Drug Discovery

- It is a lengthy and a highly expensive process
- For the pharmaceutical industry, the number of years to bring a drug from discovery to market is approximately 15 years, costing up to US$500 million per individual drug.
- A total of 40% of the compounds fail due to poor pharmacokinetics and 11% due to preclinical toxicity.
- It requires variety of tool from diverse fields.
- Several biotechnologies, including genomics, proteomics, cellular and organismic methodologies have been developed.

Genomic and Proteomic technologies

- Genomic and Proteomic technologies have been developed over the last several years.

These methods are aimed at:

a) **discovering new genes and proteins**

b) **quantifying and analyzing gene and protein expression**

c) **assigning functionality**

- Being able to compare levels of gene and protein expression between diseased and normal cells or cells treated with compounds, which vary in their efficacy and toxicity, could prove valuable:

a) **identifying new drug targets**

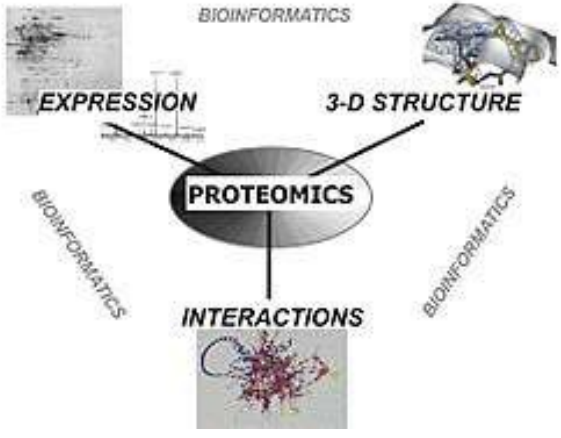
b) **optimizing the choice of lead compound candidates by more closely predicting their success or failure**

Genomics

- Study of genes and their function
- Aims to understand the structure of the genome (mapping of genes and sequencing the DNA)
- Seeks to exploit the finding from the sequencing of the human and other genomes to find new drug targets

Proteomics

- Proteomics is essentially protein analysis
- Could be described as a broad classification for a set of technology and bioinformatics platforms aimed at the comprehensive molecular description of the actual protein complement of a given sample.
- Presently, it is typically associated with systems biology.
- Progress in characterizing rapid posttranslational protein modifications



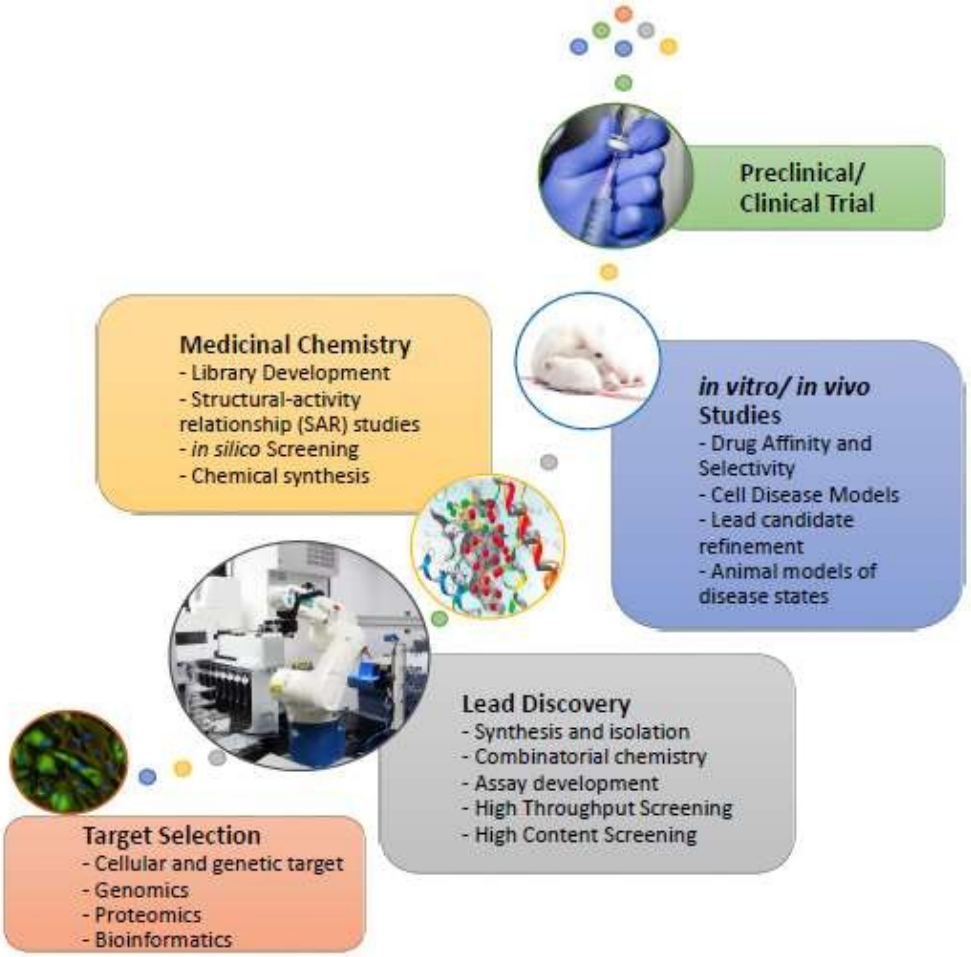
Difference between Genomics and Proteomics

- Genome- **relatively static**
- Proteome- **not static** (it changes constantly in response to tens of thousands of intra- and extracellular environmental signals).

- The proteome varies with various factors like health or disease, the nature of each tissue, the stage of cell development, and effects of drug treatments.

- Proteomics runs parallel to genomics in many ways:
  - Genomics starts with the gene and makes inferences about its products (proteins)
  - Proteomics begins with the functionally modified protein and works back to the gene responsible for its production

