Proteomics Course

LECTURE-3
Genomics and Transcriptomics: Why proteomics?

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Lecture outline

- Genomics
- Transcriptomics
- Why proteomics?

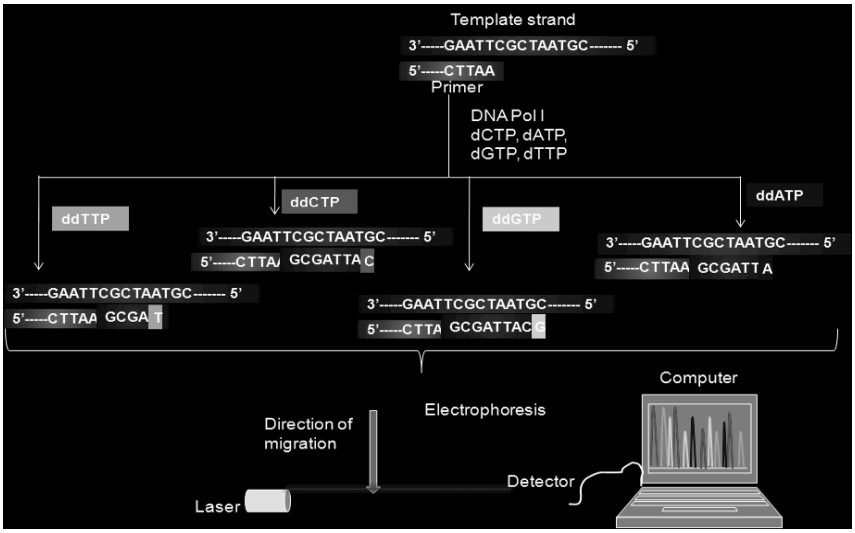
(I) Genomics

Genomics

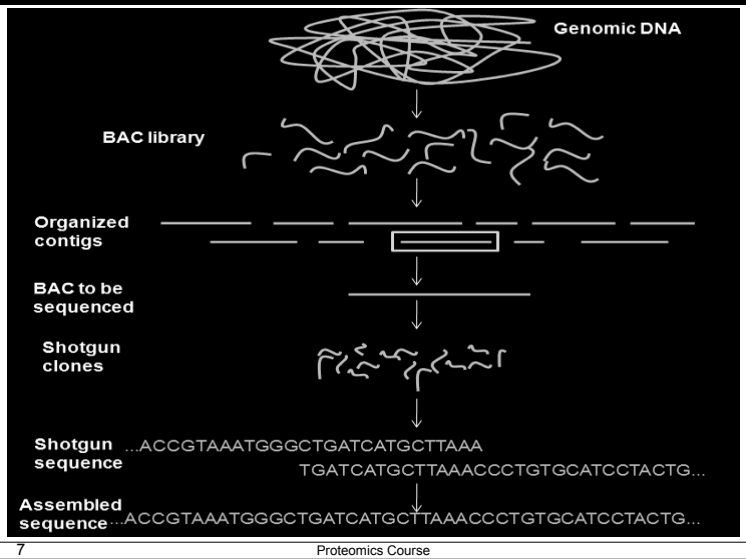
- Genome: The entire sequence of an organism's hereditary information, including both coding and non-coding regions, encoded in DNA is known as "genome".
- Studying genome of an organism by employing sequencing and genome mapping is known as "genomics".

