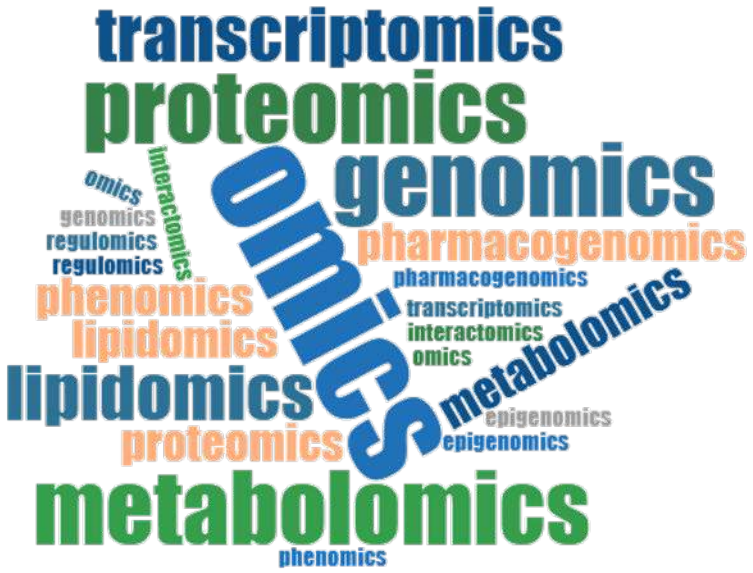
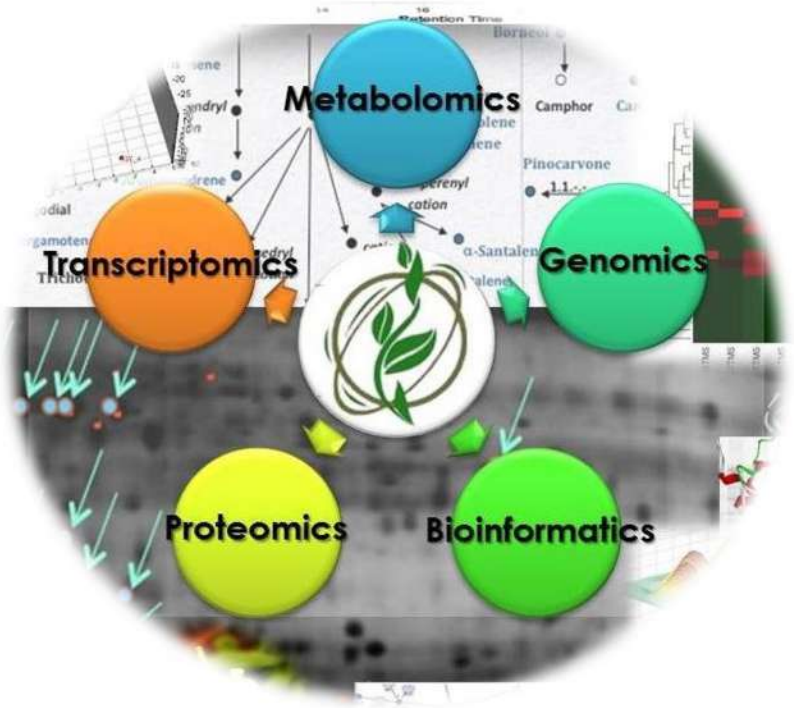


Omic Technologies

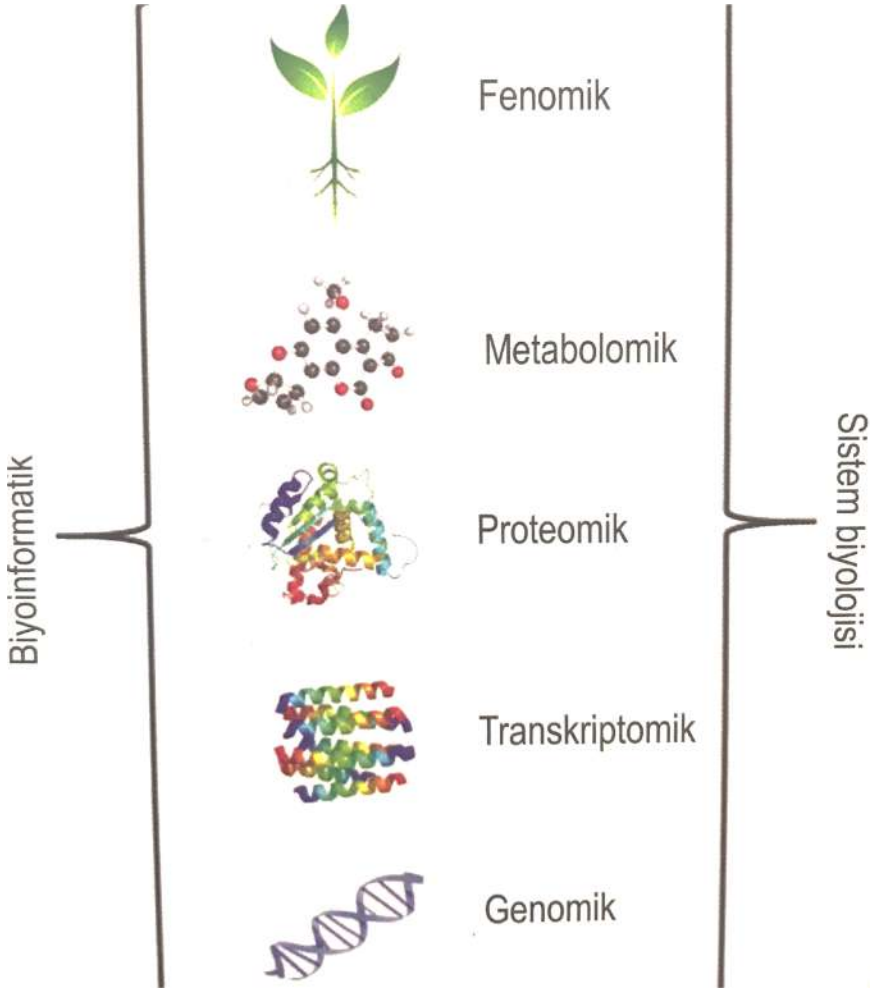
31.03.2020

Assoc. Prof. Dr. İlker BÜYÜK



Omics?

It can be defined as the detection of all functional cellular molecules such as gene, RNA, protein and metabolit in a biological sample with the help of high output technologies.



Omic technologies, which basically seem to consist of genomics, transcriptomics, proteomics and metabolomics, have now turned into a field of study that combines all the information that can be used even at the level of lipidome and phenome under system biology with bioinformatics approaches.

1. Genomics

Genomics is the use of high output technologies to understand the complex biological functions of the genome, that is, the genetic material of the organism. The first stage of genomic studies is to obtain the genome sequence.

Next Generation Sequencing Technologies

The next generation sequencing is performed by three different platforms, which are generally developed by different companies, called **454 / Roche**, **ABI / SOLID** and **Illumina / Solexa**, with their own positive and negative sides.

