معهد بحوث ودراسات البيولوجيا الجزيئية
Molecular Biology Researches & Studies Institute

جامعة أسيوط

PROTEIN ANALYSIS

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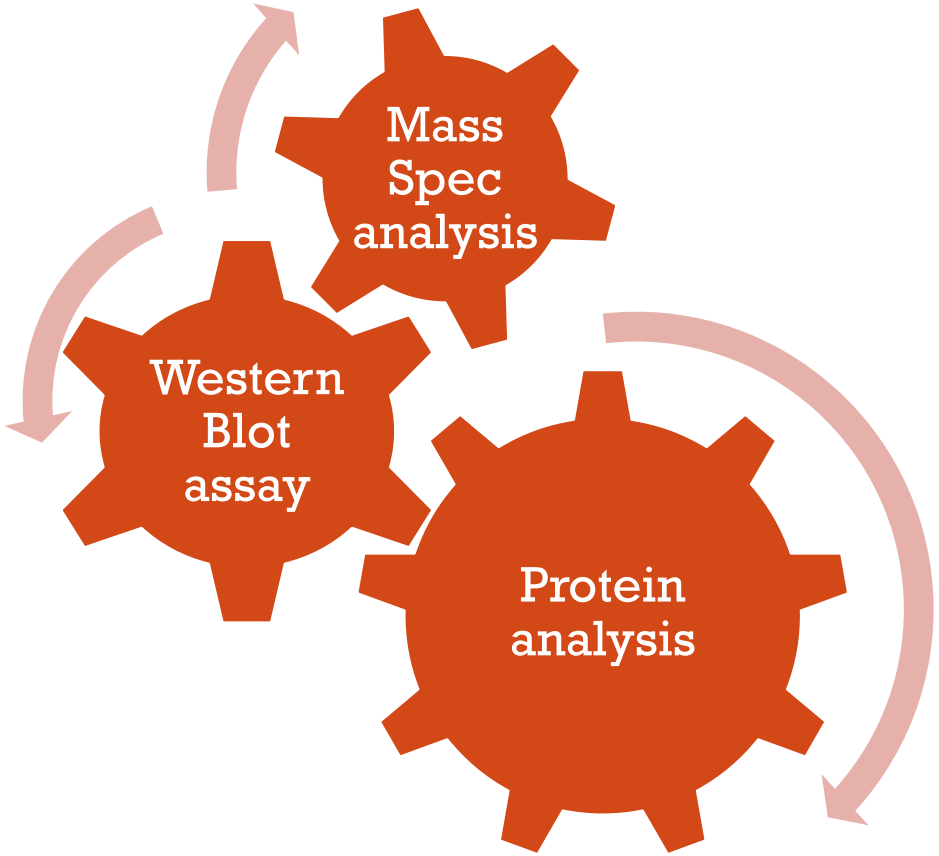
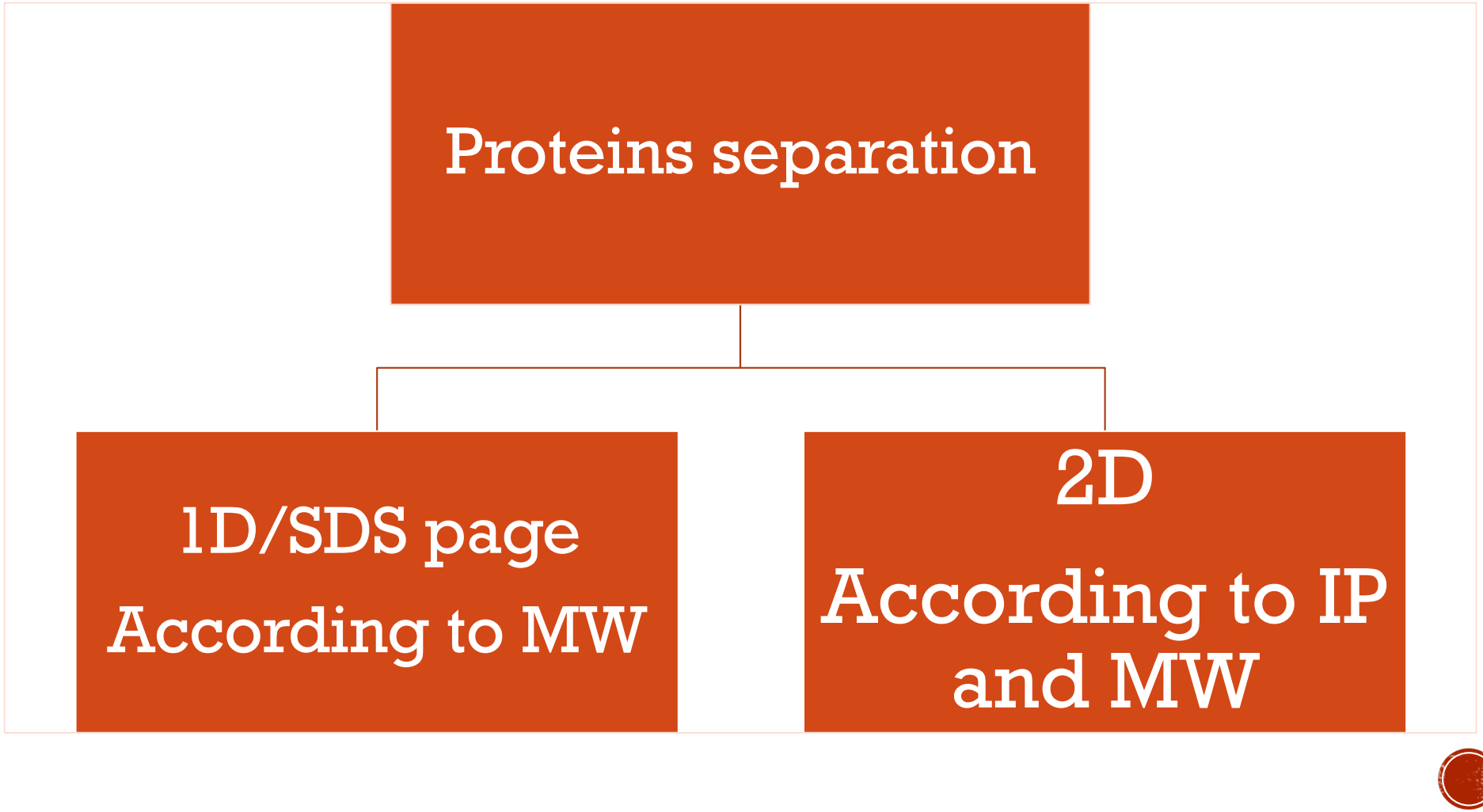