Genome Sequencing: Traditional methods

DNA sequencing – Sanger's method



Shotgun Sequencing

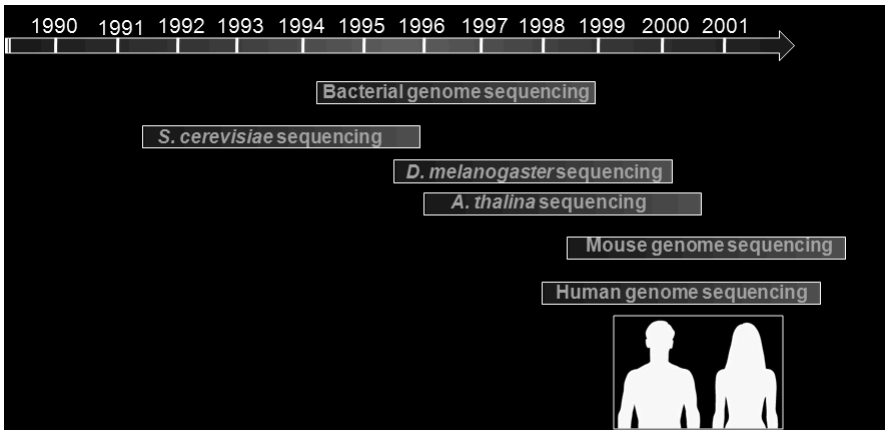


Traditional DNA Sequencing Methods

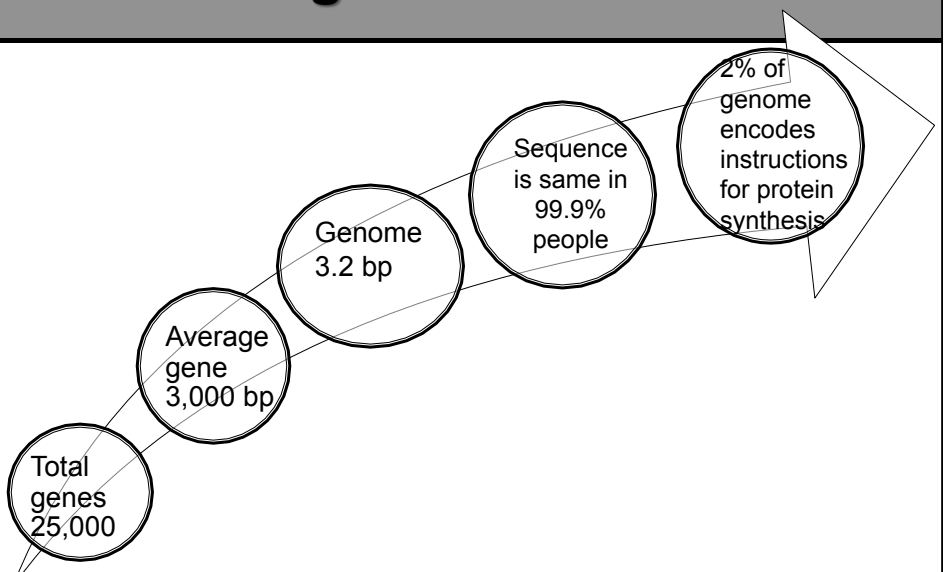
Technique	Description	Sensitivity
Chain termination method (Sanger)	Gold standard but time consuming	High
Pyrosequencing	Based on chemiluminescent detection	Very high
MALDI-TOF	Identifies variant alleles SNPs	Very high

Genome Sequencing Projects

Genome Sequencing Projects



Insights from HGP



Potential Benefits of HGP

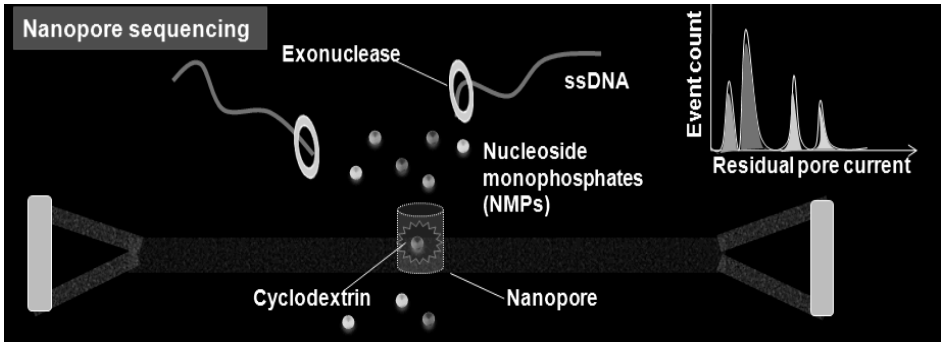
- Molecular Medicine
- Environment
- Agriculture and Livestock
- Microbial Genetics
- Risk assessment of individuals to specific allergens
- Bioarchaeology, Anthropology, Evolution, and Human Migration

