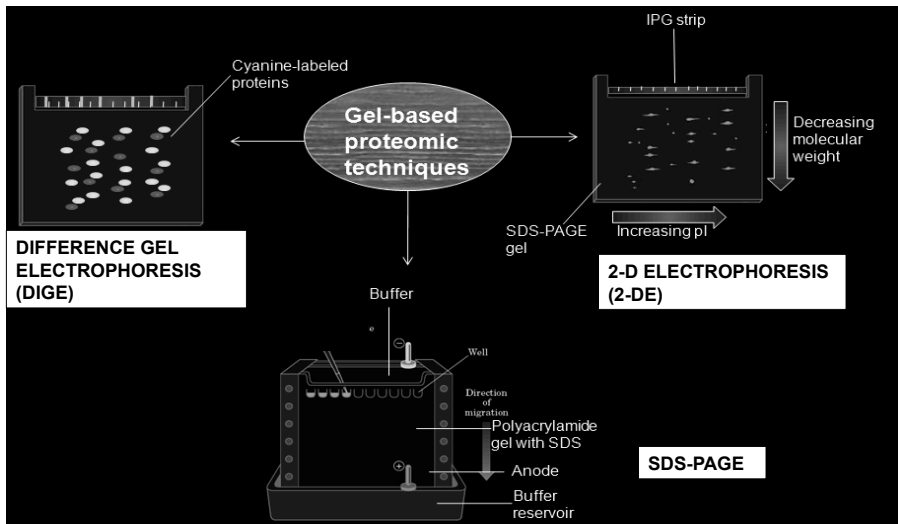
Single gene, multiple protein products indicates complexity of the proteome

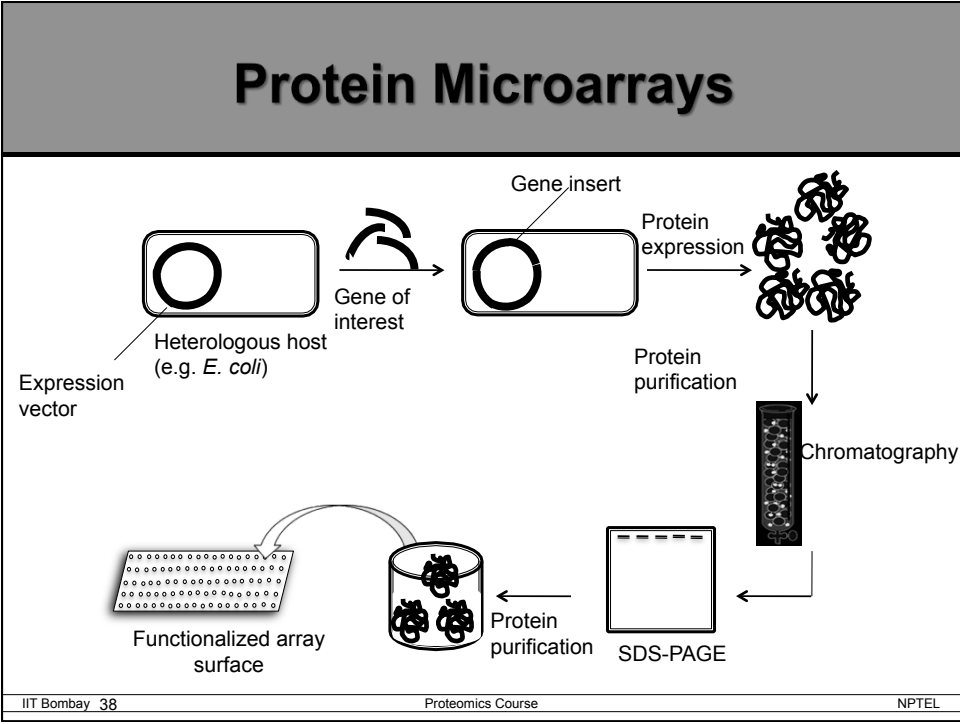
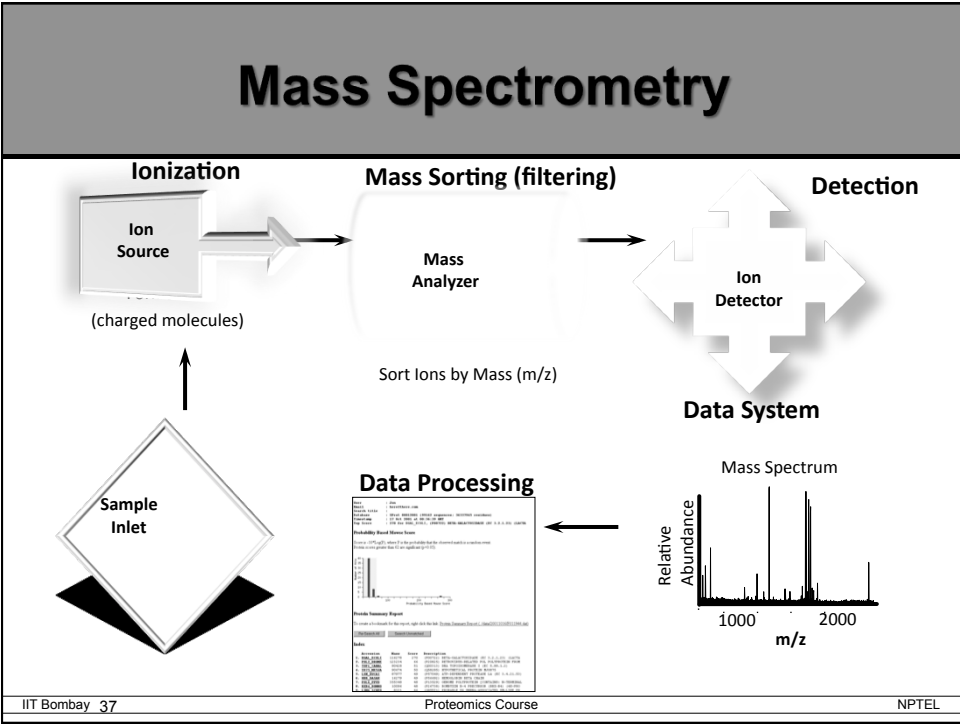
# Genomics vs. Proteomics (3)



