Electrophoresis

Electrophoresis is the motion of dispersed particles relative to a fluid under the influence of a spatially uniform electric field. It is a general term assigned to the migration and separation of charged ions/molecules under the influence of electric field.

Requirements for realization: two electrodes – the anode and the cathode – and an electrolyte, which serves as a conducting medium.

Electrophoresis is widely used in laboratories to separate macromolecules based on size: DNA, RNA and proteins.

The electrokinetic phenomenon of electrophoresis was observed for the first time in 1807 by Russian professors Peter Strakhov and Ferdinand Reuss at Moscow State University

Виды электрофореза

Зональный электрофорез - электрофорез при постоянном pH буфера.

Изоэлектрическое фокусирование – электрофорез, при котором в среде для электрофореза создается градиент pH., а белки разделяются по величине pI.

По цели: аналитический; препаративный

По сохранности структуры молекул: в нативных условиях; в денатурирующих условиях.

По количеству стадий: одномерный; двумерный

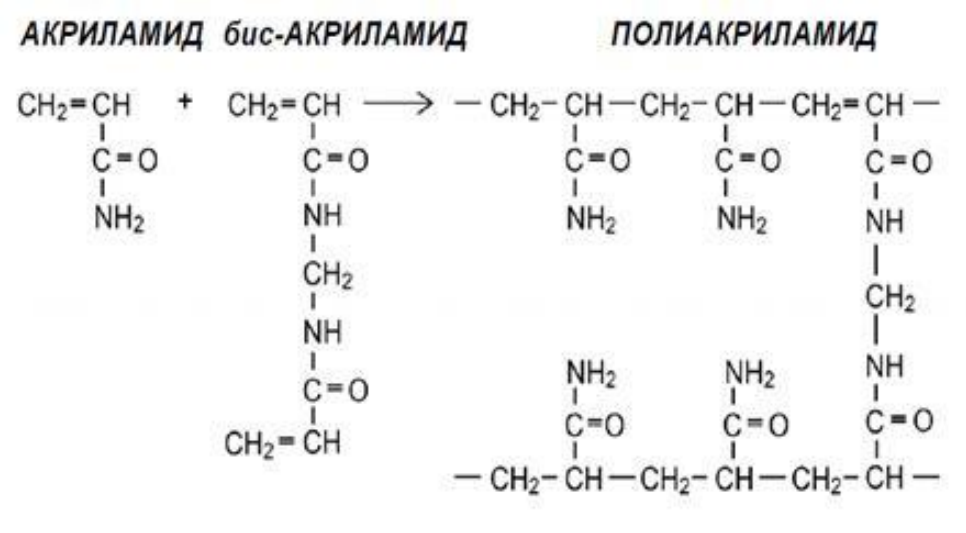
По ориентации камеры для электрофореза: горизонтальный; вертикальный.

Polyacrylamide gel electrophoresis (PAGE)

PAGE is a technique widely used in biochemistry, forensic chemistry, genetics, molecular biology and biotechnology to separate biological macromolecules, usually proteins or nucleic acids, according to their electrophoretic mobility.

Electrophoretic mobility is a function of the length, conformation and charge of the molecule.

Acrylamide monomer is in a powder state. It is soluble in water and polymerizes in water resulting in formation of polyacrylamide. Increased concentrations of acrylamide result in decreased pore size after polymerization. Small pores helps to examine smaller molecules.



Polyacrylamide gel electrophoresis (PAGE)

There are two types of polyacrylamide gel electrophoresis:

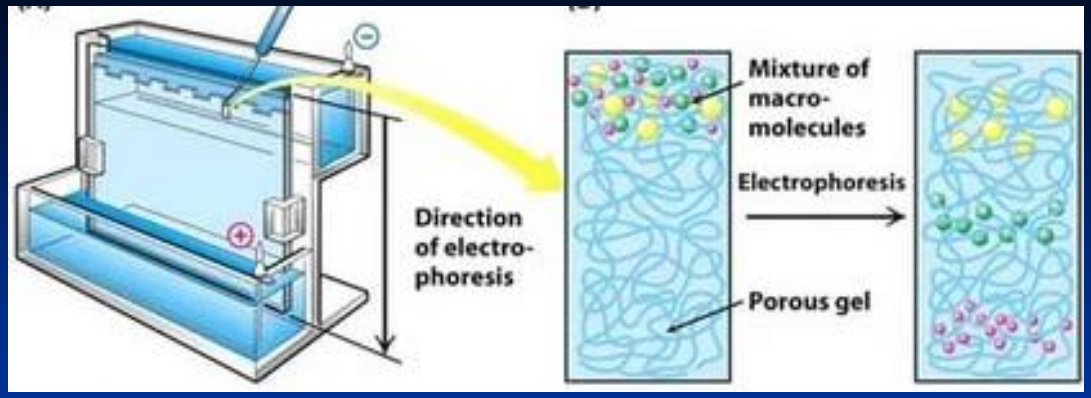
native-PAGE and **denaturating-PAGE**

native-PAGE - molecules are separated preserving their higher-order structure, and separation depends on the **length, conformation and charge** of the molecule.

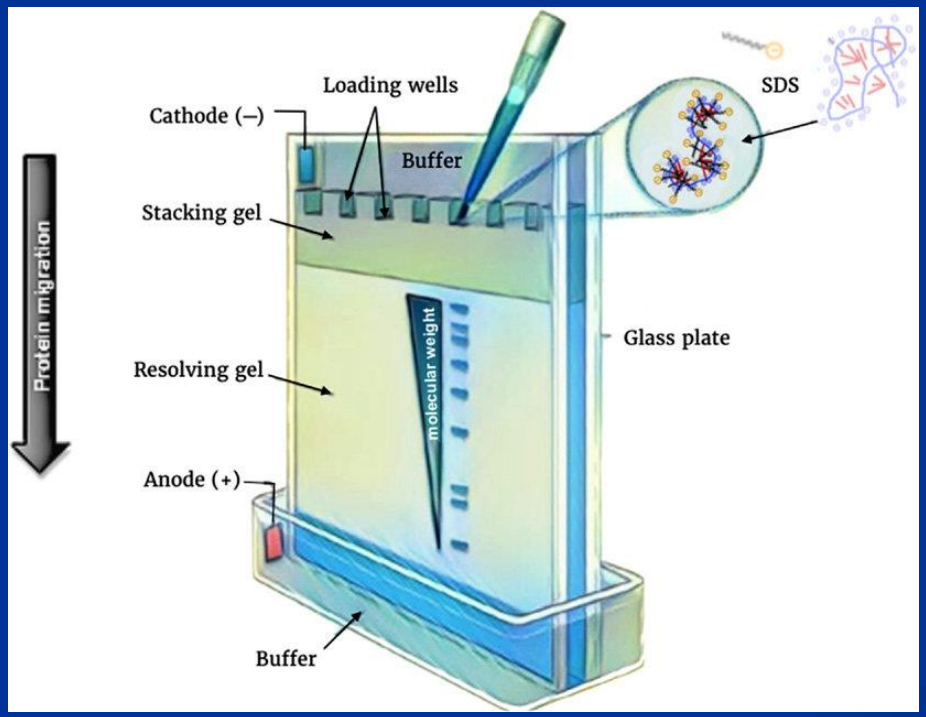
denaturizing-PAGE is applied to unstructured molecules (proteins), and mobility depends only on their length.

Denaturation of proteins is realized with SDS, and the protein-SDS complexes all have a similar mass-to-charge ratio (1 SDS molecule is bound to 2 amino acid residues).
This procedure is called **SDS-PAGE**.
The detergent destroys their secondary, tertiary and/or quaternary structure denaturing them and turning them into negatively charged linear polypeptide chains.
Mobility, or the distance traveled by molecules, is inversely proportional to the logarithm of their molecular weight