Next Generation Sequencing (NGS)

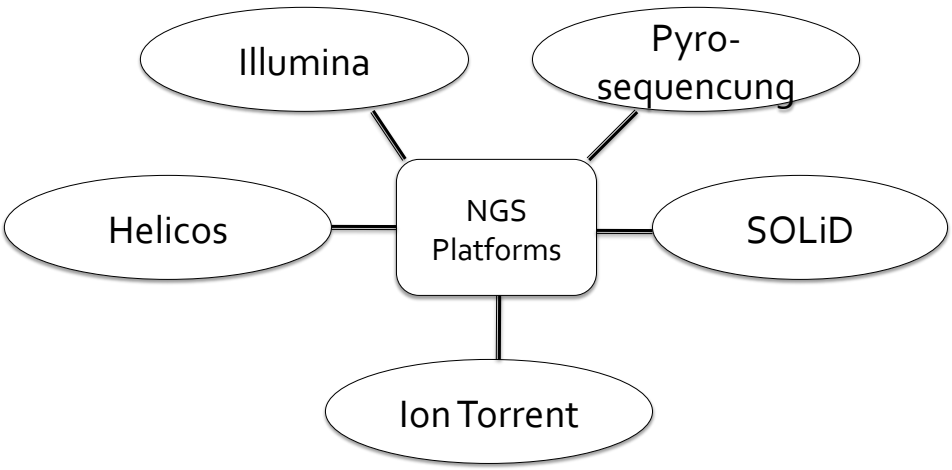
Next Generation Sequencing

- 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



NGS Platforms (Commercial)



