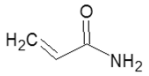
Proteomics

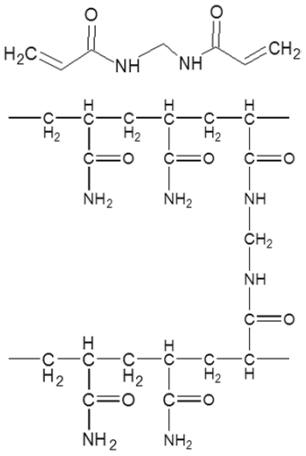
The process of identifying, characterizing, and quantifying all expressed proteins in an organism under one or several conditions.

Electrophoresis

- **Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE)**
- **Isoelectric Focusing (IEF)**
- **2 Dimensional Gel Electrophoresis**
- **Free-Flow Electrophoresis**
- **Capillary Electrophoresis**



SDS-PAGE



- Separates proteins by size
- Denaturing gels
- Resolution dependent on
  - Size of polyacrylamide gel
  - Concentration of acrylamide
    - one concentration or a gradient
  - Stacking of sample
- Stain to visualize proteins
  - Multiple stains available with varying sensitivity
    - Deep Purple, sypro ruby, sypro orange, silver

IEF

- Separation by charge
- pH gradient established by ampholytes
- Gel matrix
  - polyacrylamide strips with immobilized pH gradient.
  - pH gradients in ranges from 3-11 NL to one pH unit (i.e. 6-7) in lengths of 7cm to 28 cm strips
  - Tube gels
- Run times
  - Immobilized IEF 6-24 hours
  - Vertical/tube gels IEF 2-6 hours

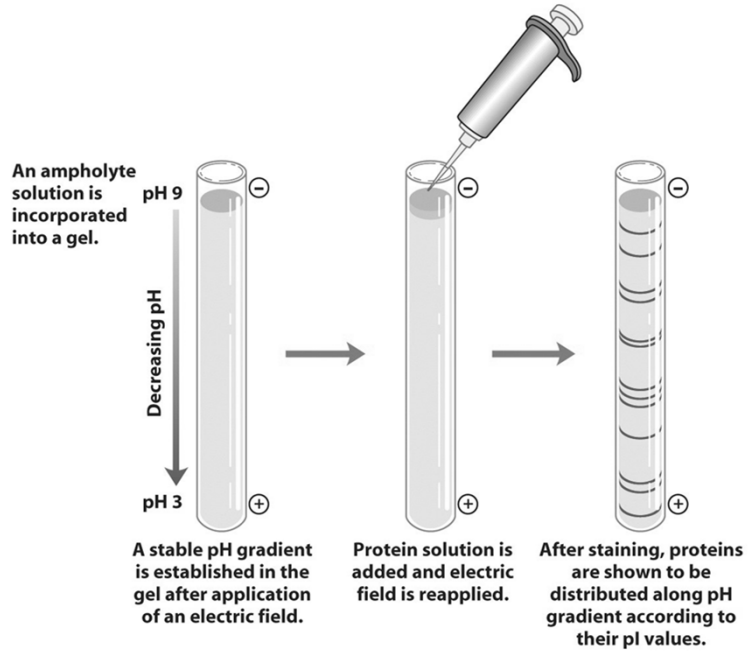
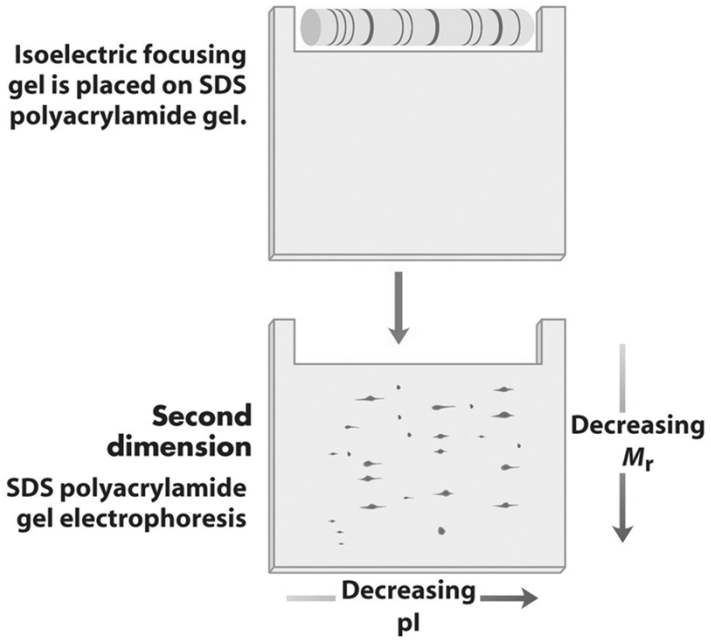