$$L=K/\lg(M.w.)$$



native-PAGE

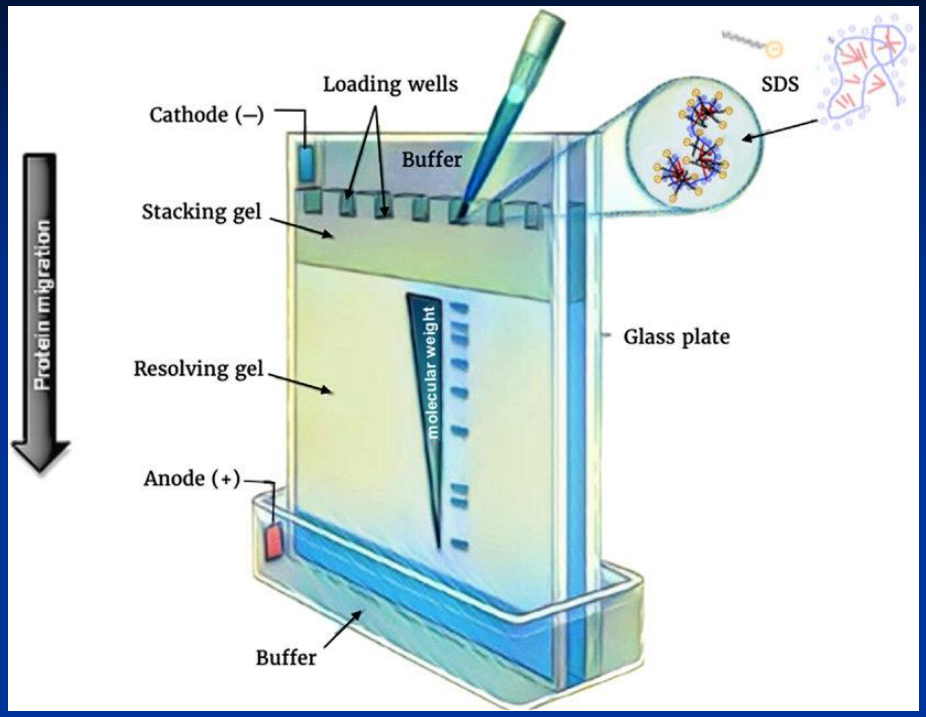


SDS-PAGE

The gels typically consist of acrylamide, bisacrylamide, the optional denaturant (SDS or urea), and a buffer with an adjusted pH.

The polymerization reaction creates a gel because of the added bisacrylamide, which can form cross-links between two acrylamide molecules.
Bisacrylamide/acrylamide=1/35
Acrylamide concentration =5-25%

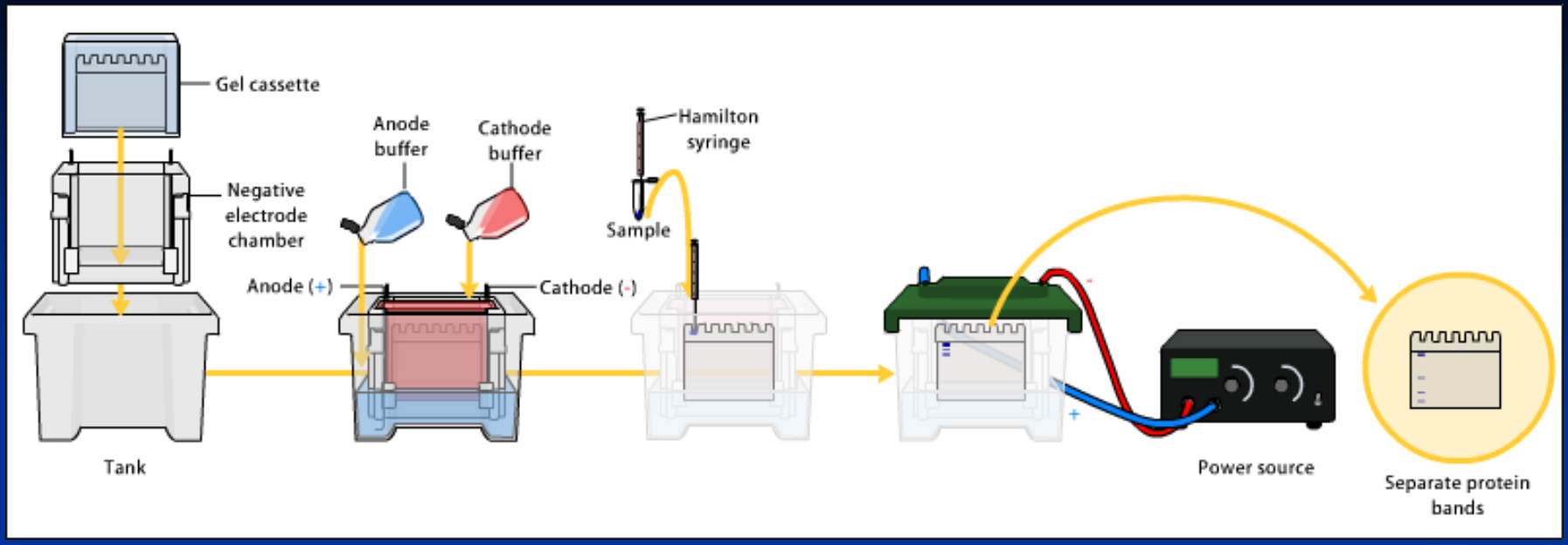
Gels are usually polymerized between two glass plates in a gel caster, with a comb inserted at the top to create the sample wells. After the gel is polymerized the comb can be removed and the gel is ready for electrophoresis.