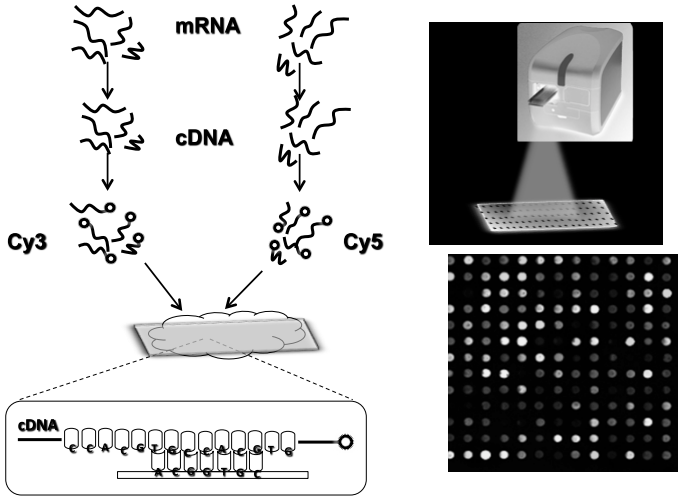
Ref: Am J Clin Pathol 2011;136:527-539

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



(II) Transcriptomics

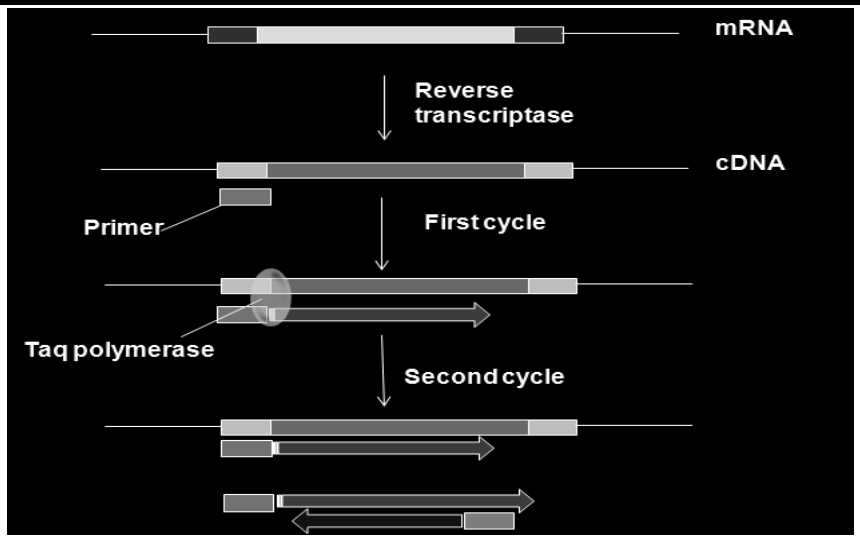
Transcriptomics

- 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

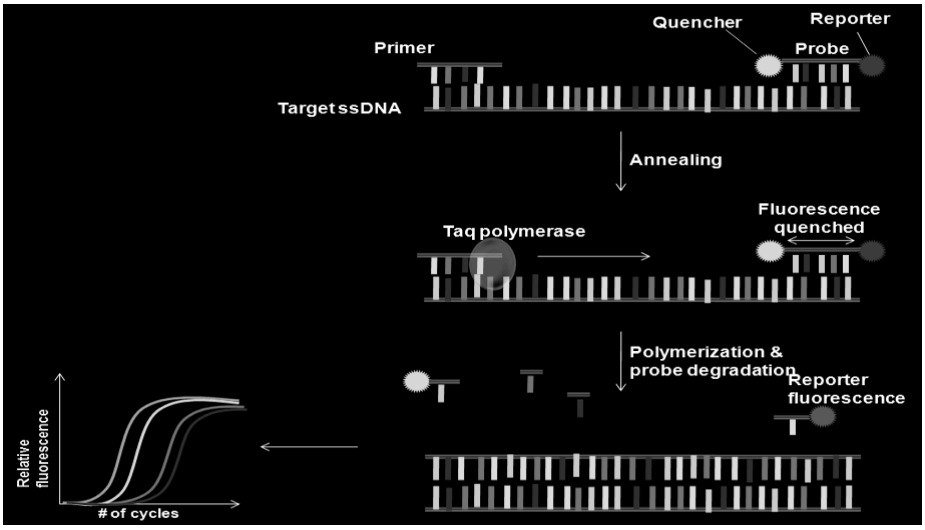
Techniques for evaluating gene expression