454 Sequencing Platform

- It was developed by one of the branches of Roche, 454 Life Sciences.

- It performs sequencing with a **pyrosequencing** approach.

- The phylogram results obtained by detecting the pyrophosphate signal released during the nucleotide synthesis performed after the binding of single chain DNA molecules to the beads by the CCD cameras are then converted into DNA sequences.

454 Sequencing Platform

Since the method is based on detecting DNA polymerase activity with a chemiluminescent enzyme, it is called sequencing by synthesis.

It has a long reading capacity that can reach up to 1000 bp.

Reading error rate is the highest and most expensive platform with 1-3 false readings in 100 bp.

ABI/ SOLID Platform

SOLID: Sequencing by Oligonucleotide Ligation and Detection; Sequencing based on the detection of oligonucleotide ligation

Just like the 454 platform, it starts with fixing the sequencing samples prepared by binding DNA particles to the beads to the flow cell.

Unlike the 454 platform, after this stage, ligation is performed with fluorescently labeled probes, not DNA synthesis.

ABI/ SOLID Platform

Fluorescent radiation detected after ligation is converted to DNA sequences by chromatogram

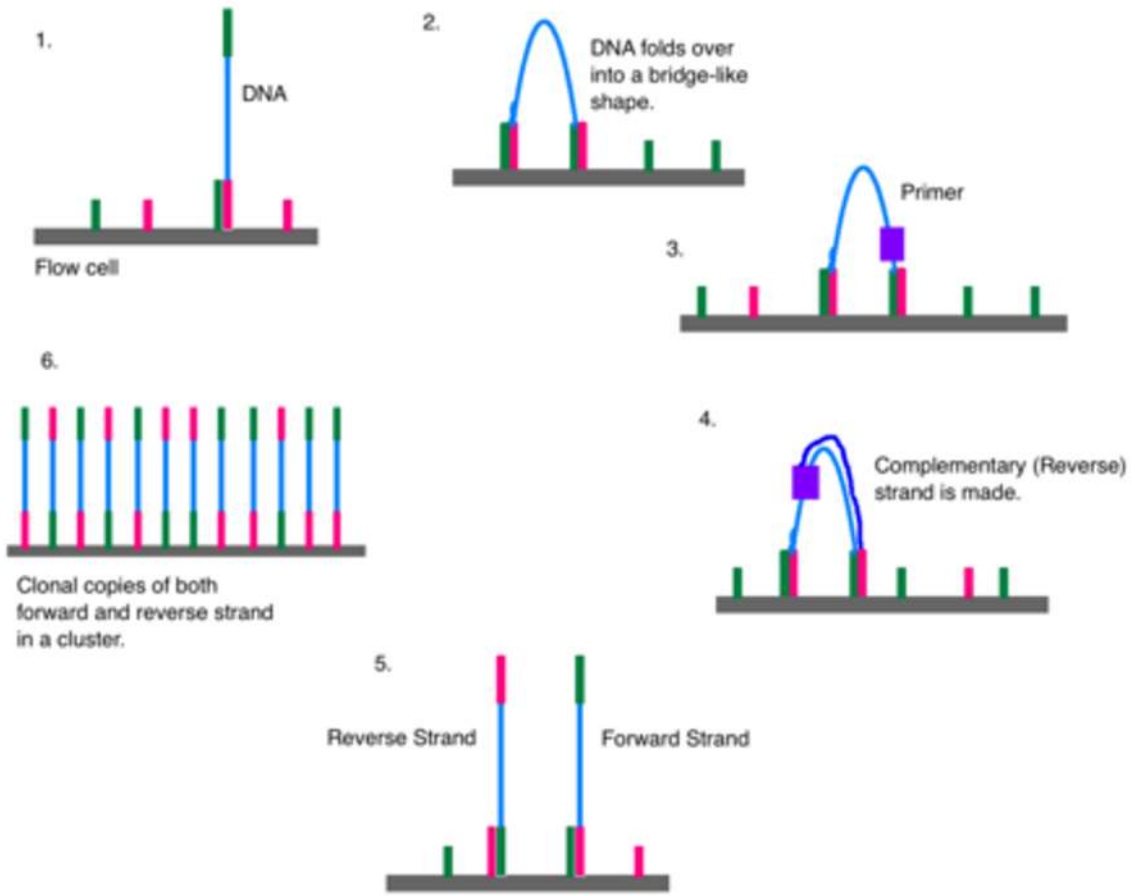
In this respect, SOLID is a sequencing method based on ligation that is carried out through DNA ligase, not synthesis.

A short reading capacity of 75 bases, 1 error reading in 10000 base pairs, Low cost…

Illumina/Solexa Platform

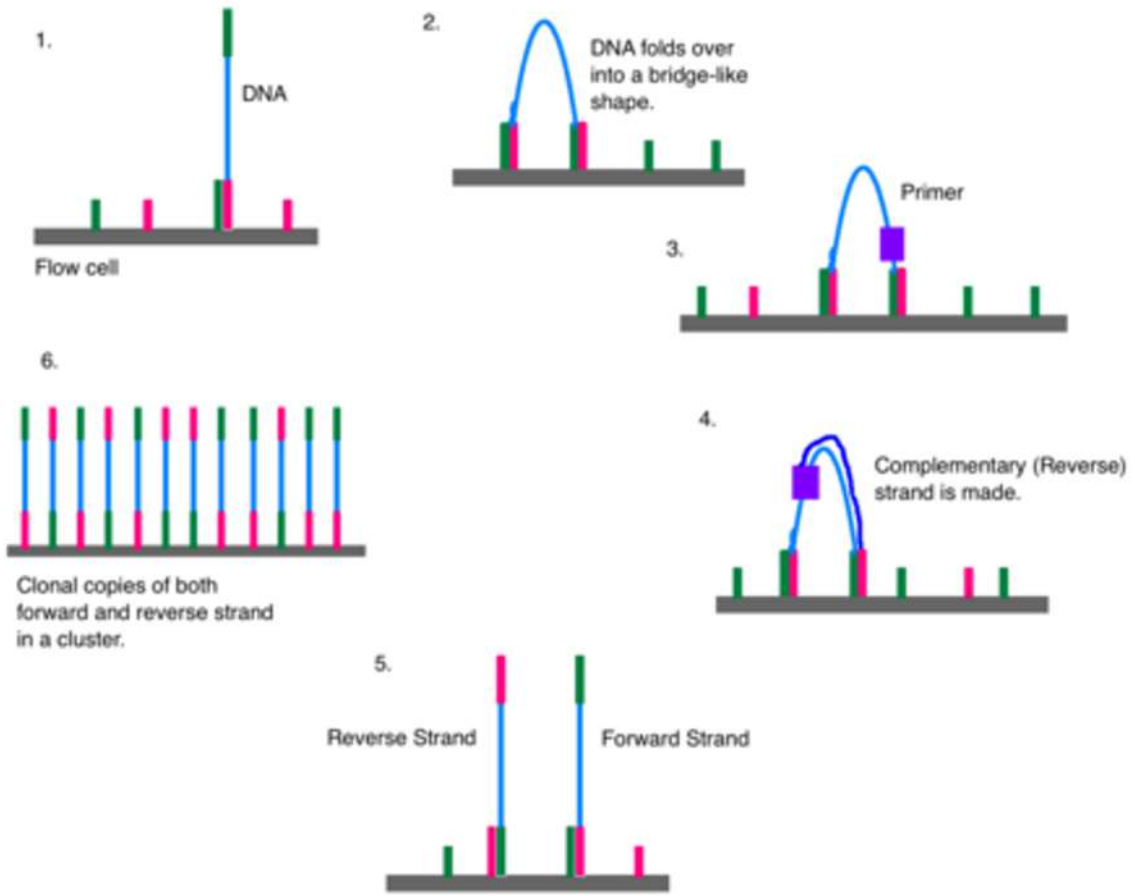
Third Next Generation Sequencing Platform