Process of drug discovery and development

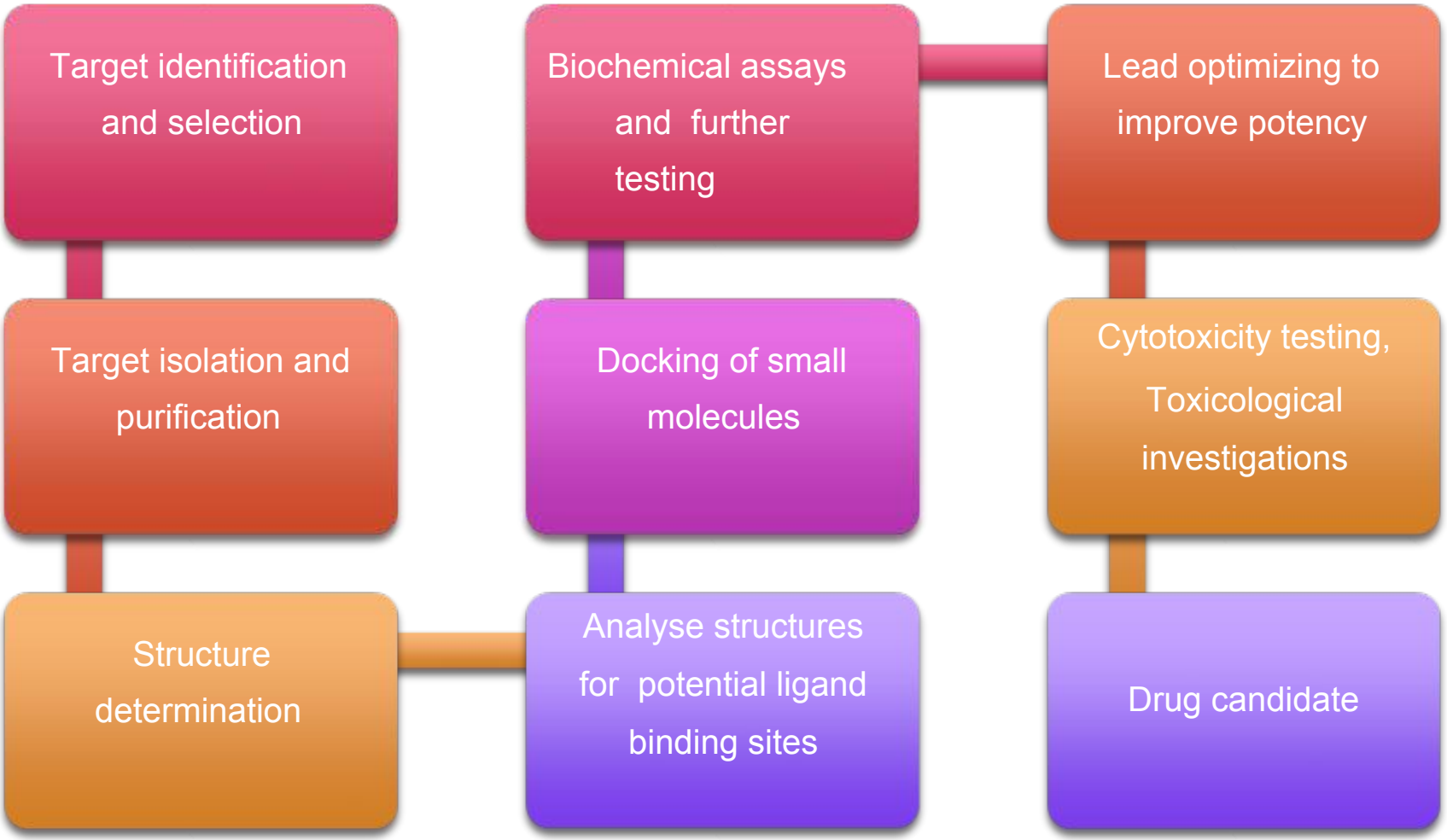


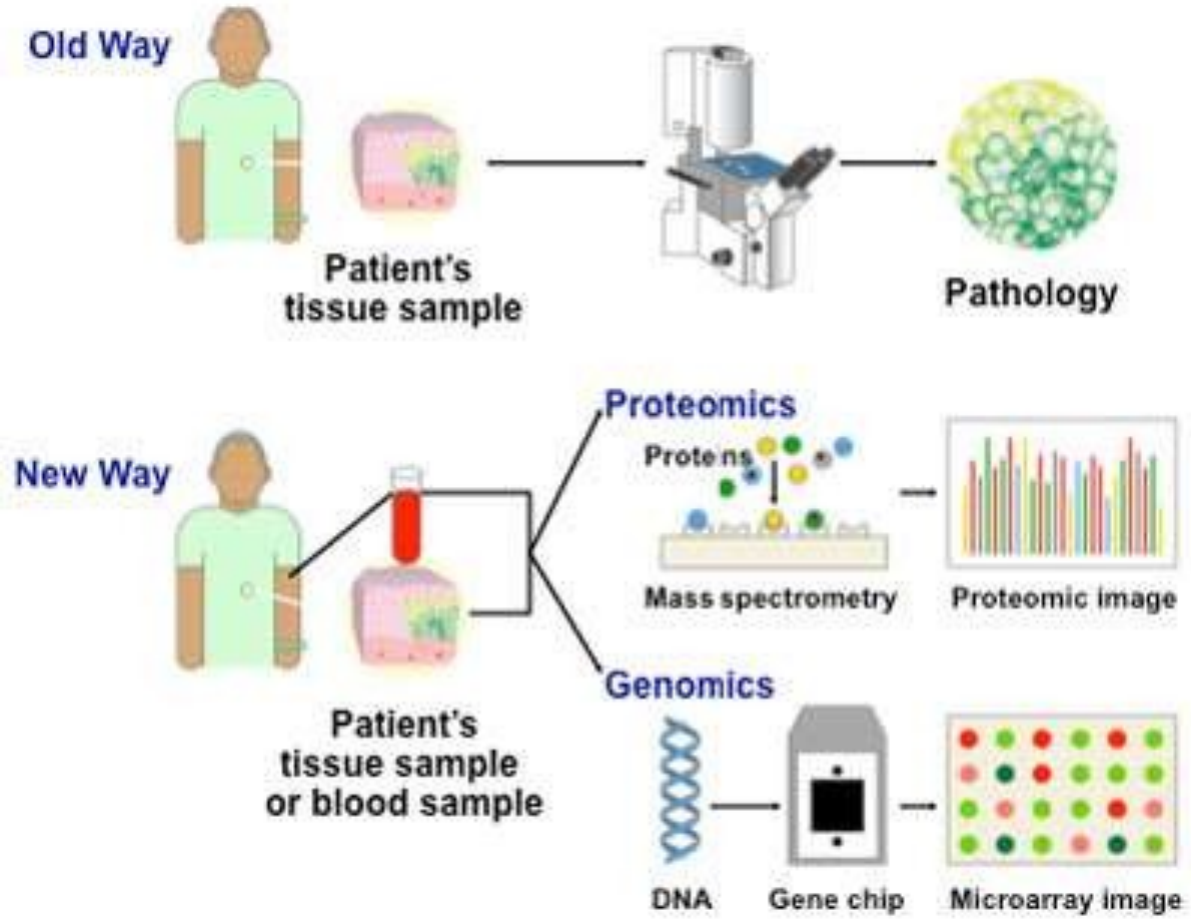


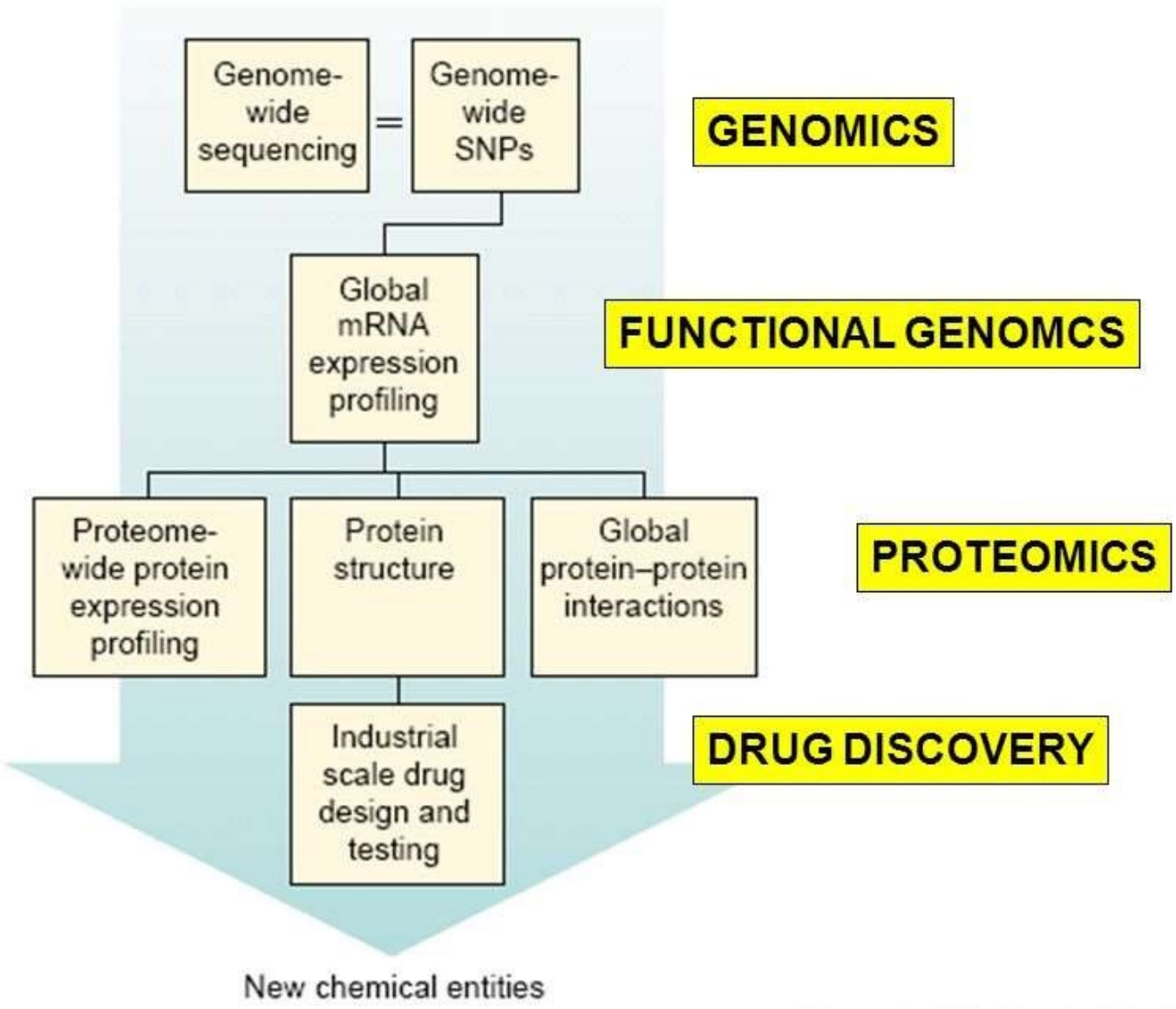



Target Selection	Lead Discovery	Medicinal Chemistry	In Vitro Studies	In Vivo Studies	Clinical Trials and Therapeutics
• Cellular and Genetic Targets	• Synthesis and Isolation	• Library Development	• Drug Affinity and Selectivity	• Animal models of Disease States	
• Genomics	• Combinatorial Chemistry	• SAR Studies	• Cell Disease Models	• Behavioural Studies	
• Proteomics	• Assay development	• In Silico Screening	• MOA	• Functional Imaging	
• Bioinformatics	• High-Throughput Screening	• Chemical Synthesis	• Lead Candidate Refinement	• Ex-Vivo Studies	