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

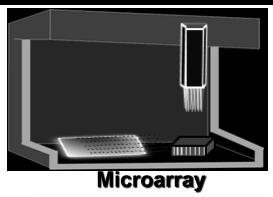
Reverse transcription PCR



Real-time PCR

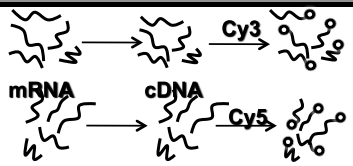


cDNA Microarrays

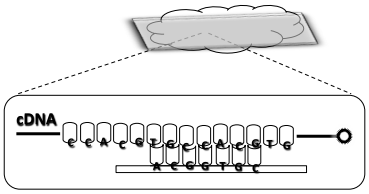


Microarray

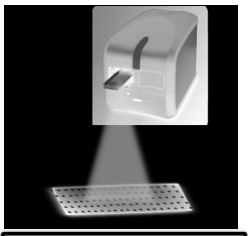
1. DNA Array printing



2. Reverse transcription & labeling



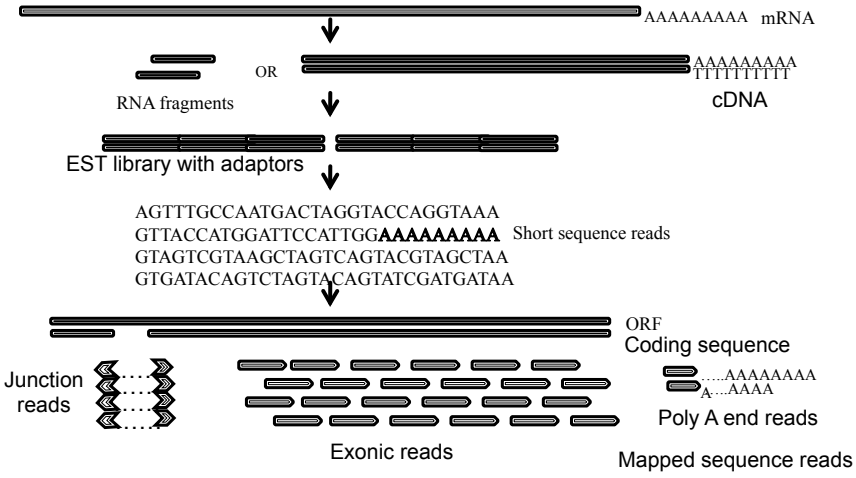
3. cDNA hybridization



4. Array scanning

RNA Sequencing

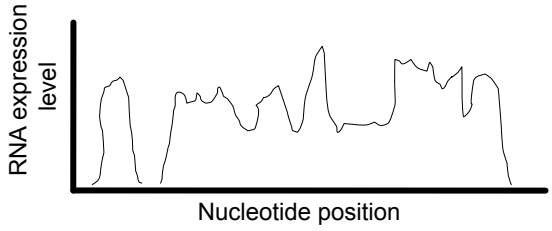
RNA-Seq



Ref: Nat Review Genetics 2009;10:57-63

RNA-Seq (2)

Base resolution expression profile



(III) Why proteomics?

Proteomics

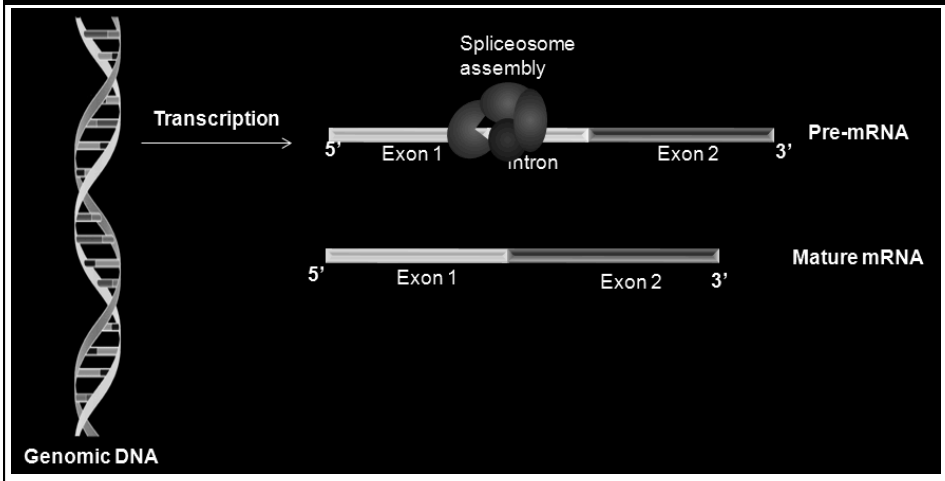
- "Proteome" entire complement of proteins expressed by genome of an organism under defined conditions
- The study of entire compendium of proteins encoded by a genome is known as "proteomics"