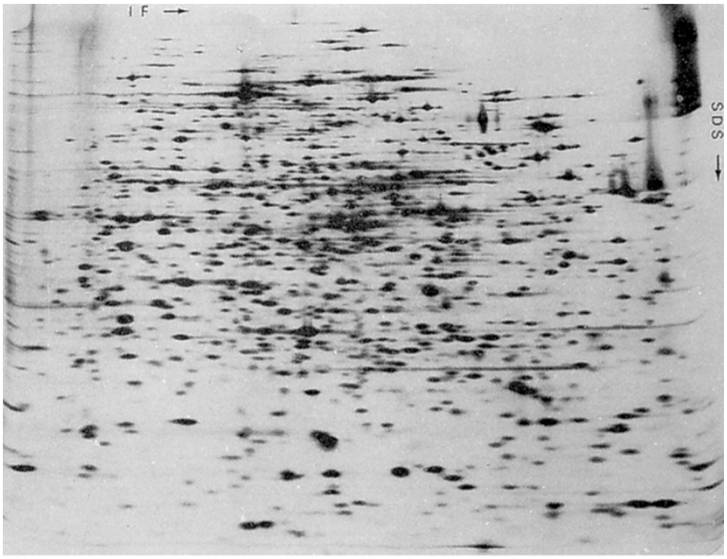
Figure is from "Principles of Biochemistry" Lehninger, Fourth Edition

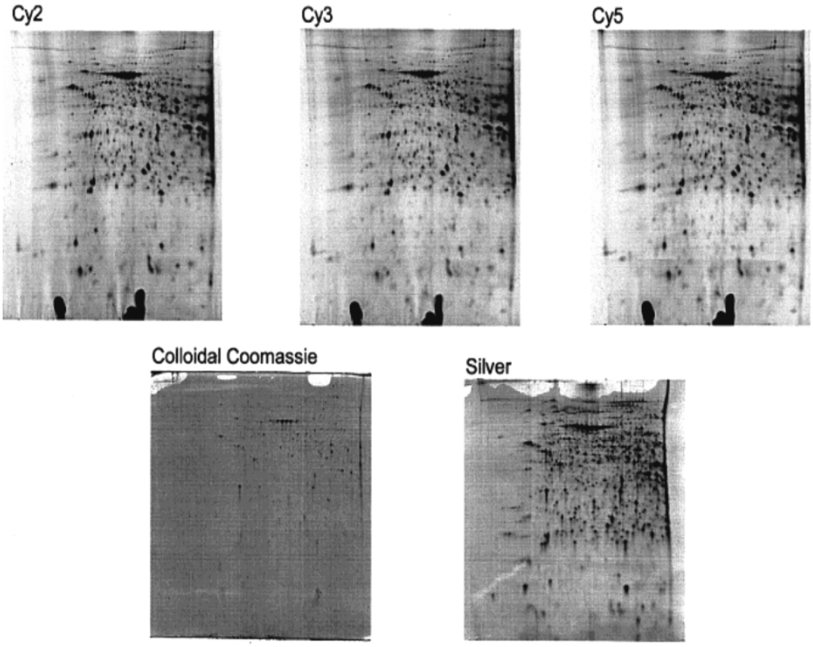
2D gel electrophoresis

- **Perform IEF**
- **Place IEF gel in large well of SDS gel and perform electrophoresis**
- **Stain**
- **Cut out spots to identify by mass spectrometry**