It works with Sanger chain termination method.



First, genomic DNA is split into small pieces and loaded on a fixed surface that acts as a kind of bridge. Amplification is performed with fluorescently labeled terminator dNTPs (ddNTP) on this surface called flow cell.

The presence of ddNTPs allows the fluorescent radiation read after each cycle to express the nucleotide added to the sequence in that cycle, due to only one base elongation at a time of amplification.



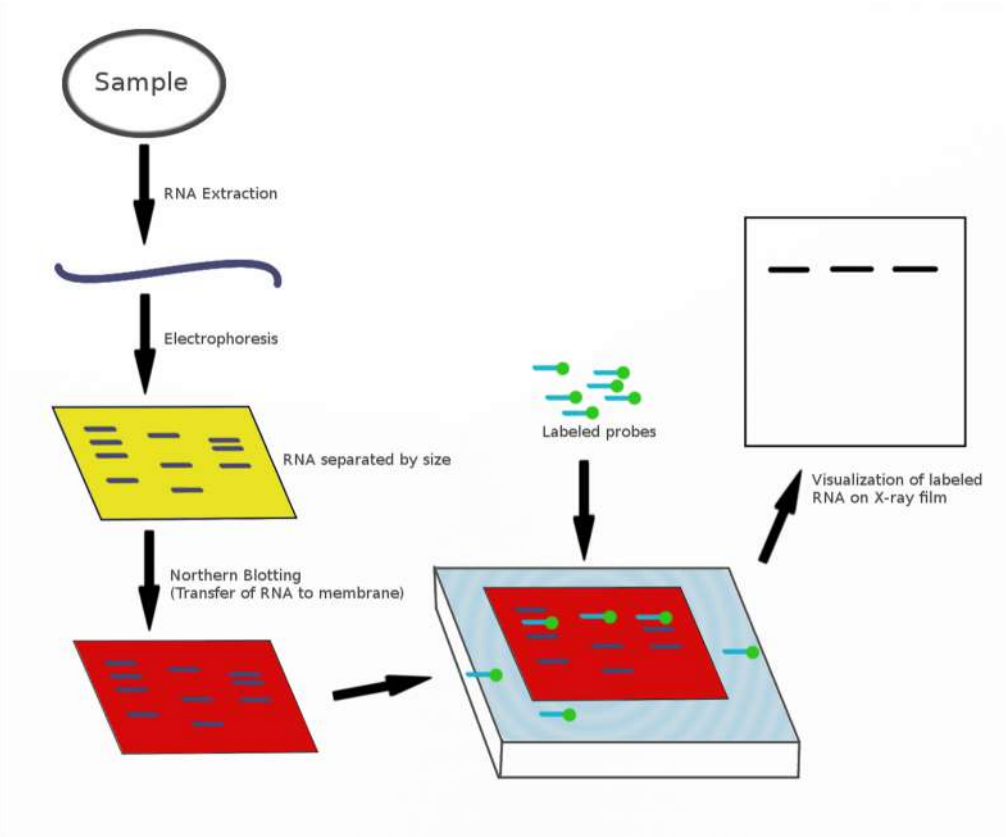
Illumina new generation sequencing platform, based on synthesis sequencing like the 454 platform, has 1 error rate in 1,000 base pairs and the cheapest cost among the existing platforms.

There are also new generation sequencing methods developed by other companies. Ion Torrent from ThermoFisher Scientific, PacBio produced by Pacific Biosciences and **Chip-Seq** provided by different companies such as Illumina and Epigenie can also be counted.

2. Transcriptomics

It is the detailed and / or comparative examination of all RNA molecules in the transcript.

Comparison of gene expression profiles in transcriptomic approaches is actually the implementation of **Northern blotting** technique in a larger scope.



Northern blotting has been replaced by the following techniques in which more data can be obtained;