Steps of Drug Discovery Process



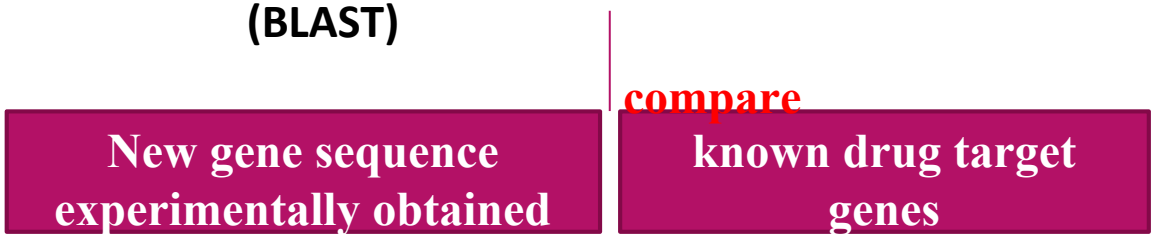

Old Way
Patient's
tissue sample
Pathology
New Way
Proteomics
Proteins
Mass spectrometry
Proteomic image
Genomics
Patient's
tissue sample
or blood sample
DNA
Gene chip
Microarray image


Genome-wide sequencing
=
Genome-wide SNPs
GENOMICS
Global mRNA expression profiling
FUNCTIONAL GENOMCS
Proteome-wide protein expression profiling
Protein structure
Global protein–protein interactions
PROTEOMICS
Industrial scale drug design and testing
DRUG DISCOVERY
New chemical entities

ROLE OF GENOMICS

Identity comparison for new nucleic acid sequences

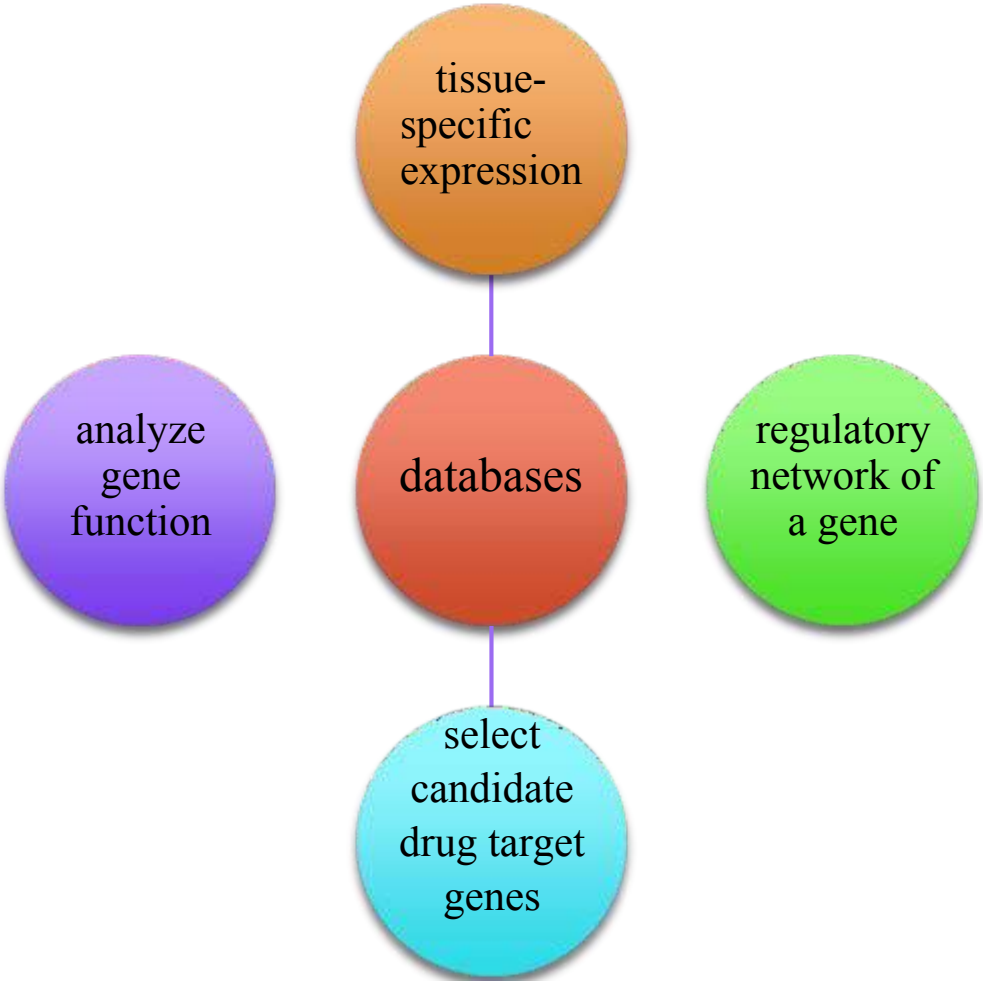
Basic Local Alignment Search Tool (BLAST)