Genomics Vs. Proteomics

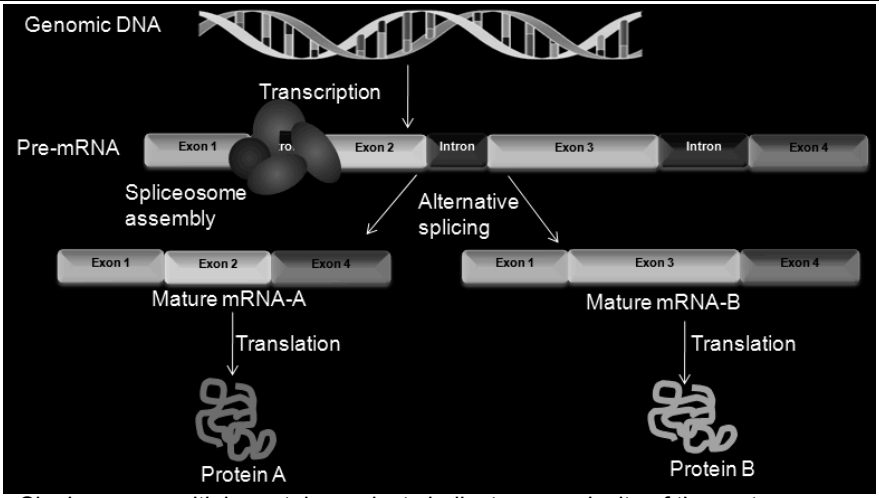
Genomics vs. Proteomics

- 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

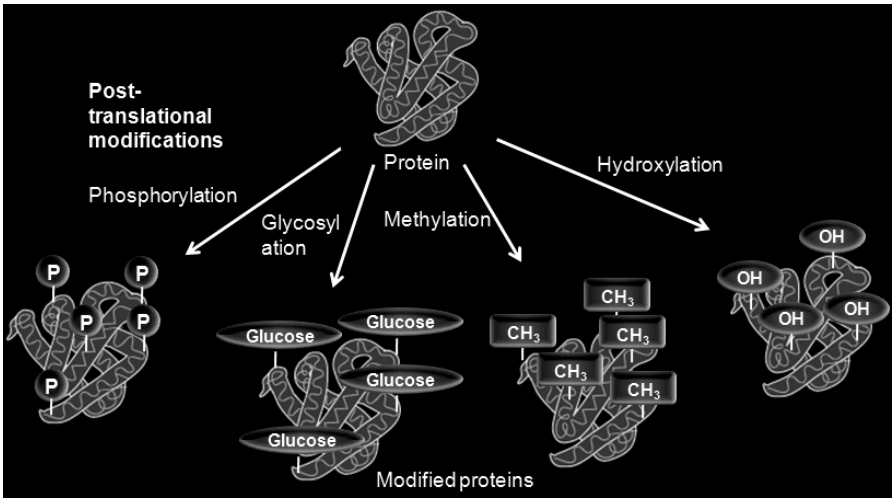


Genomics vs. Proteomics (2)