Differential Display

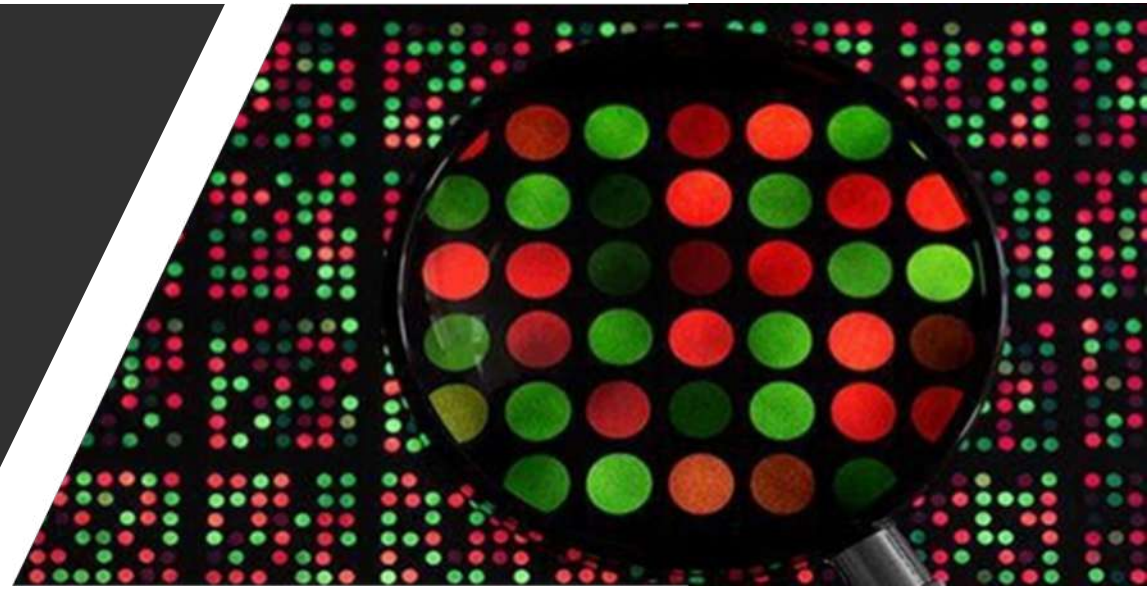
ESTs, expressed sequence tags

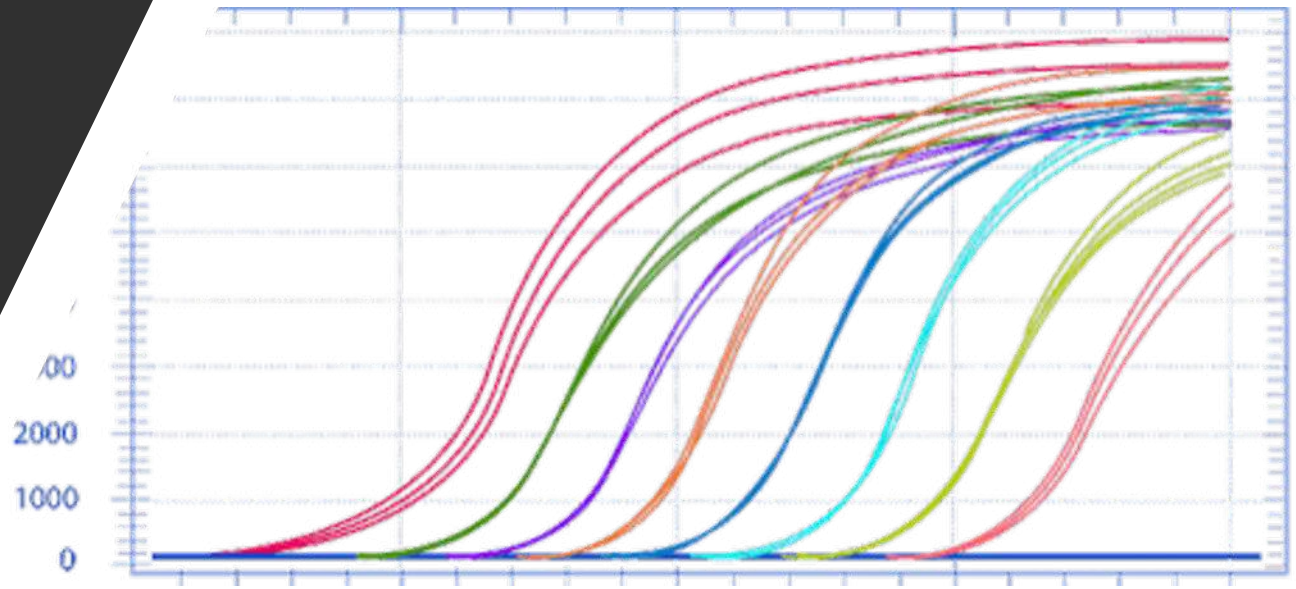
SAGE, serial analysis of gene expression

SSH: Supression Subtractive Hybridization

Microarray

Quantitative PCR (qPCR)