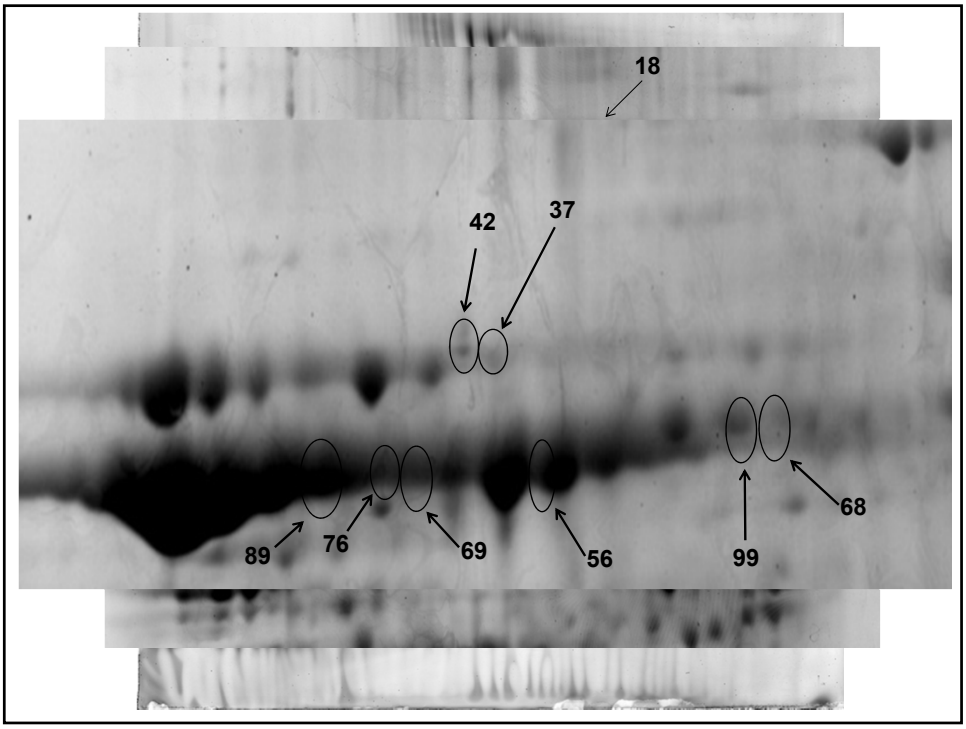


2D Gel Electrophoresis





R Tonge, J Shaw, B Middleton, R Rowlinson, S Rayner, J Young, F Pognan, E Hawkins, I Currie, M Davison (2001) Validation and development of fluorescence two-dimensional differential gel electrophoresis proteomics technology . Proteomics 1:377-396



Spot	Identification	Avg. ratio	T-test	% Cov	Accession #
31	Mitochondrial Chaperonin 60 (Zea Mays)	2.68	0.002	51	AAA33452.1
27	Similar to Heat-shock protein precursor	1.88	0.046	20	NP_001066882.1
242	Not analyzed	1.69	0.022	----	--------
18	Victorin Binding Protein, Avena sativa (glycine decarboxylase P subunit)	1.58	0.040	33	AAA63798.1
37	Dihydrolipoamide dehydrogenase family protein (glycine decarboxylase L subunit)	1.38	0.001	14	NP_001042918.1 AK330954
38	Not analyzed	1.35	0.014	----	--------
76	Serine hydroxymethyltransferase	1.31	0.002	25	AAA33687.1
69	Serine hydroxymethyltransferase	1.28	0.008	23	AAA33687.1
89	Serine hydroxymethyltransferase	1.28	0.044	30	AAA33687.1
42	Chloroplast ATP Synthase α-subunit T. aestivum	1.27	0.007	16	AAA84725.1
42	Dihydrolipoamide dehydrogenase family protein				AK330954
68	heat shock protein Hsp90	1.21	0.042	20	Os12g0514500
99	T-cytoplasm male sterility restorer factor 2 (mitochondrial aldehyde dehydrogenase 2)	1.15	0.031	23	AAG43988
56	Rubisco large sub unit	-1.39	0.022	32	ABR01438
56	Serine hydroxymethyltransferase			23	AAA33687.1

Limitations

- A single protein can make multiple spots so number of proteins less than spots
- Usually see only most abundant proteins
- Separation limited by gel concentration and size
- Basic and membrane bound proteins are not well separated by 2D gel electrophoresis.