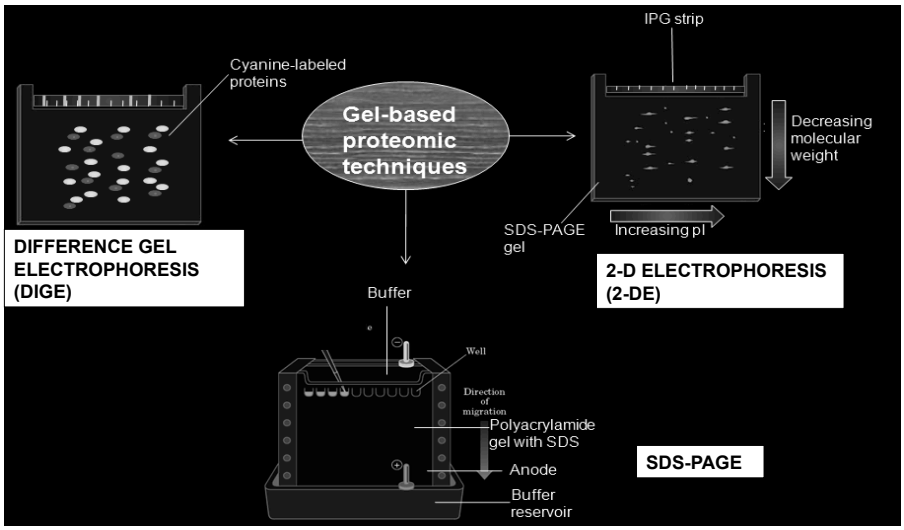


Single gene, multiple protein products indicates complexity of the proteome

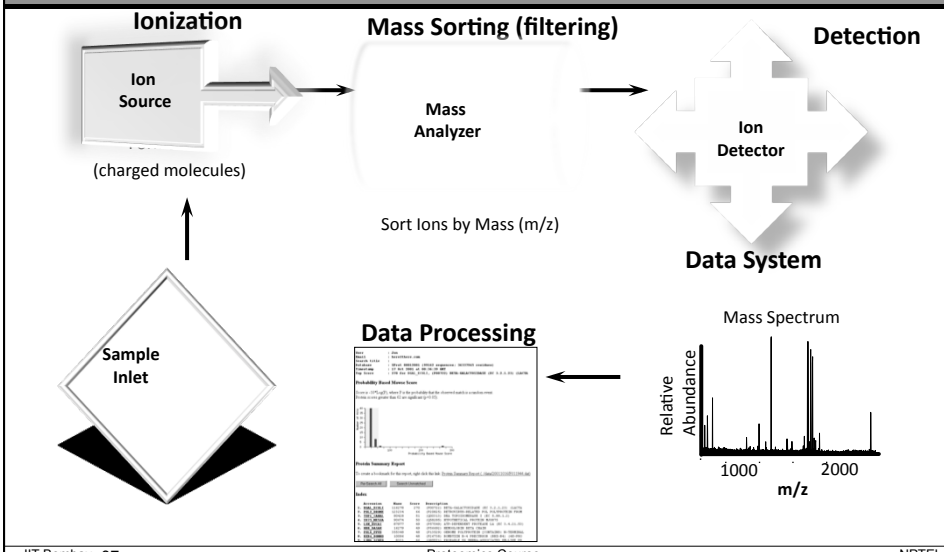
Genomics vs. Proteomics (3)



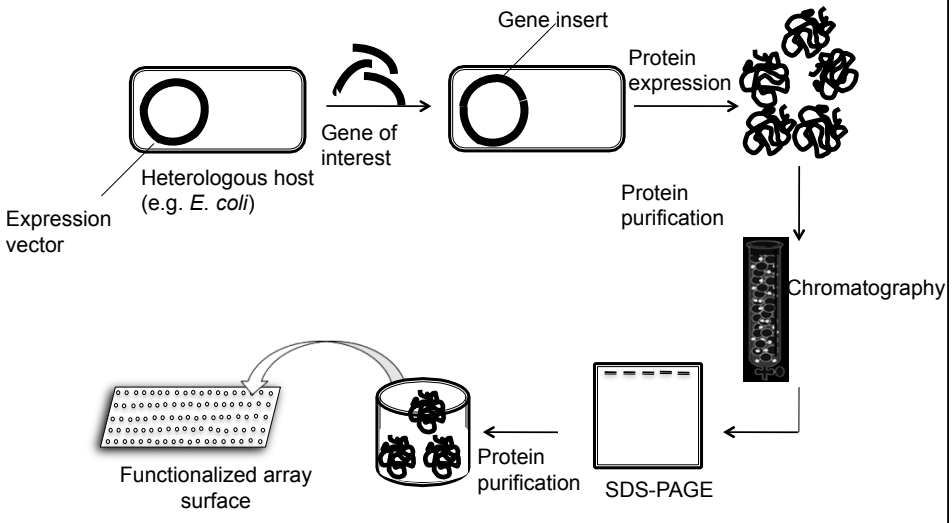
Gel-based proteomic techniques



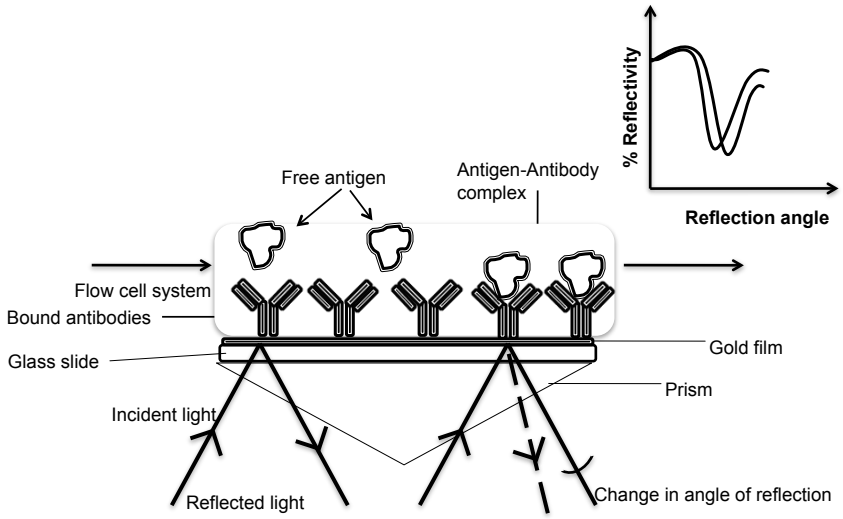
Mass Spectrometry



Protein Microarrays



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



Summary

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

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