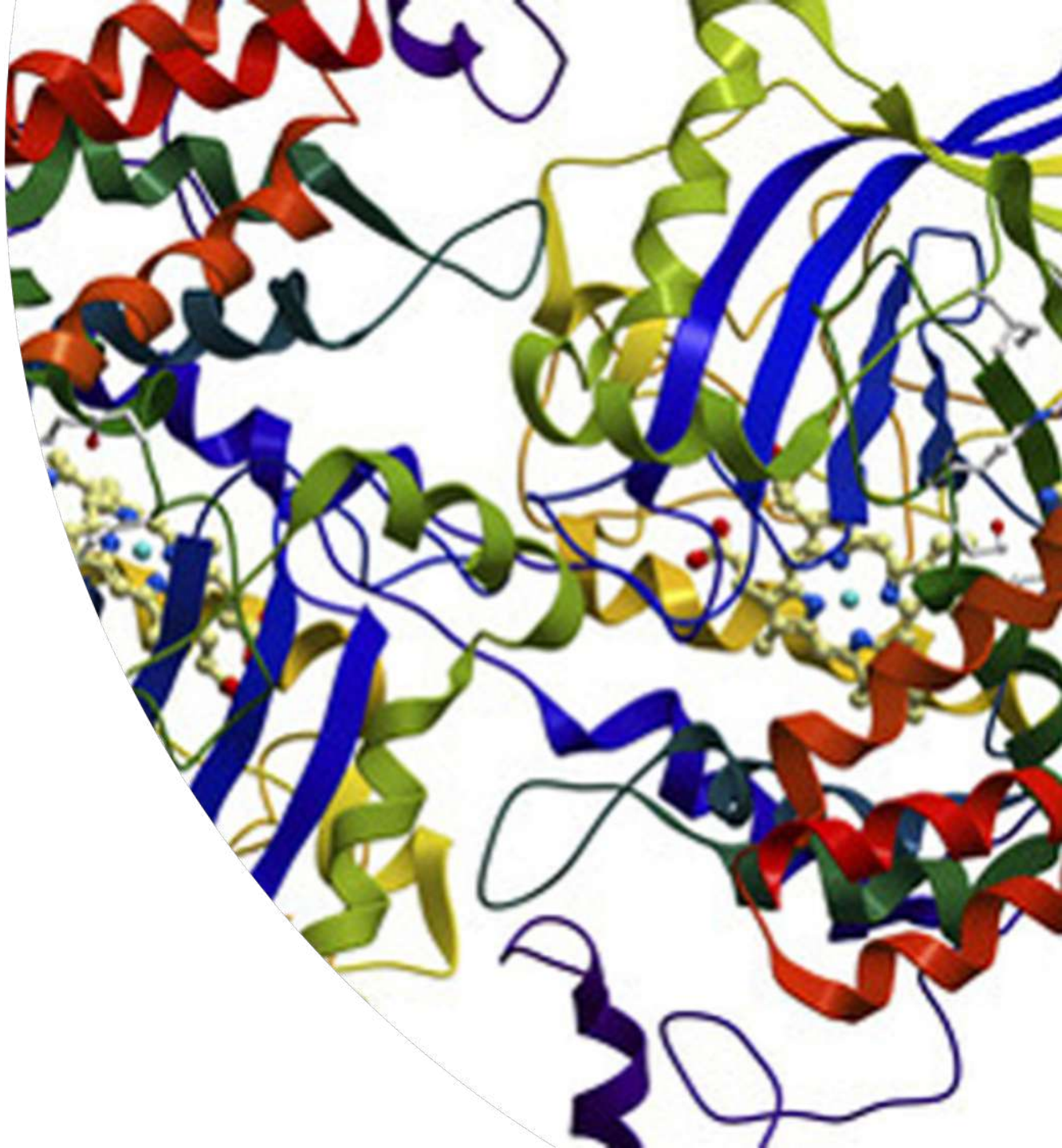


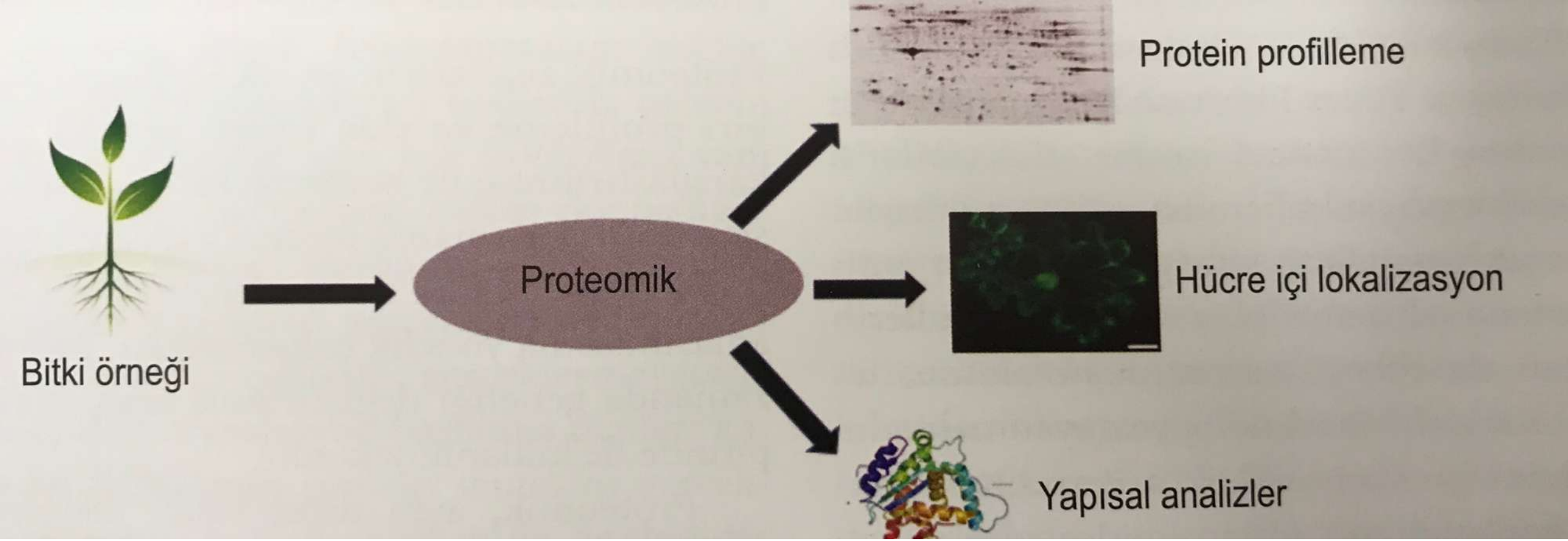
3. Proteomics

Whichever approach is used, transcriptom studies allow the relative calculation of different amounts of mRNA in a cell.

However, the amount of mRNA in the cell does not represent the amount of protein it encodes, nor is there any guarantee that every mRNA, that is, the transcript is converted into a functional protein.

Therefore, it is possible with **proteomics** to accurately detect metabolic activities that regulate the growth, development and interaction with the environment taking place in a cell.





Protemic analysis in plants targets 3 things

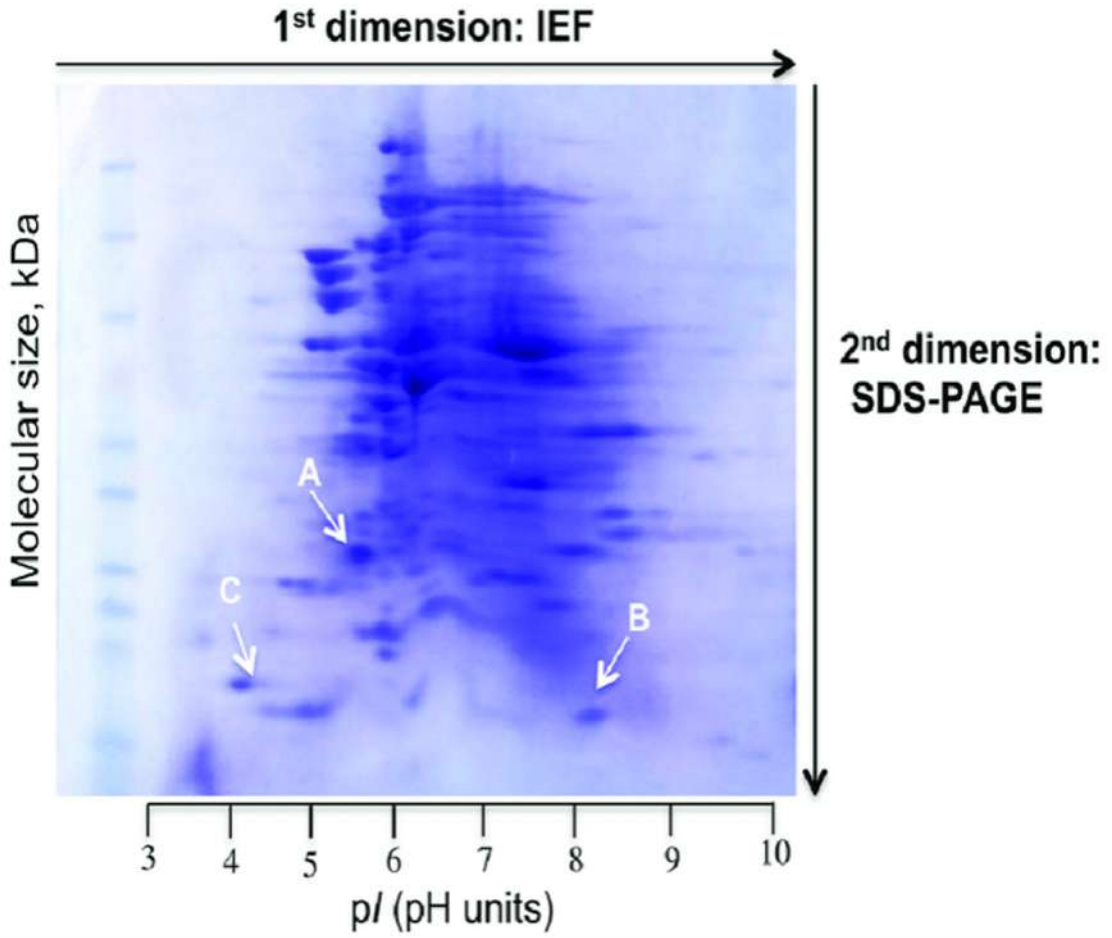
Identifying all the proteins of the plant in certain tissues and their modifications and interactions with each other is a rather difficult task.

Profiling of all of the proteins involved in cellular activities in a cell or tissue, at a certain developmental stage and / or in a certain environmental condition is usually done by electrophoresis-based methods.

No matter how the amino acid sequences or three-dimensional structures are similar, each protein has unique isoelectric point (pI, ph value of which the net charge is zero) and molecular weight.