For example, tumor necrosis factor (TNF)-related apoptosis-inducing ligand (TRAIL), a known member named apoptosis ligand 2 (Apo2L) in the TNF family, was discovered by searching homologs of the TNF family proteins in the expressed sequence tag database.

Databases of model organisms

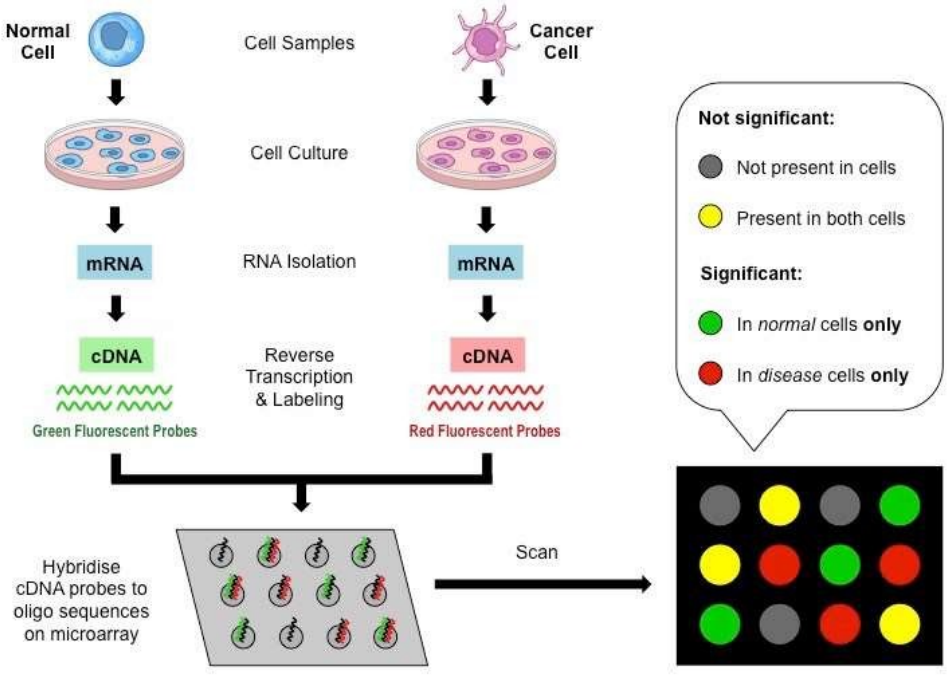
- There are over 30 model organisms for which whole genomes were sequenced, and more than hundreds of organisms are being sequenced.
- The obtained data resources are helpful for functional predictions of genes, especially for pathogenic organisms, and are useful for screening drug targets.
- For instance, this kind of whole-genome sequence information, integrated from multiple pathogenic organisms, can be used to screen:
  - **race-specific genes**
  - **virulence genes**
  - **common genes of pathogenic organisms (conserved genes) specific bacterial or viral enzyme genes**
  - **bacterial membrane-translocation proteins**


tissue-specific expression
analyze gene function
databases
regulatory network of a gene
select candidate drug target genes

Analysis of gene expression profiles

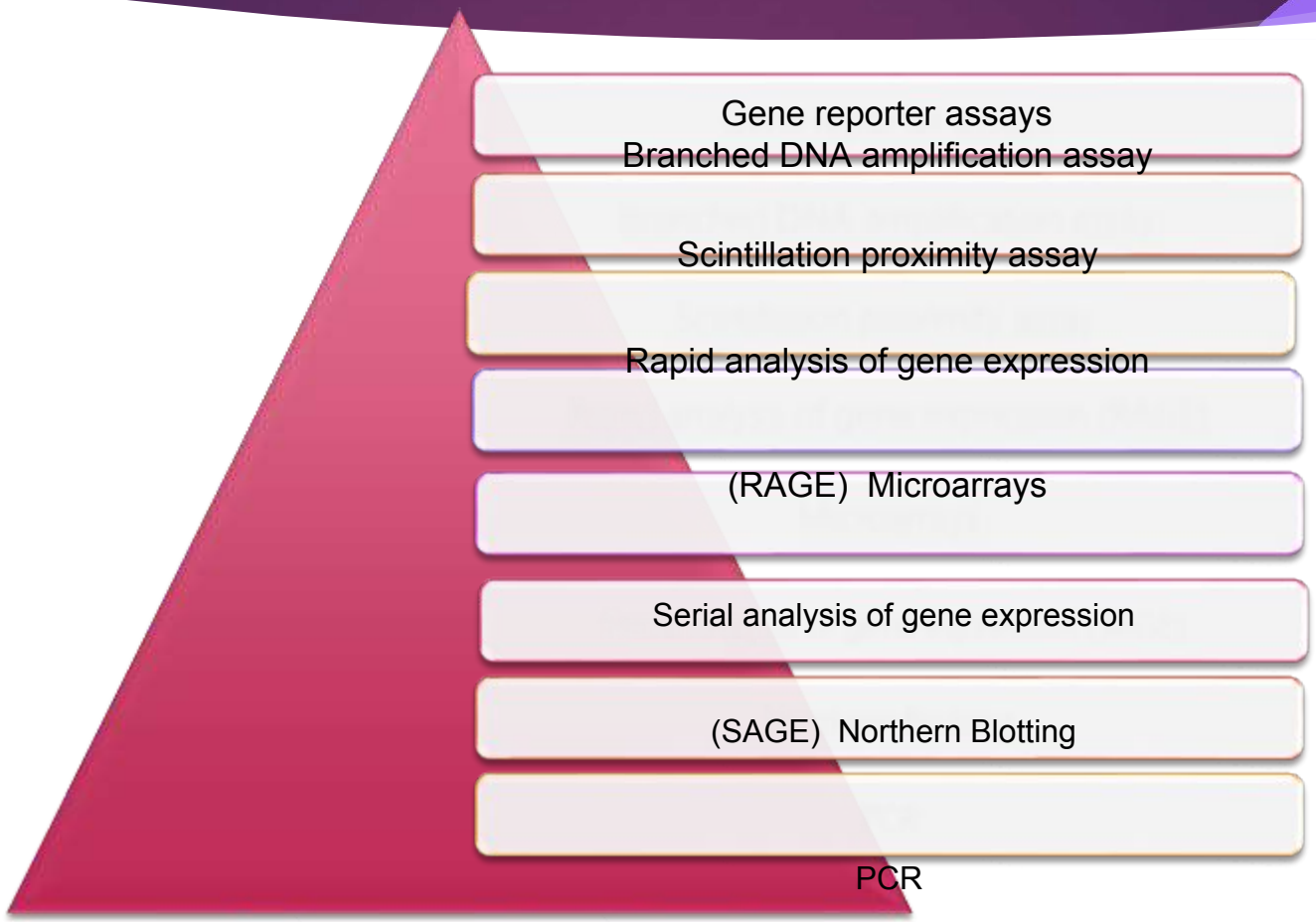
Gene microarray – gene expression levels between two group of samples.