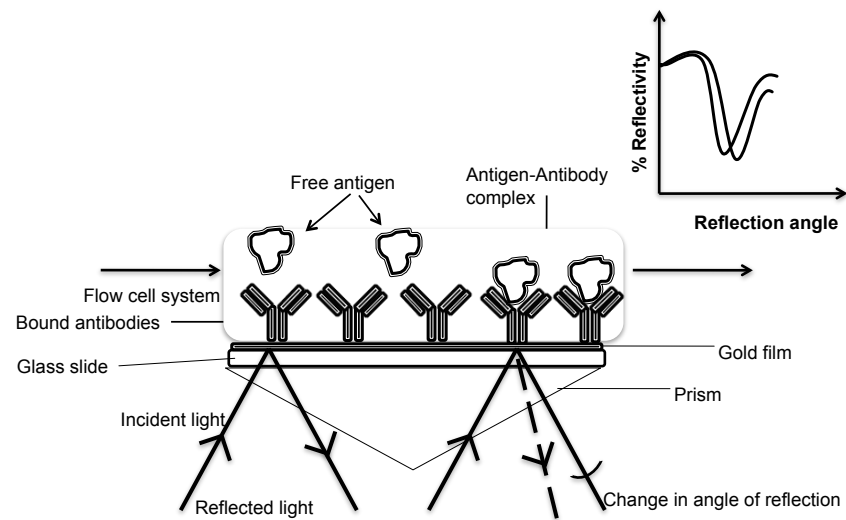
# Gel-based proteomic techniques

IPG strip





## Label-free detection techniques: Surface Plasmon Resonance (SPR)



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## Summary

- Genomics
- Transcriptomics
- Why Proteomics?
- Need for Systems Level investigation

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## REFERENCES

- Berg J., Tymoczko J. & Stryer L., Biochemistry fifth ed., W. H. Freeman & company, 2002. ISBN: 0716746840.
- Link A. J. & Joshua LaBaer, Proteomics: A laboratory Cold Spring Harbor course manual first ed., Cold Spring Harbor laboratory press, 2008. ISBN: 0879697873.
- Simpson R. J. Proteins and Proteomics: a laboratory manual ed., Cold Spring Harbor laboratory press, 2002. ISBN: 0879695544.
- Voet D. & Voet J., Biochemistry fourth ed., Wiley, 2000. ISBN: 047158651X.
- Zhong Wang, Mark Gerstein, Michael Snyder, RNA-Seq: a revolutionary tool for transcriptomics. Nat Review Genetics 2009;10:57-63.
- Jeffrey S. Ross, MD,1,2 and Maureen Cronin, Whole Cancer Genome Sequencing by Next-Generation Methods. Am J Clin Pathol 2011;136: 527-539.
- Wang et al. RNA-Seq: a revolutionary tool for transcriptomics. Nat Review Genetics 2009;10:57-63

## REFERENCES

- Science Genome Map. Science 16 February 2001. Vol. 291 no. 5507 p. 1218
- Pareek et al. Sequencing technologies and genome sequencing. Journal of Applied Genetics. November 2011, Volume 52, Issue 4, pp 413-435,
- Lawler et al. 2000. Harbinger of a Litigious Future? Science 10 November 2000: Vol. 290 no. 5494 p. 1076