Mass Spec analysis
Western Blot assay
Protein analysis


Proteins separation
1D/SDS page
According to MW
2D
According to IP
and MW

ONE DIMINTIONAL PROTIEN ANALYSIS SDS-PAGE

In this case the protien separtaed according to its Molecular weight

Technique:

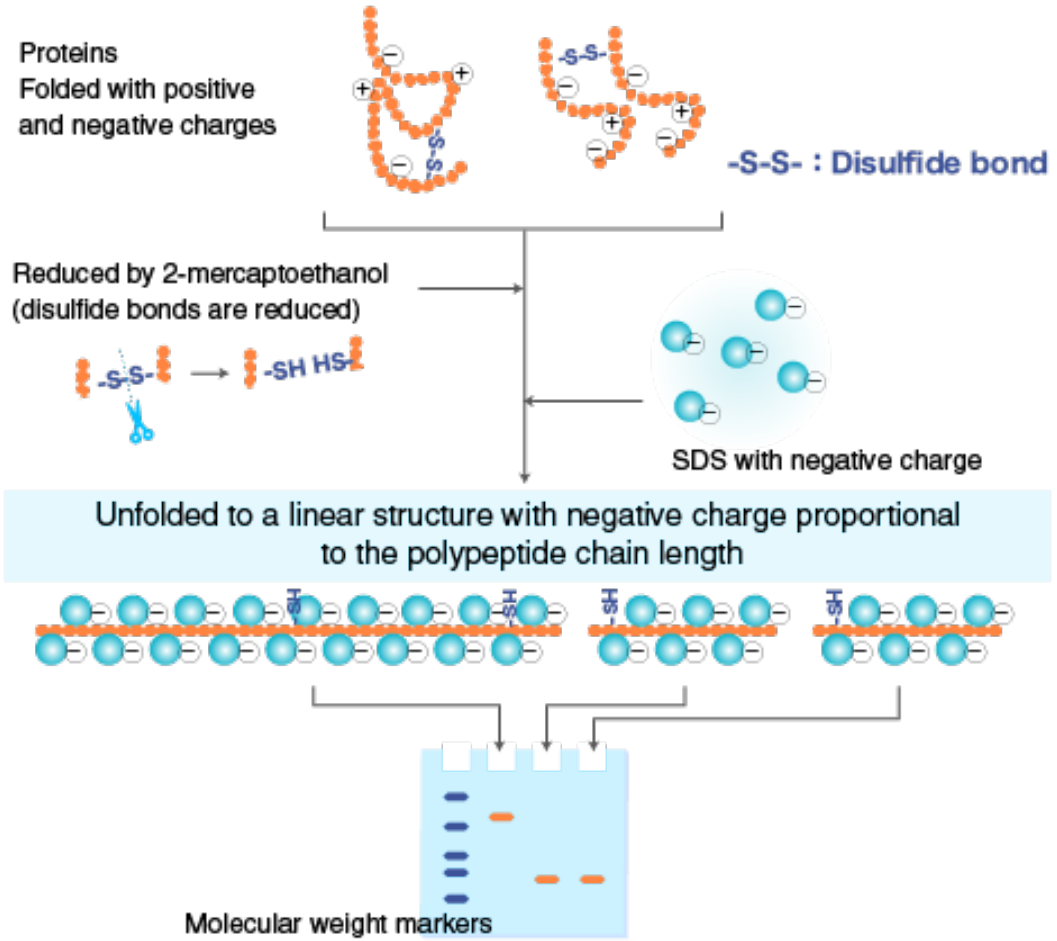
1. Cell lysis to extract protein:

Protein can be extracted from different kind of samples, such as tissue or cells.

After extracting the protein, it is very important to have a good idea of the extract's concentration.

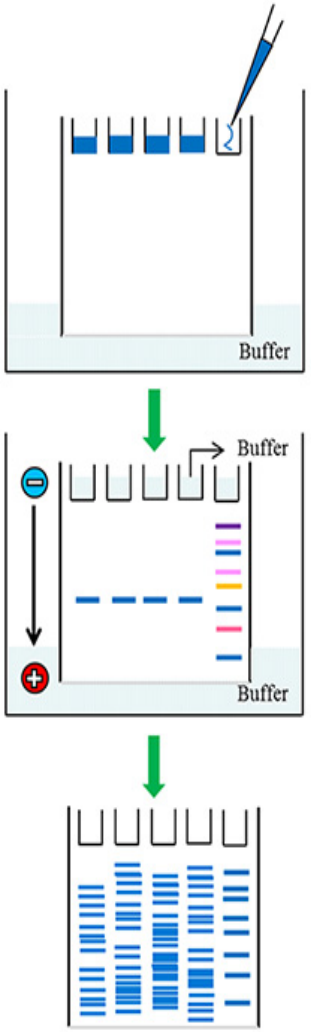
2. Protein preparation with Loading dye:

Incubate the sample with loading dye for 10 min at 100 c to denature the sulfide bridges/bonds.