Analyze different cellular mRNA or reverse transcription products that are derived from different individuals (healthy persons and patients), tissues, cell cycles, development periods, differentiation periods, pathological change conditions, or stimulation conditions (including different induction and treatments).



Analysis of the genes related to drug action

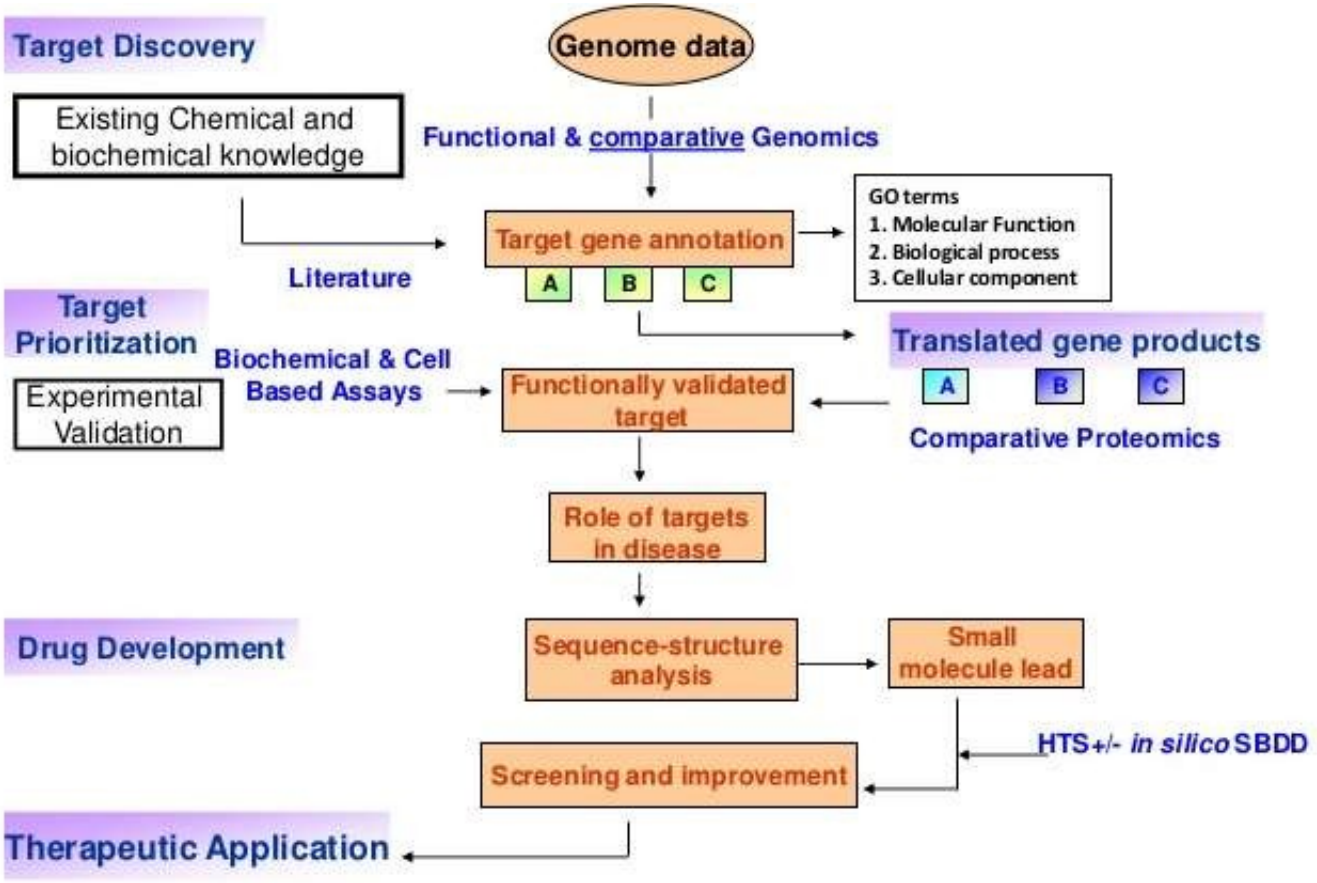
- DNA microarray containing 97% of the predicted open reading frames to monitor changes in *Mycobacterium tuberculosis* gene expression in response to the anti-tuberculosis drug isoniazid.
- The results showed that isoniazid induced several genes that encode proteins that are physiologically relevant to the drug's mode of action, including an operonic cluster of 5 genes encoding type II fatty acid synthase enzymes and fbpC, which encodes trehalose dimycolyl transferase.
- Other genes, not apparently within directly affected biosynthetic pathways, also were induced. These genes, efpA, fadE23, fadE24, and ahpC, likely mediate processes that are linked to the toxic consequences of the drug.
- Insights gained from this approach may define new drug targets.


Gene reporter assays
Branched DNA amplification assay
Scintillation proximity assay
Rapid analysis of gene expression
(RAGE) Microarrays
Serial analysis of gene expression
(SAGE) Northern Blotting
PCR

CONCLUSION

- Knowledge of all the human genes and their functions may allow effective preventive measures.
- The cause of common fatal diseases has been identified by genomics and it shows the potential to identify individuals who are particularly susceptible to a given disease long before that disease becomes apparent.
- It has positively impacted the drug research strategy and drug discovery development processes.
- The process has been made simpler and economical. Further innovations in this area are expected, which should take drug discovery research to a new level.

Genomic Approach to Drug Discover
y



ROLE OF PROTEOMICS

Application of proteomics in drug target discovery

- Proteomics is the large-scale study of the proteins in a cell, tissue, or entire organism.
- Compare changes in protein levels in normal and diseased tissue.
- One established technique for comparative proteomics is based on labelling proteins from normal and diseased tissues with different fluorescent dyes (Cy3 and Cy5), mixing the proteins together, and then separating them by isoelectric point and molecular weight (difference in-gel electrophoresis).
- The sequencing of the human genome has increased interest in proteomics because while DNA sequence information provides a static snapshot of the various ways in which the cell might use its proteins, the life of the cell is a dynamic process.
- This new data increase the interest of proteomics in the field of science, medicine, and most notably – pharmaceuticals

- Proteomics can analyze biomarkers by quantifying individual proteins and show the separation between one or more protein “spots” on a scanned image from two-dimensional gel electrophoresis; for example, proteomic differences between early and advanced stages of an illness can be observed.