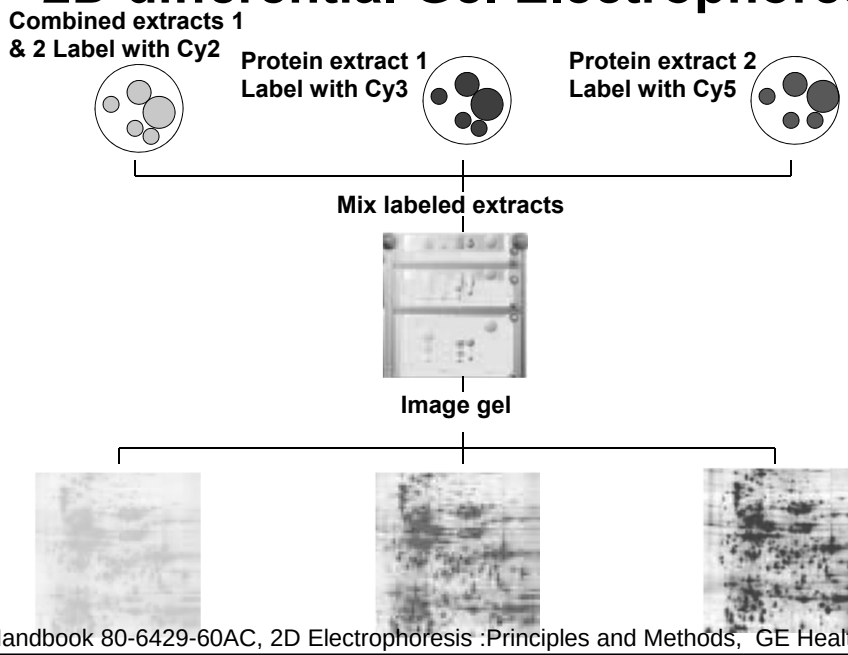
Other 2D Gel Methods

- Blue Native Gel followed by SDS gel
  - Used for organelles such as mitochondria and chloroplasts
  - Keeps electron transport complexes together during native gel process
- Non denaturing followed by denaturing
  - Can allow for complexes to move together
  - Then separates subunits of complexes
- Differential gel electrophoresis

Differential Gel Electrophoresis

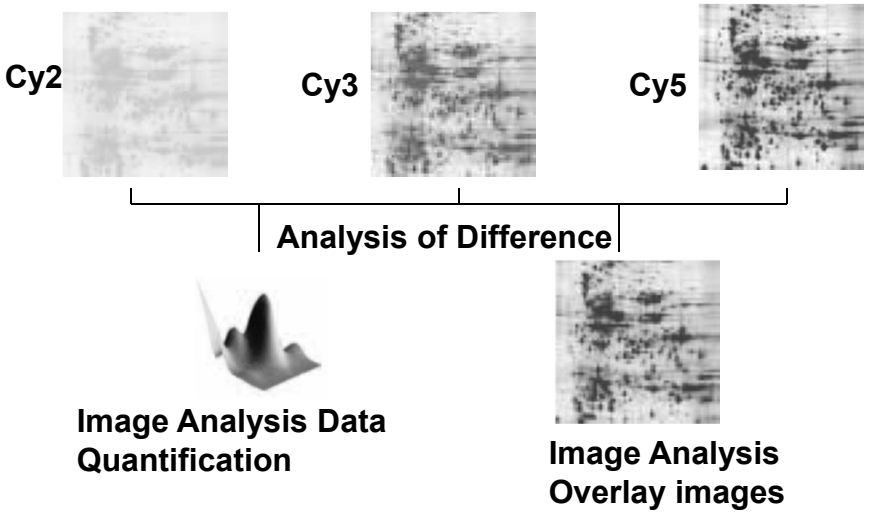
- Allows measurement of the relative concentration of proteins
- Method
  - Isolate proteins from test and control
  - Label test proteins with one dye
  - Label control protein with second dye
  - Make third sample of mixed control and test and label with third dye.
  - Combine all three samples and separate by 2D gel electrophoresis
  - Analyze the intensity of the test and sample relative to the combined sample.