Proteins
Folded with positive
and negative charges
-S-S-
-S-S- : Disulfide bond
Reduced by 2-mercaptoethanol
(disulfide bonds are reduced)
-S-S-
-SH HS-
SDS with negative charge
Unfolded to a linear structure with negative charge proportional
to the polypeptide chain length
-SH
Molecular weight markers

4. Electrophoresis:

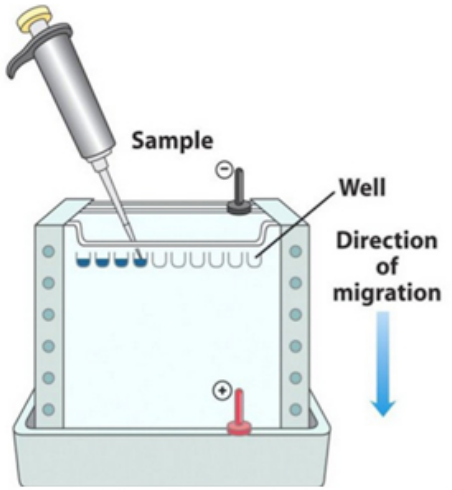
1. Pour the running buffer into the electroporator.
2. Place gel inside the electroporator and connect to a power supply. (When connecting to the power source always connect red to red, and black to black).
3. Make sure buffer covers the gel completely, and remove the comb carefully.
4. Load marker (6 µL) followed by samples (15 µL) in to each well.
5. Run the gel with low voltage (70 V) for approximately an hour, or until the dye fro runs off the bottom of the gel.

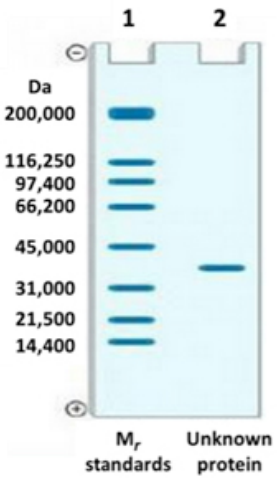


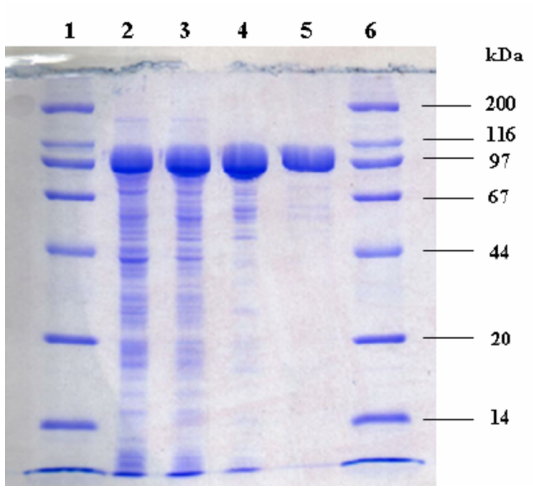
Protein samples and marker loaded in vertical SDS-PAGE system

Direction of migration of samples in vertical SDS-PAGE system

SDS-PAGE gel after Coomassie blue staining



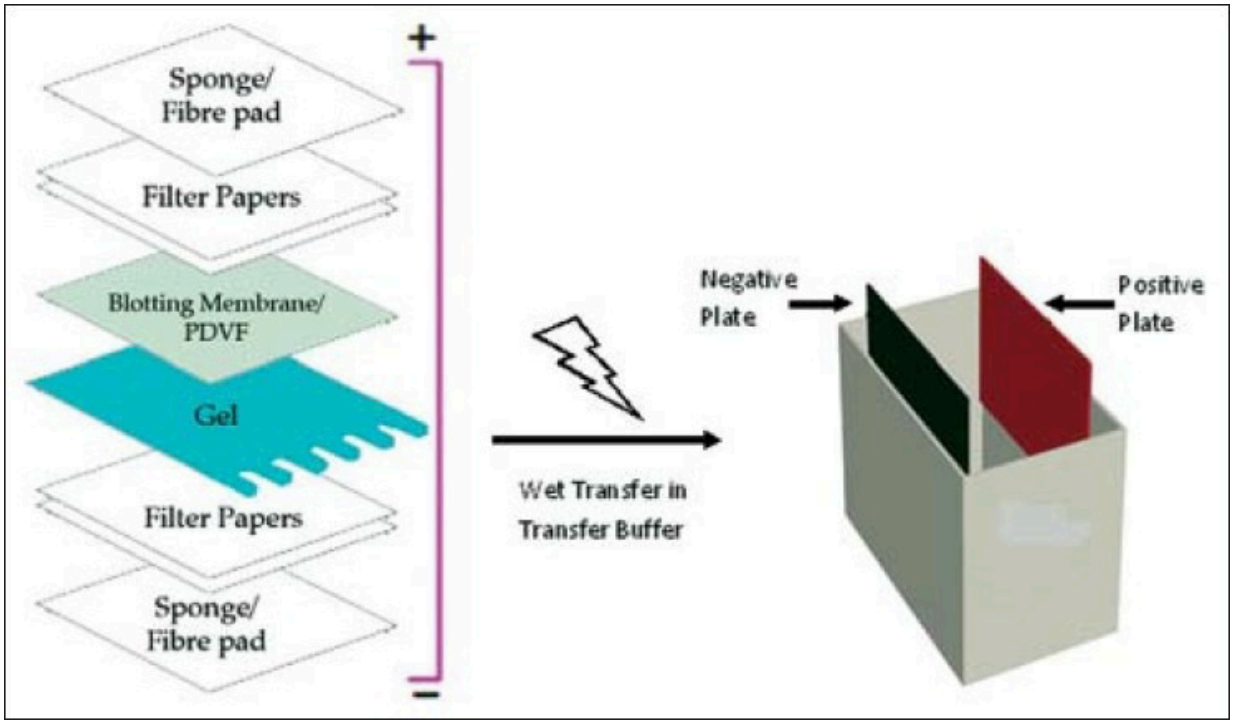




Here I just seperateted protiens according to their MW?