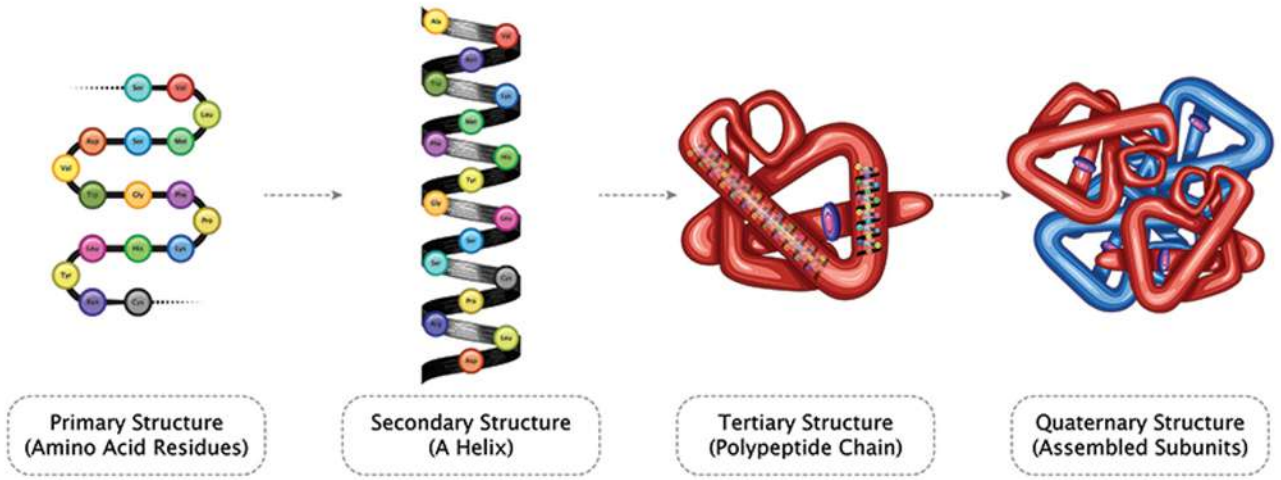
Based on these properties of two-dimensional gel electrophoresis (2-Dimensional Polycacrylamide Gel Electrophoresis, 2D-PAGE), it separates the proteins according to pI value in the first gel.

In the second polyacrylamide-based gel, the proteins walk according to their molecular weight, thereby allowing the proteins in the sample to dissociate at high resolution.

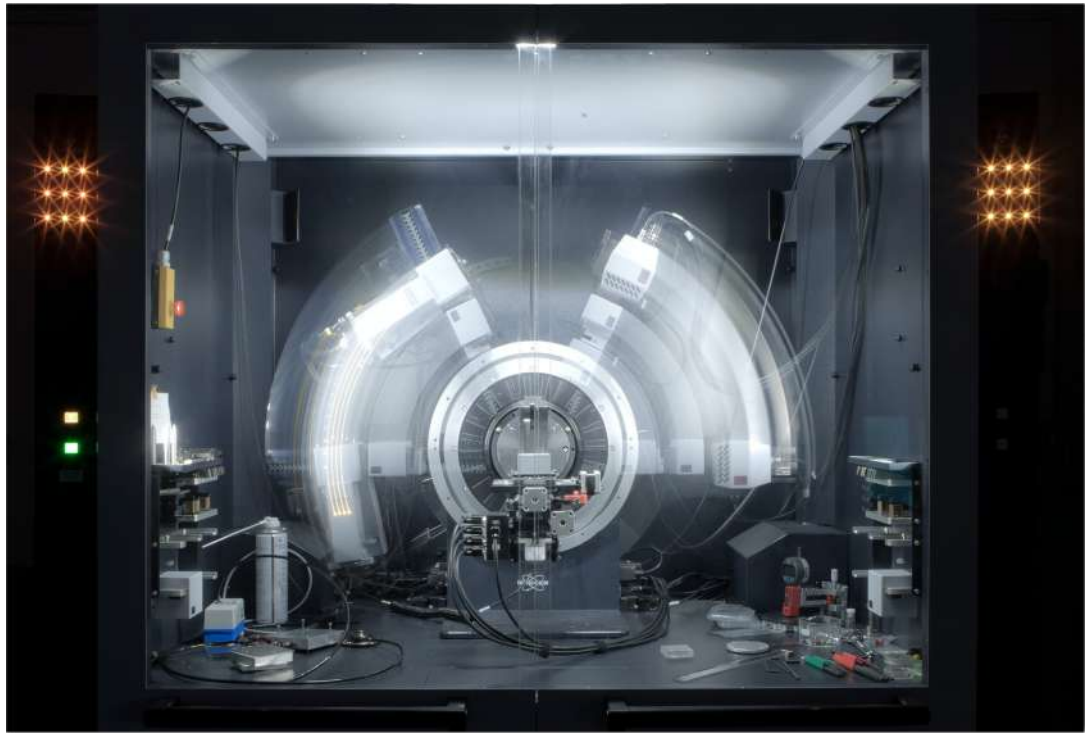


The traditional method of investigating protein-protein and protein-DNA interactions is the Yeast Two Hybrid System (Y2H) system. Although large scans can be performed, it takes time to detect all interactions of a protein in the cell with this method.

Protein microarrays, Affinity Chromatography-Mass Spectrophotometry, Fluorescence Resonance Energy Transfer (FRET) and Surface Plasma Resonance (SPR) are new techniques used to detect these interactions.

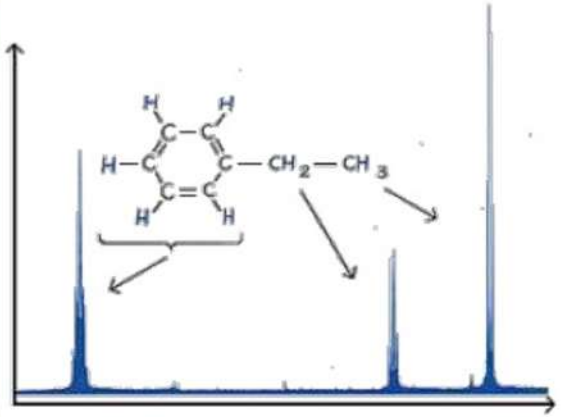


Besides the sequences of proteins and their interactions with other proteins, their three-dimensional structures and their location within the cell are among the things that need to be known for a better understanding of their role in metabolic activities.