2D differential Gel Electrophoresis



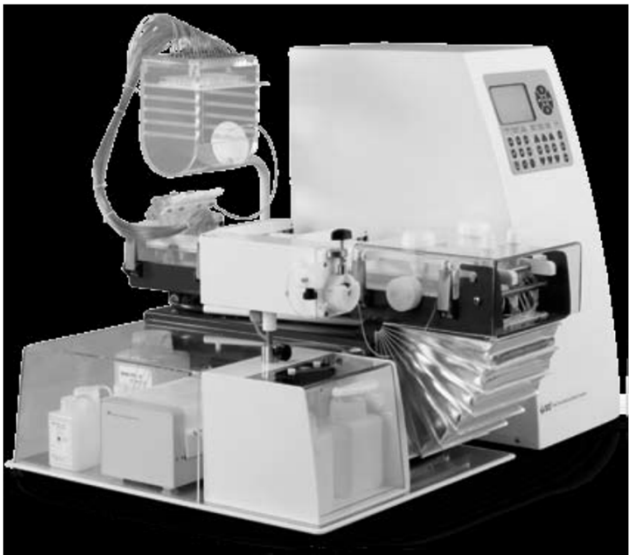
Handbook 80-6429-60AC, 2D Electrophoresis :Principles and Methods, GE Healthcare

2D differential Gel Electrophoresis

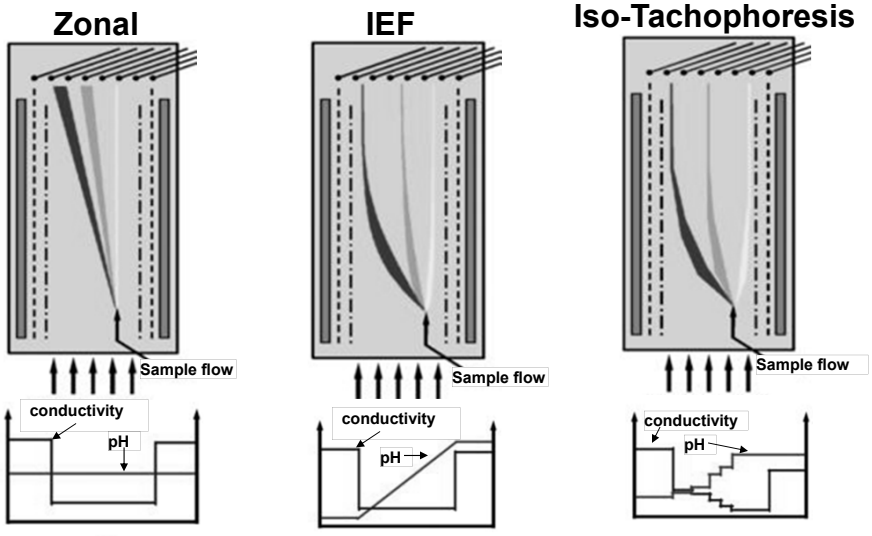


Handbook 80-6429-60AC, 2D Electrophoresis :Principles and Methods, GE Healthcare

Free Flow Electrophoresis



Forms of Free-Flow Electrophoresis



http://www.bd.com/proteomics/products/ffe/technology.asp

FFE

- **FFE can be used to separate any charged item that can be suspended in and aqueous solution.**
  - **Cells (zonal, isotacho-)**
  - **Organelle (zonal, isotacho-)**
  - **Proteins (IEF)**
  - **Subcellular fragments (zonal, isotacho-)**
  - **Nanoparticles**
- **Uses low molecular weight weak acids and basis to establish pH**.

2 Dimensional Chromatography

- Alternative means to reduced protein complexity.
- Consists of performing two or more usually orthagonal chromatographic steps prior to LC-MSMS
- Process sometimes called Multidimensional protein identification technology (MuDPIT)

2D Chromatography

Types of chromatography

Strong cation/anion exchange SCX/SAX	
Weak cation/anion exchange	WCX/WAX
Size exclusion chromatography	SEC
Hyroxyapatite chromatography	HA
Chromatofocusing	
Hydrophobic interaction	HIC
Reverse Phase	RP
Mixed bed	

2D Chromatography

- Advantages
  - Reduces complexity for LC-MS/MS and 2D gels
  - Can concentrate low abundance proteins
- Disadvantages
  - Typically up to 20% loss at each chromatographic step
  - Longer experiment times

Gel Electrophoresis

What you need to know

- Types of gel electrophoresis
  - Most common -- SDS-PAGE, IEF, 2D
  - Other methods (FFE, blue native, differential, etc.)
  - How differential gel electrophoresis works.
  - How each method separates proteins
  - Limitations
- 2 dimensional chromatography
  - How each method separates proteins
  - Limitations