Next step will be identification

Western blot



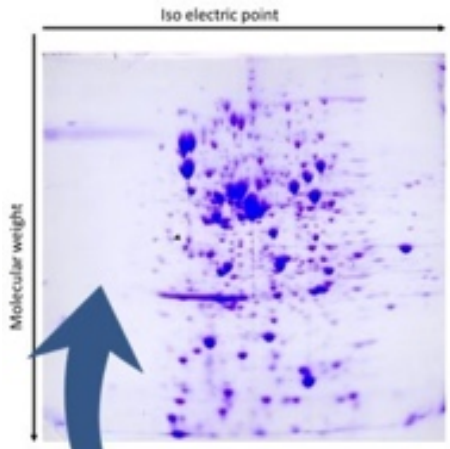
TWO DIMINTIONAL PROTIEN ANALYSIS

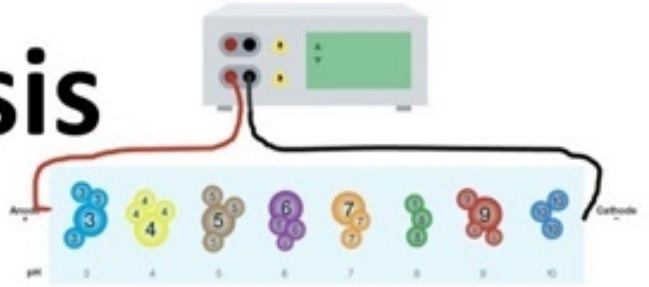
2D gel electrophoresis (2DE):

In the first step, protein is separated into its IEP (Separation of the proteins by isoelectric point is called isoelectric focusing (IEF)).

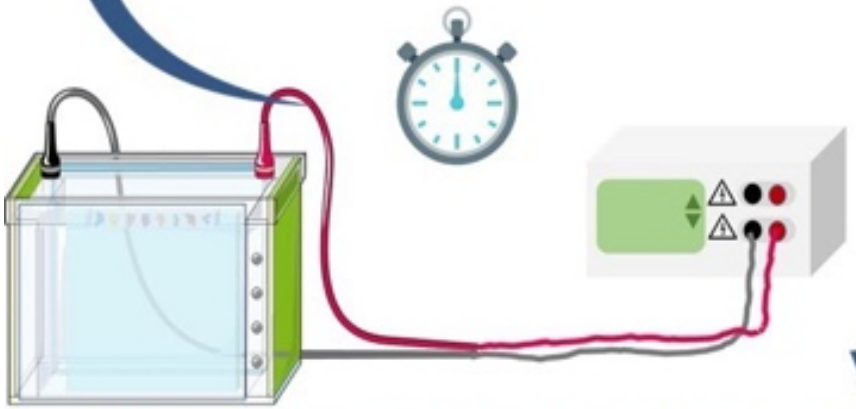
In the second step, the protein is separated according to its mass (MW=Molecular weight).

2D gel electrophoresis





1st dimension is separation based on isoelectric point



2nd dimension is separation based on molecular weight

2D gel electrophoresis (2DE):

- **It is a key technique for purifying individual proteins from complex samples based on their isoelectric points and molecular weights.**
- **It usually coupled with mass spectrometry for protein identification and analyze protein expression patterns.**
- **This technique not only used for full-length protein expression, but expression of modified, splice variant, cleavage product, and processed proteins. Any protein modification leads to a change in overall protein charge and/or molecular weight (MW) will generate a different spot on the 2D gel.**

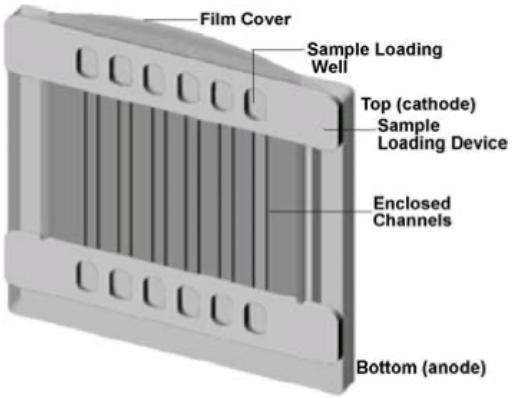
Preperation of strip

1. Protein sample

2. Sample Rehydration Buffer.

3. ZOOM® IPGRunnerTM Mini-Cell

4. • ZOOM® Strips



Rehydrating IPG Strips

1. Prepare your protein sample at a suitable concentration using rehydration buffer, sample in a final volume of 155 µL.
2. Remove the ZOOM® IPGRunnerTM Cassette from the box.
3. Set the ZOOM® IPGRunnerTM Cassette on a level surface with the Sample Loading Wells facing upwards.
4. Load 155 µL of the Sample Rehydration Buffer containing the protein sample into the Sample Loading Wells located at the rounded edge of the ZOOM® IPGRunnerTM Cassette.

5. Remove the Strip card from its pouch and peel a strip away from the card backing using forceps.

6. Each strip has a gel side and a side with printed markings on it. Hold the ZOOM® Strip at the basic end (-) using forceps with the printed-side facing *down* (i.e., gel side up). Using your fingers to guide the strip, gently slide the acidic end (+) of the strip into the sample well at the curved end of the ZOOM® IPGRunner™ Cassette until the acidic end (+) of the strip touches the end of the channel slot (see the following figure). Avoid introducing large air bubble.