Quantitative Proteomics

- Technique to determine the amount of proteins in the sample.
- Mainly performed by 2D- gel electrophoresis and Mass spectrometry.

Quantification using spectrophotometry

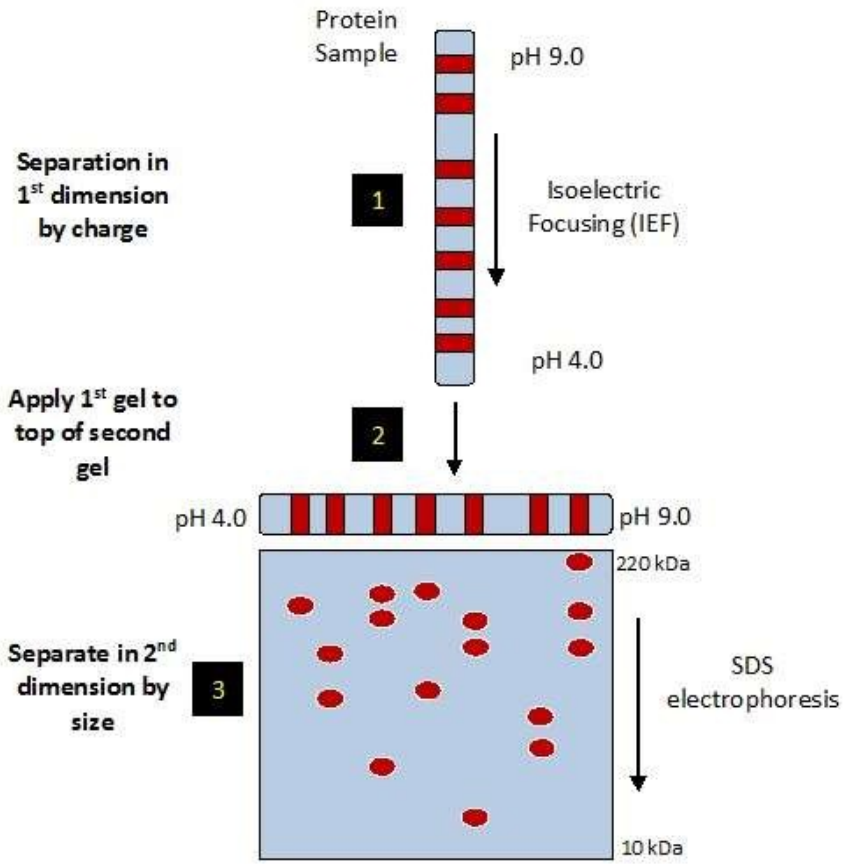
- Concentration of a certain protein in a sample may be determined
- Concentration of a protein can be determined by measuring the OD at 280 nm on a spectrophotometer, which can be used with a standard curve assay to quantify the presence of Tryptophan, Tyrosine, and Phenylalanine.

DISADVANTAGES:

- Not the most accurate technique: composition of proteins can vary greatly and this method would not be able to quantify proteins that do not contain the mentioned amino acids.
- This method is also inaccurate due to the possibility of nucleic acid contamination.
- Other more accurate spectrophotometric procedures for protein quantification include the Biuret, Lowry, BCA, and Bradford methods.

Quantification using two dimensional electrophoresis

- 2-DE provides information about the protein quantity, charge, and mass of the intact protein.
- It has limitations for the analysis of proteins larger than 150 kDa or smaller than 5kDa and low solubility proteins.


Protein Sample
pH 9.0
Separation in 1st dimension by charge
1
Isoelectric Focusing (IEF)
pH 4.0
Apply 1st gel to top of second gel
2
pH 4.0
pH 9.0
220 kDa
Separate in 2nd dimension by size
3
SDS electrophoresis
10 kDa

Quantification using mass spectrometry

- Mass spectrometry (MS) represents one of the main technologies for quantitative proteomics with advantages and disadvantages.
- Quantitative MS has higher sensitivity but can provide only limited information about the intact protein.
- Quantitative MS has been used for bothdiscovery and targeted proteomic analysis to understand global proteomic dynamics in cells, tissues or organisms.