X-Ray Crystallography

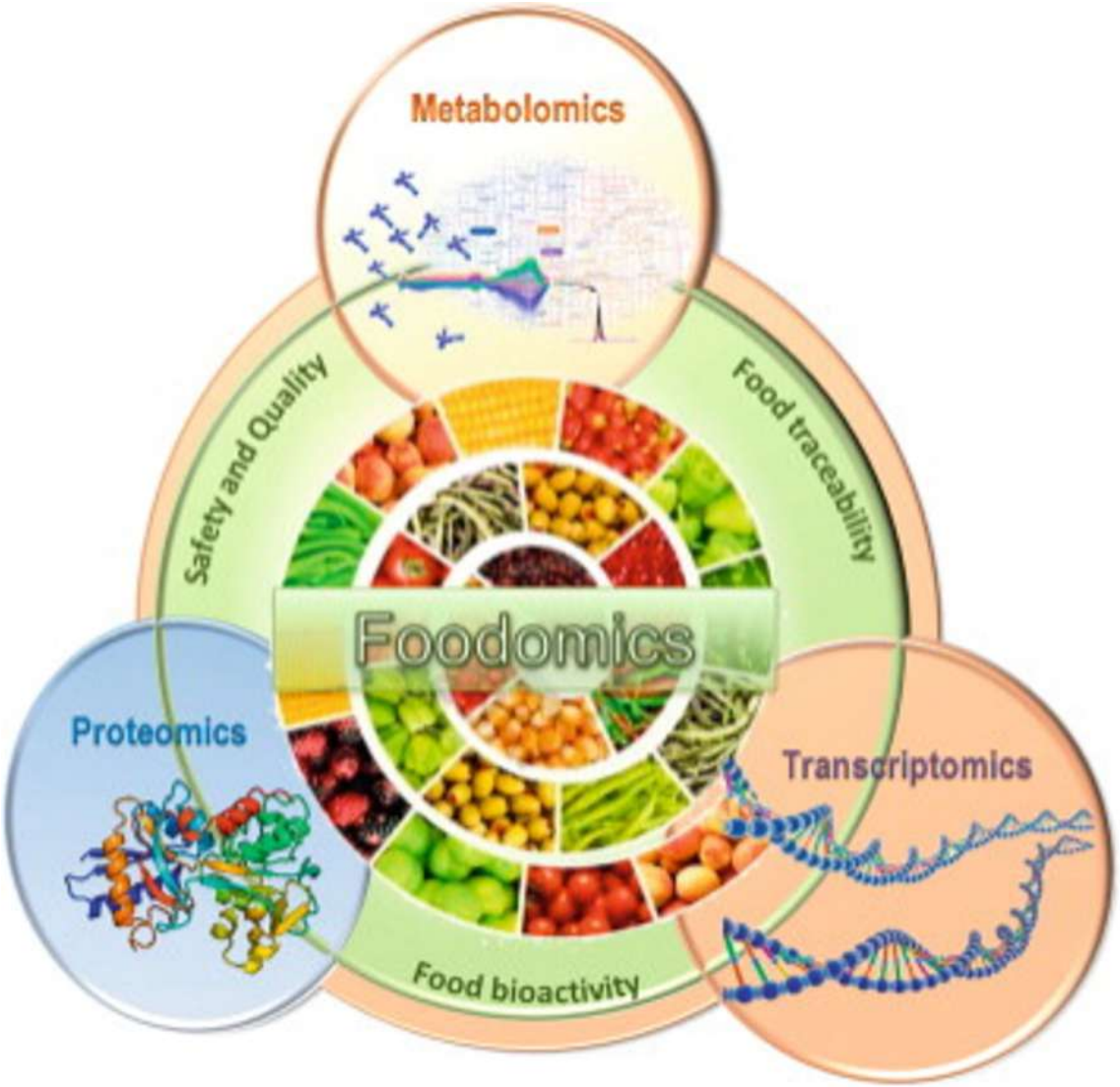
NMR - NUCLEAR MAGNETIC RESONANCE SPECTROSCOPY



A proton NMR spectrum of a solution containing a simple organic compound, ethyl benzene. Each group of signals corresponds to protons in a different part of the molecule.

Nutriproteomics ?

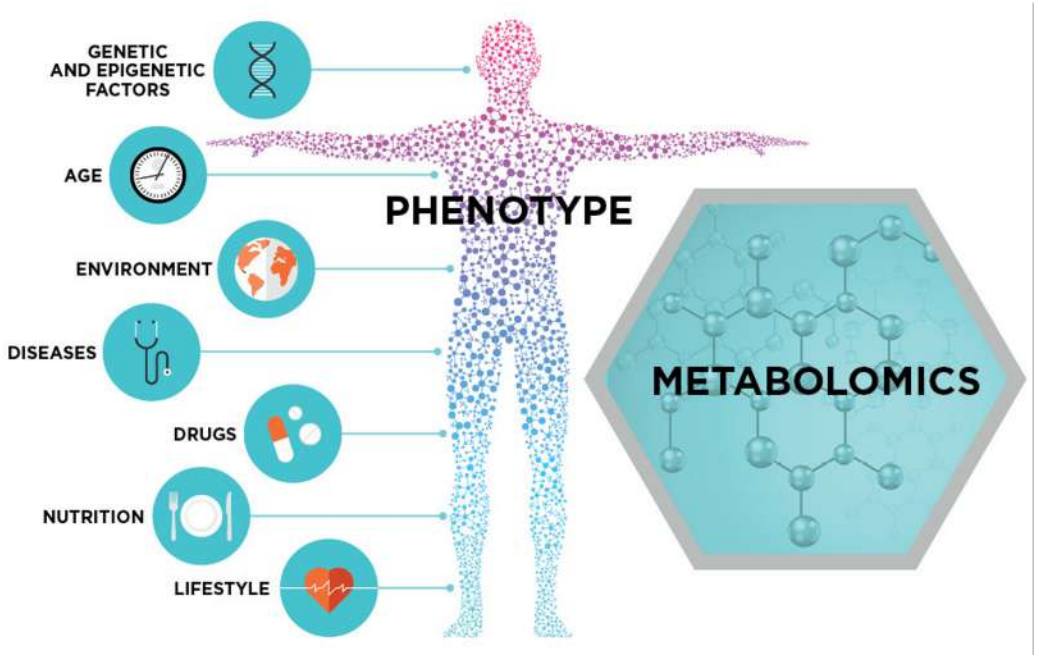
It is a proteomic approach based on the examination of the effects of nutrition on plant health in plants.



4. Metabolomics

Metabolomic, or metabonomic, as used in some sources, is the detailed study of all metabolic intermediates, hormones, signal transduction molecules and secondary metabolites present in a biological sample.

It is one of the most difficult subjects to study among ohmic technologies as it changes very rapidly depending on the growth stage and environmental factors.



Metabolomic Techniques

Metabolomic methods allow the identification and quantification of metabolites known to be included in a sample.

- Metabolom analysis mass spectrophotometry (MS)
- Gas chromatography-mass spectrometry (GC-MS)
- Liquid chromatography-mass spectrometry (LC-MS)
- Capillary electrophoresis-mass spectrometry (CE-MS)
- Fourier Transform Ion Cyclotron Resonance-mass spectrometry (FI-ICR-MS) NMR

devices are used.

5. Phenomics

Phenomic, or field-omics, as it appears in sources from time to time, are actually studies to reveal the role of the environment in the relationship between genotype and phenotype.

In this context, it should be emphasized that the newly applied phenomic approaches have a more important role in plant studies than all other omic approaches.