7. Seal all of the Sample Loading Wells (including the unused wells) with the Sealing Tape provided in the kit. Make sure that the tape fully seals all wells.

8. Incubate the ZOOM® IPGRunner™ Cassette with ZOOM® Strips for 8–16 hours at room temperature for rehydration.

9. The next day, proceed to Assembling the ZOOM® IPGRunner™ for Focusing (refer to the ZOOM® IPGRunner™ System Manual).

10.Run the isoelectric focusing.

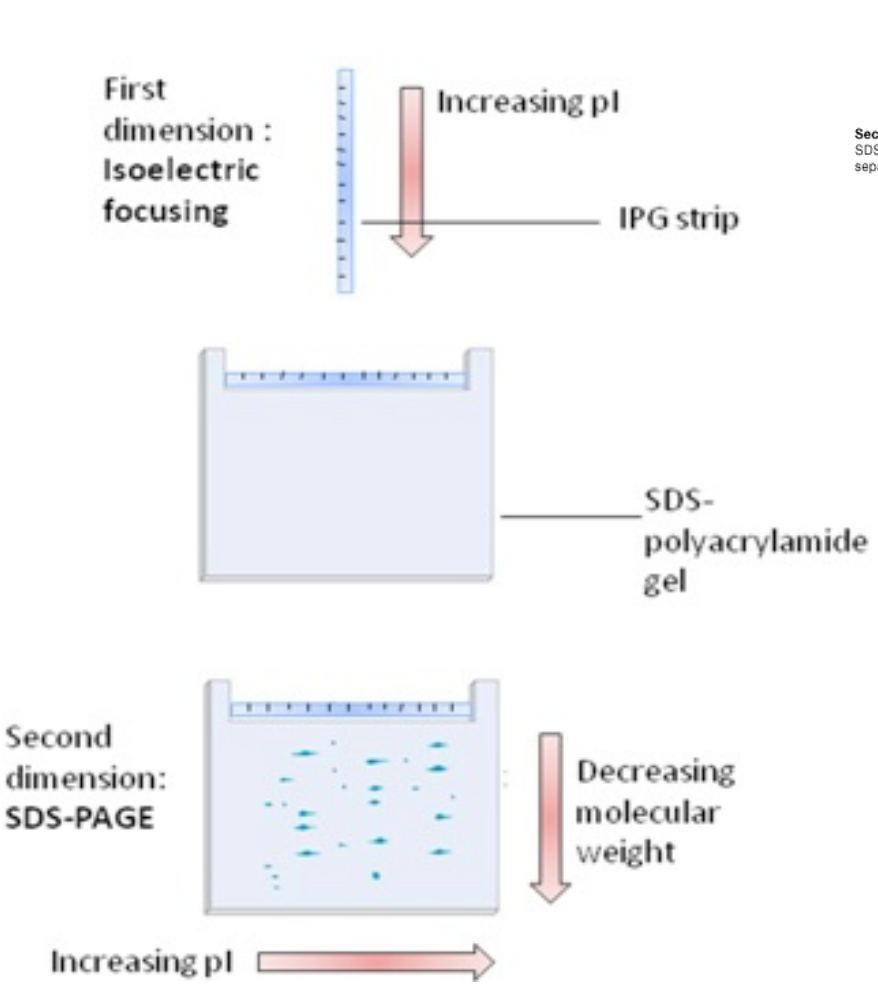
11. After running, remove the assembly, remove the electrode wicks and freeze at -80 in a ziplock bag until you are ready to run the next step.

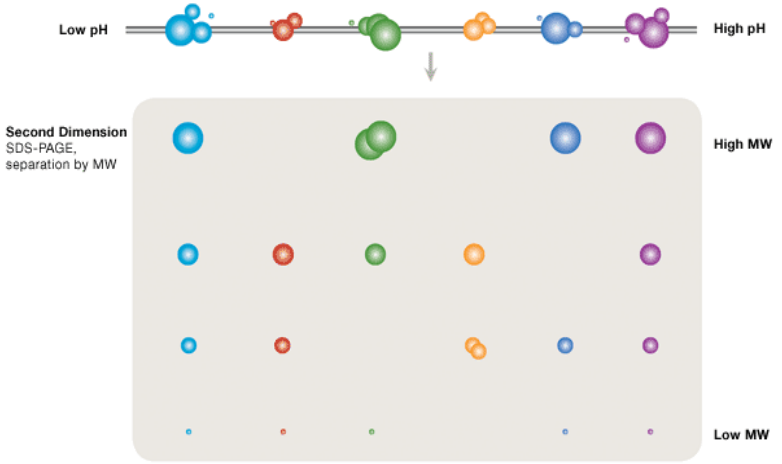
12. Remove strips 1 and 2 by peeling back the plastic only partially for the first 1 and 2. Then put these strips in a container with 20mL of the equilibration buffer for 15 min.

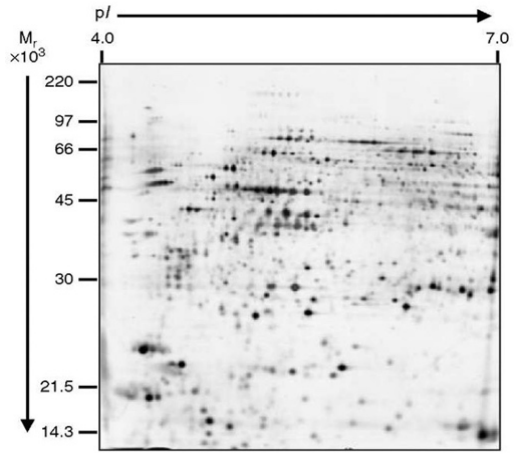
13. After equilibration is complete, microwave the 0.5% agarose in running buffer to get it as a liquid.