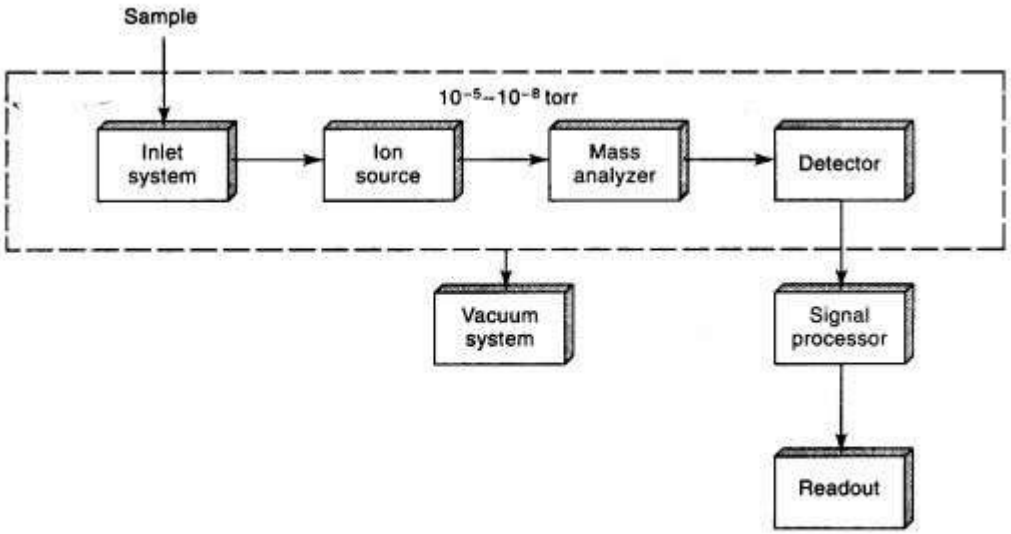
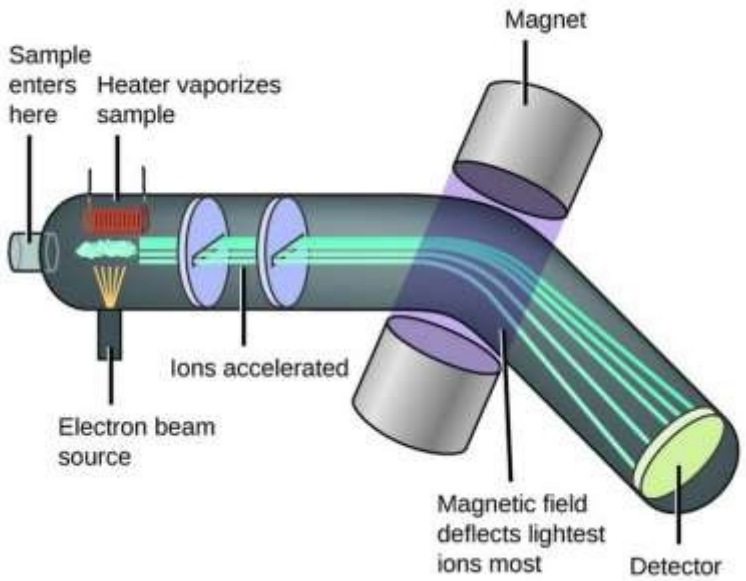
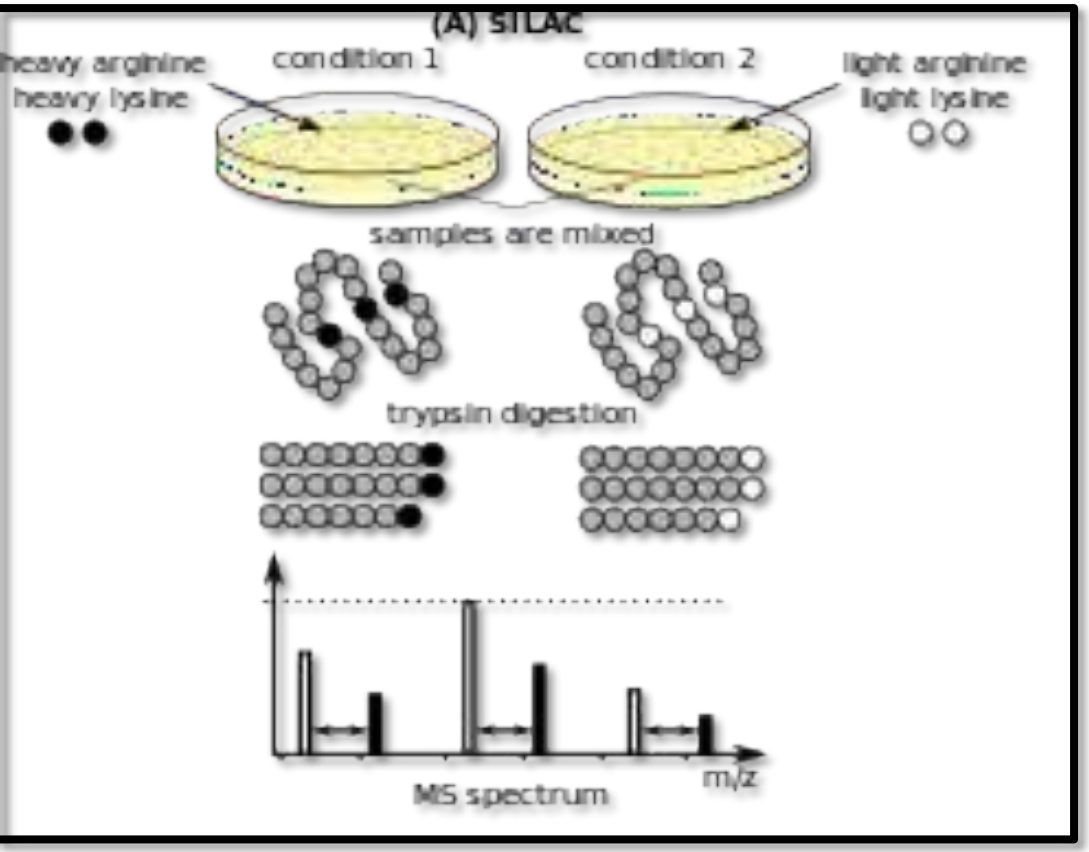


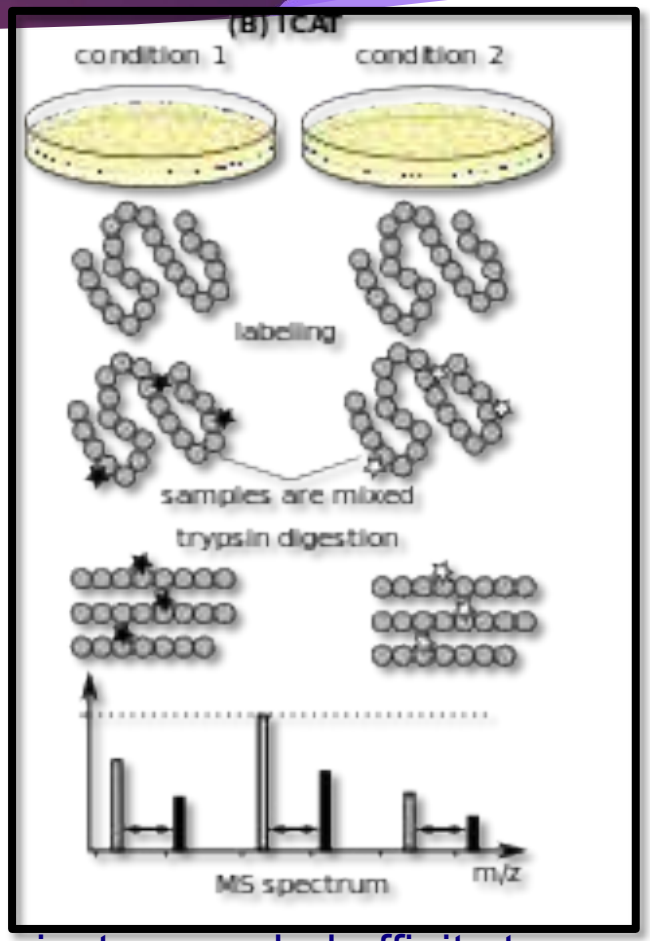
FIGURE 18–1 Components of a mass spectrometer.