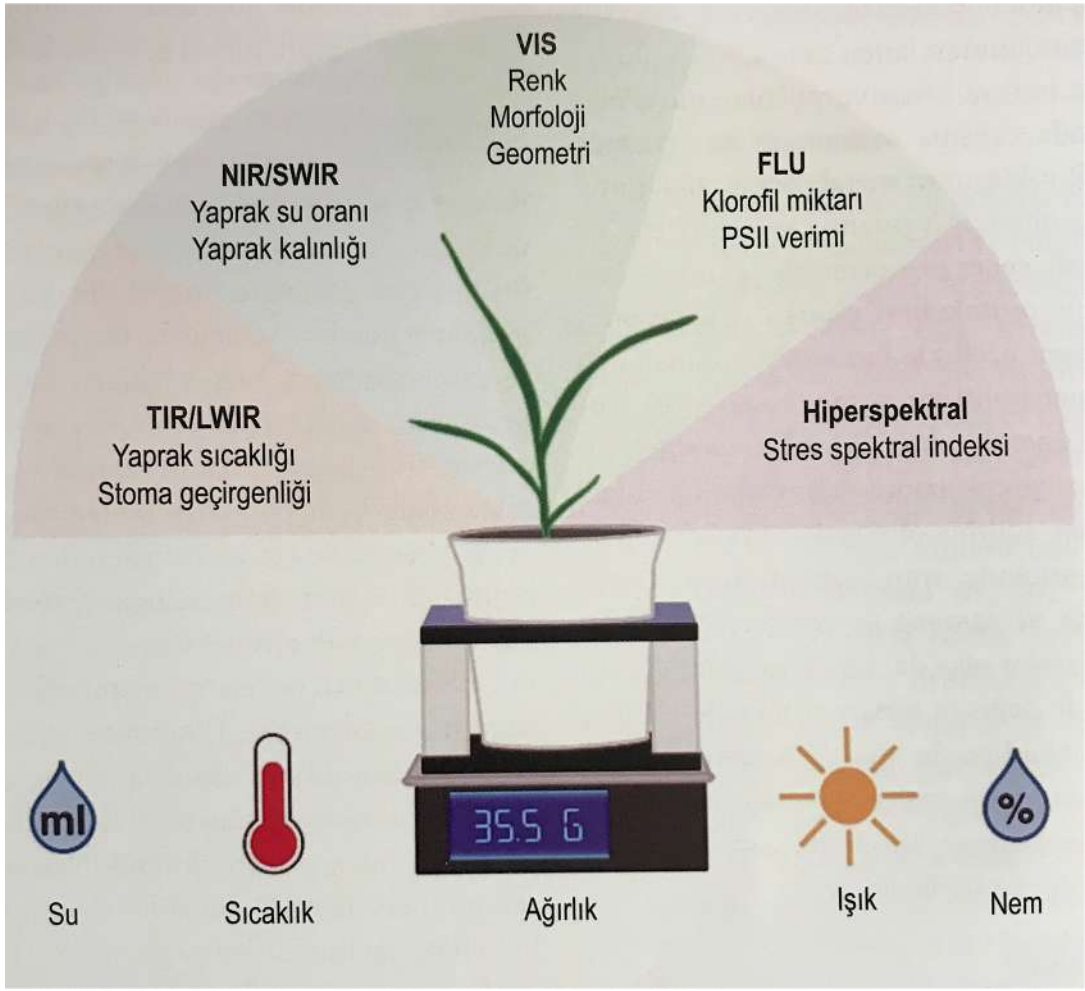
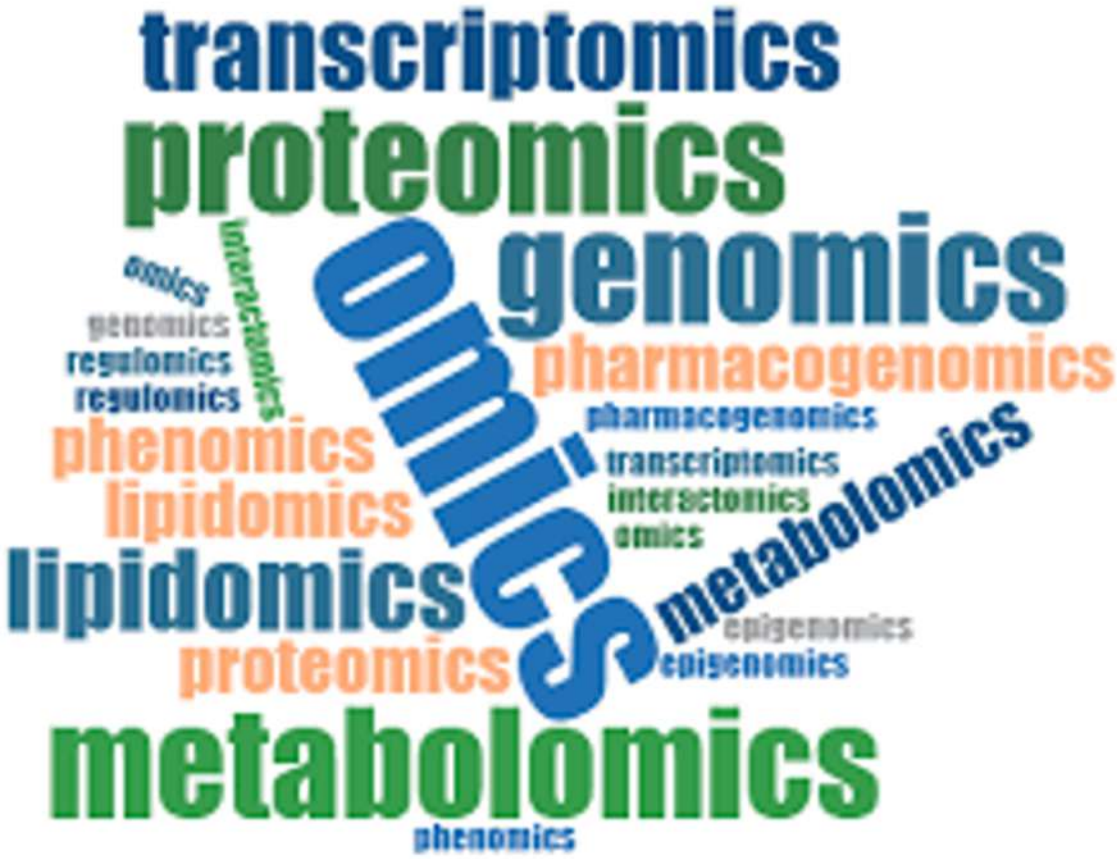


Figure. Data collection methods in phenomic studies conducted under controlled conditions

During the studies, one of the parameters such as the amount of water added to the pot, ambient temperature, light and humidity is changed, the others are kept constant and the phenotypic changes created by this change in the plant are observed**.**

Other omic technologies

- Epigenomic
- Ionomic
- Lipidomic
- Glykomic
- Hormonomic
- Cytomic
- Metallomic
- Ribonomic
- Regulomic



Thanks for listening