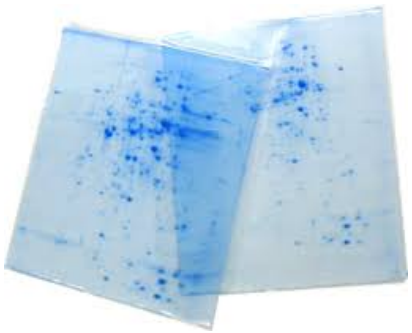
14. Cut the plastic on the positive (+ acidic) end of the strip and insert this WITH GEL SIDE FACING FORWARD on an angle into the 12% Polyacrylamide gel. Then add agarose and be sure it isn't too warm! 15. Use a gel loading tip to gently cut out some of the agarose on the corner furthest from the strip to put a ladder. Run at 125V for roughly 1.75 hours.

2-D Electrophoresis









After the dye front has run off remove the gel from the glass holder and put a small notch on the acidic end to keep track of which side is which and put these gels in 50mL Sypro orange diluted 1/5000 in Transfer buffer covered with foil in dark for 40 minutes then take an image to show that proteins ran nicely

Step 4: Analysis: Three different ways: The final step depends on your particular experimental endpoints: are you comparing protein expression? and/or identifying proteins?

1. **When comparing protein expression across different experimental samples:**

*The gels are typically stained with silver or Coomassie blue for total protein. Various image analysis platforms are then used to scan and compare the location and intensities of the separated proteins.

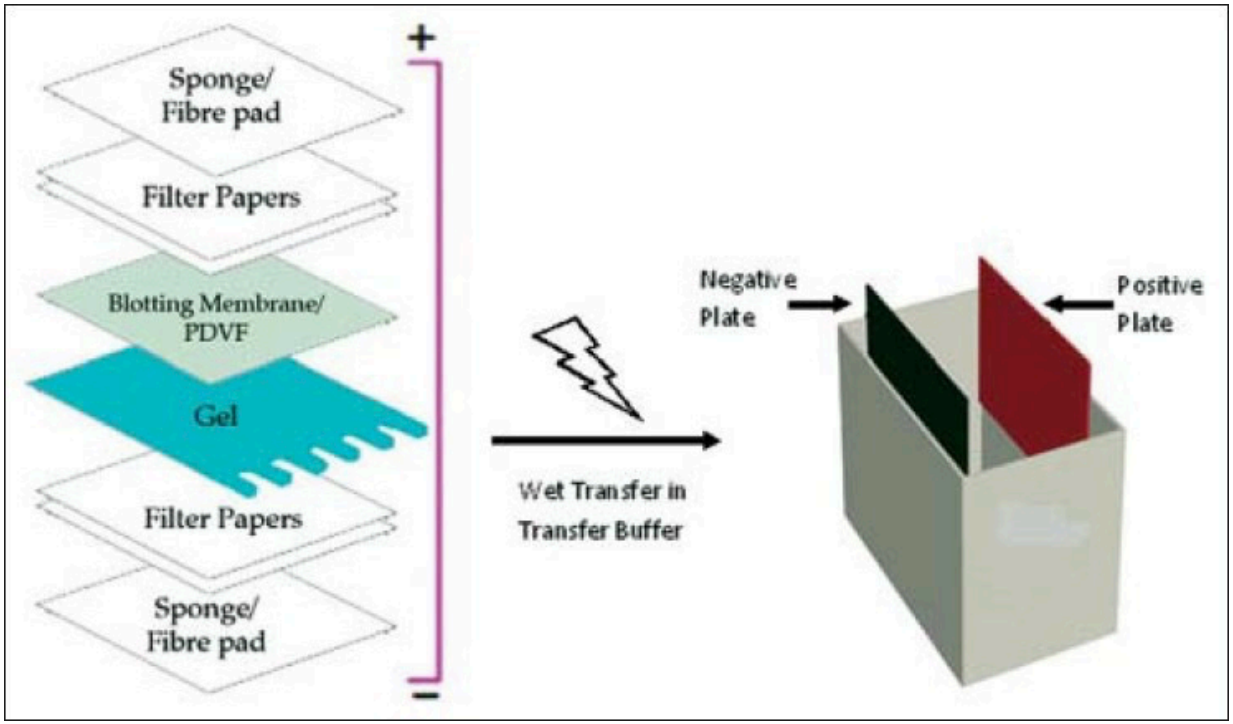
2. Western Blot assay: for identify the protein through using specific antibodies.

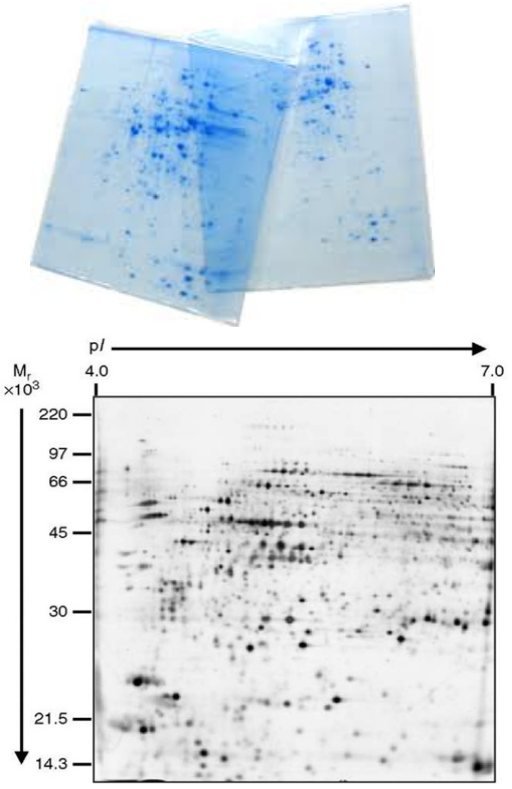
3. Interpreting that Morse-like pattern of dots and splotches requires protein identification and annotation.