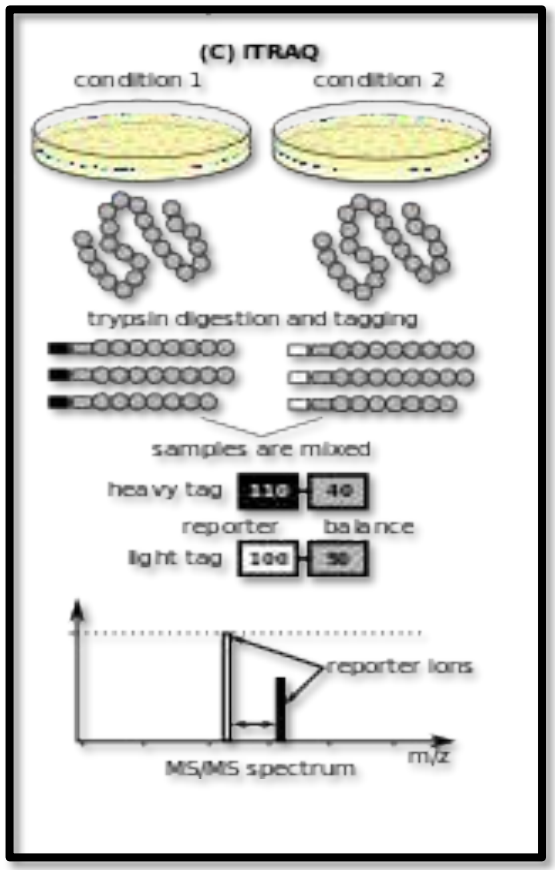
Quantitative Proteomics

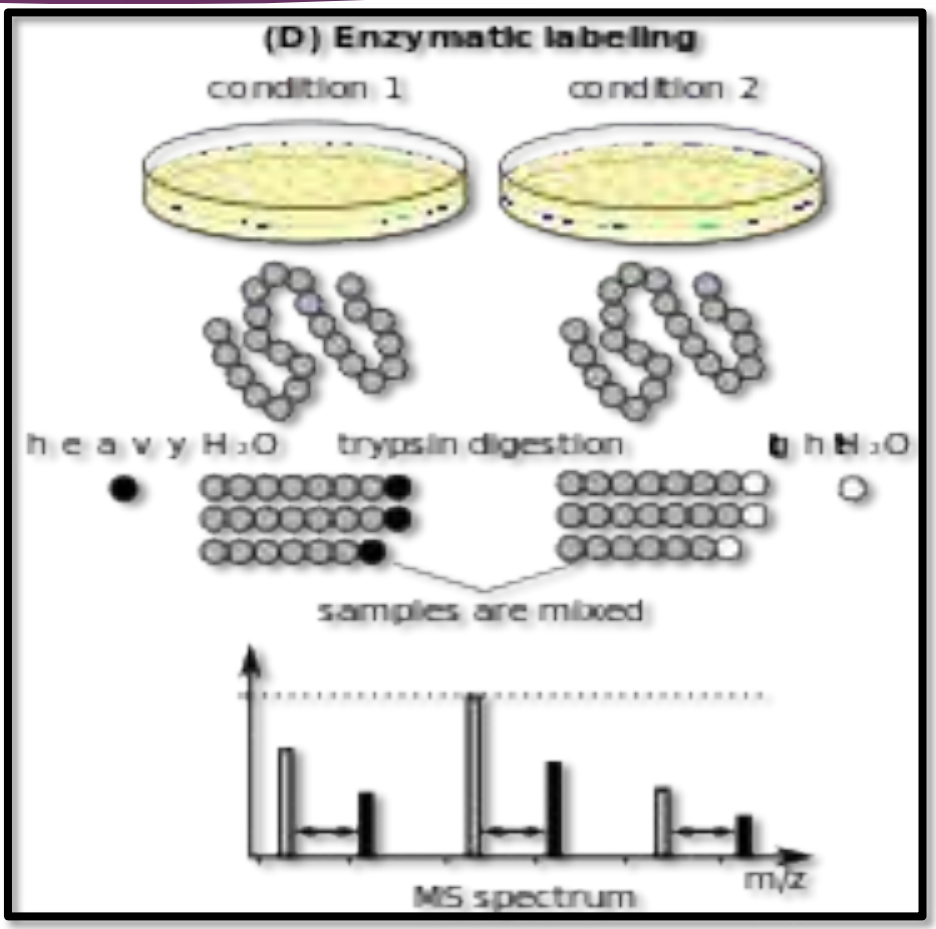


stable isotope labeling with amino acids in cell culture

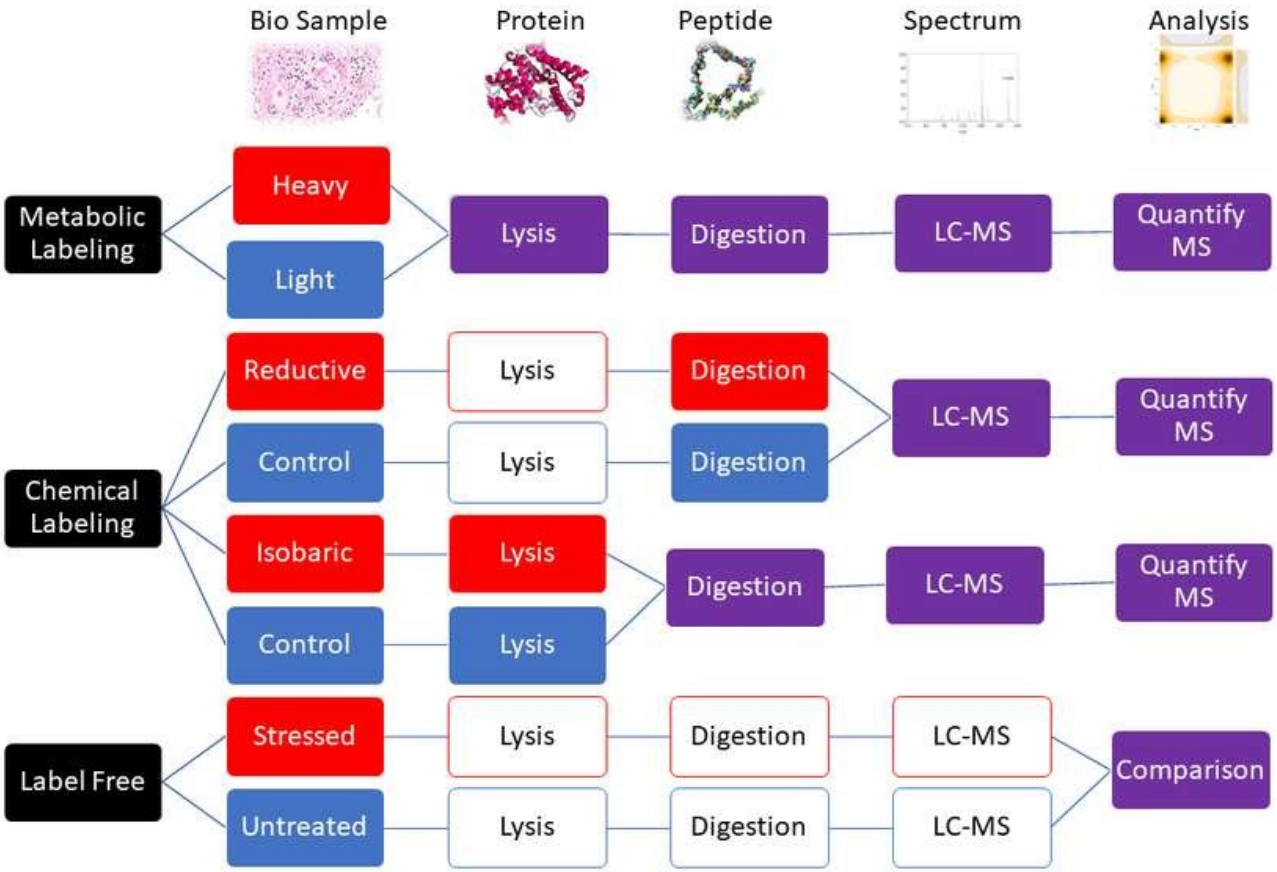


isotope-coded affinity tags





isobaric tags for relative and absolute quantification


Bio Sample
Protein
Peptide
Spectrum
Analysis
Metabolic Labeling
Heavy
Light
Lysis
Digestion
LC-MS
Quantify MS
Chemical Labeling
Reductive
Control
Isobaric
Control
Lysis
Lysis
Lysis
Lysis
Digestion
Digestion
Digestion
LC-MS
LC-MS
Quantify MS
Quantify MS
Label Free
Stressed
Untreated
Lysis
Lysis
Digestion
Digestion
LC-MS
LC-MS
Comparison

CONCLUSIO N

- Using the combination of genomics and proteomics, scientists can now see every dimension of their biological focus, from genes, m RNA, proteins and their subcellular localization.
- This will greatly assist our understanding of the fundamental mechanistic basis of human disease and allow new improved and speedier drug discovery strategies to be implemented.

THANK YOU