**This will be either through in-house efforts or via databases serach of similar samples after Mass spc.

The first and second ways:

Staining /run a western blot, transferring the proteins from the SDS-PAGE gel.





The third approach which is more powerful, laborious and expensive is to excise the proteins in the gel, digest them, and send them for identification by MS.

In this case: Clean-up steps are typically required to remove the detergents and protein stains, which often requires development with a formaldehyde solution that interfere with MS sample preparation.

Note: MS requires typically several hundred nanograms of protein per sample, which means your protein of interest must be visible by the less-sensitive Coomassie blue stain.

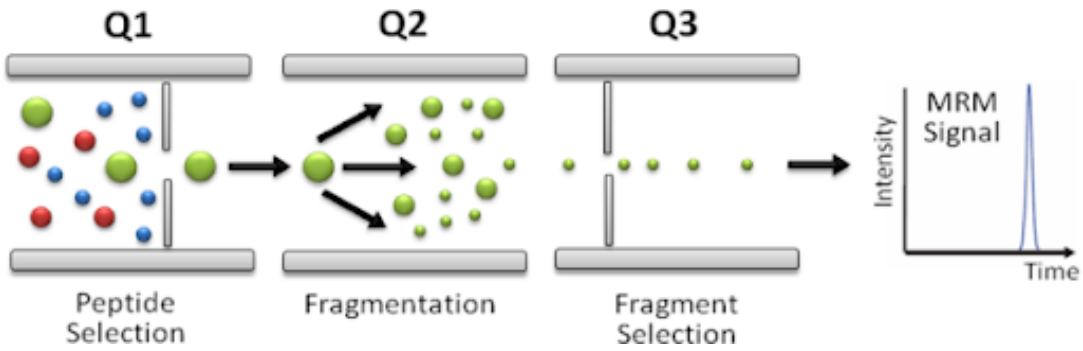
Mass spectrometry (MS):

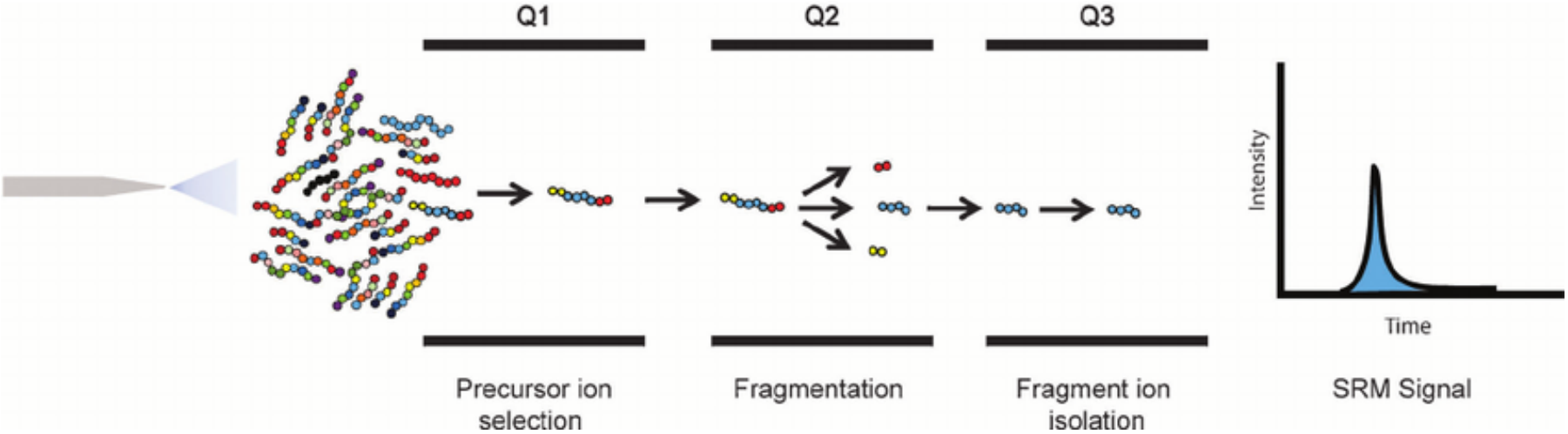
It is an analytical techniques that is used to measures the mass-to-charge ratio of ions.

In MS procedure sample will be ionized, for example by bombarding it with electrons.

This may cause some of the sample's molecules to break into charged fragments or simply become charged without fragmenting.

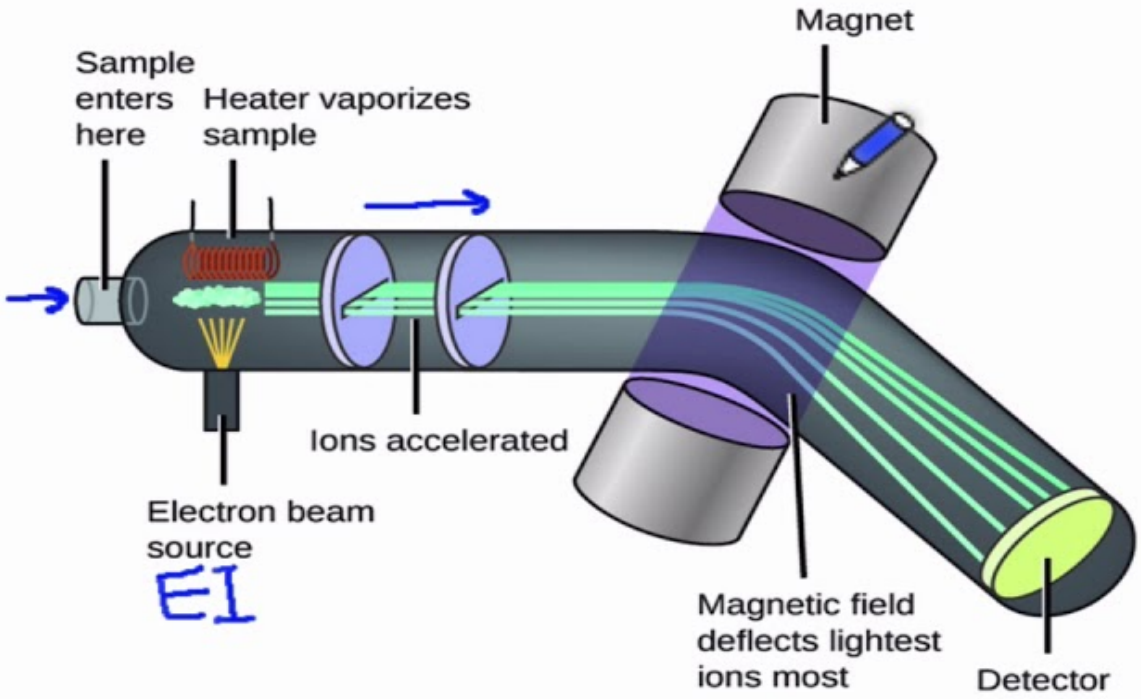
These ions are then separated according to their mass-to-charge ratio, for example by accelerating them and subjecting them to an electric or magnetic field.


Q1
Q2
Q3
Peptide Selection
Fragmentation
Fragment Selection
MRM Signal
Intensity
Time


Q1
Q2
Q3
Precursor ion selection
Fragmentation
Fragment ion isolation
Intensity
Time
SRM Signal

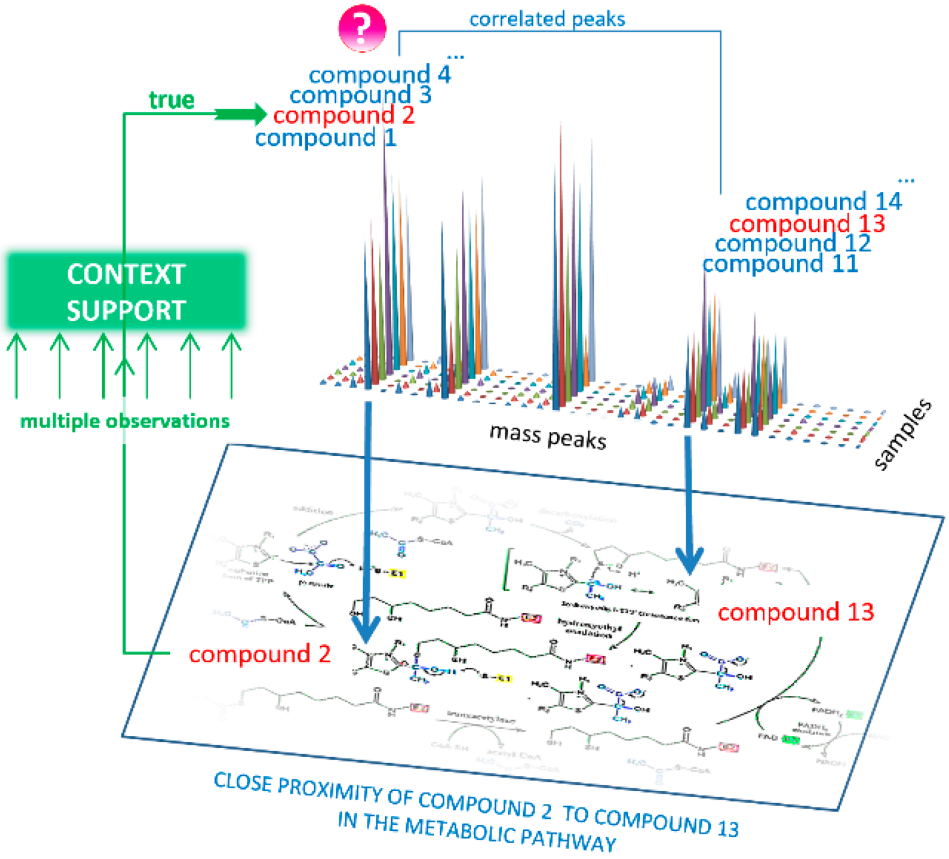
Overview of Mass Spectrometry

In a *mass spectrometry* experiment, the sample is ionized under high-energy conditions to produce the *molecular ion* ($M^{+\cdot}$). This ion fragments into smaller ions, all of which are carried to a detector while separating by *mass-to-charge ratio* (m/z).



Results are displayed as spectra of the signal intensity of detected ions as a function of the mass-to-charge ratio.

The atoms or molecules in the sample can be identified by correlating known masses (e.g., an entire molecule) to the identified masses or through a characteristic fragmentation


correlated peaks
compound 4
compound 3
compound 2
compound 1
true
CONTEXT SUPPORT
multiple observations
compound 14
compound 13
compound 12
compound 11
mass peaks
samples
compound 2
compound 13
CLOSE PROXIMITY OF COMPOUND 2 TO COMPOUND 13
IN THE METABOLIC PATHWAY

THANKS A LOT

WITH MY BEST REGARDS AND MY BEST WISHES