Polyacrylamide gels are composed of a **stacking** gel and **separating** gel. Stacking gel (5%, pH 6.8) has higher pores relative to the separating gel (>5%, pH 8.8).

Stacking gel facilitates migration of proteins in a concentrated area.

pH=8.8 slows down the mobility of proteins and improve resolution.



Various buffer systems are used in PAGE depending on the nature of the sample and the experimental objective. The buffers used at the anode and cathode may be the same or different.

The gel is run usually for a few hours depending on the voltage applied. Migration occurs more quickly at higher voltages, but accuracy is reduced as compared to low voltages.

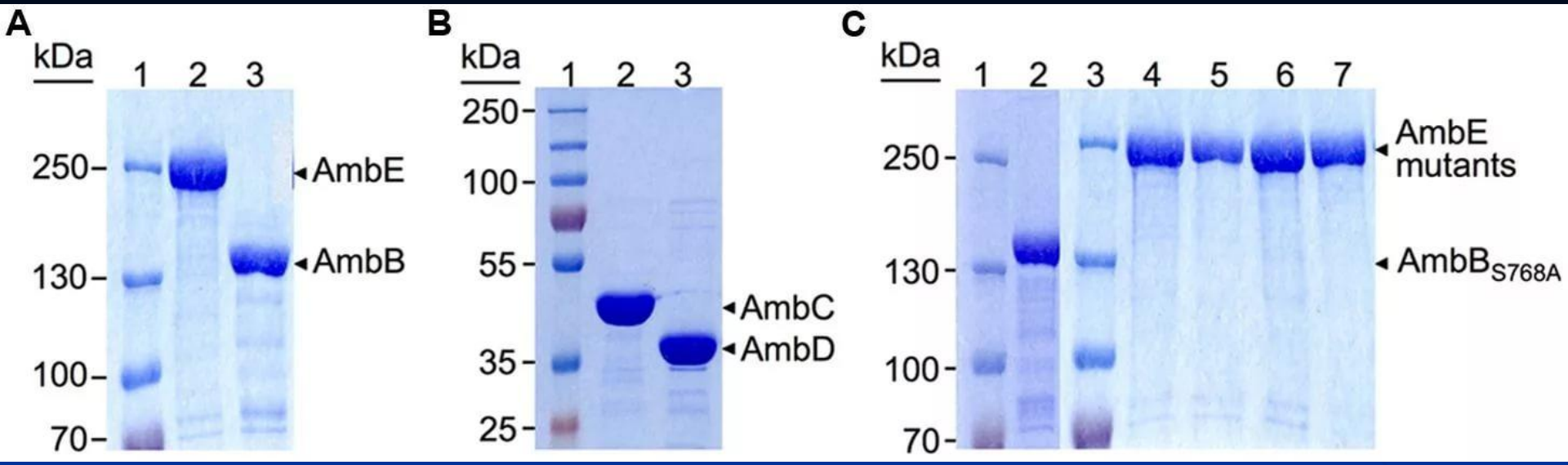
Smaller biomolecules travel farther down the gel.

Свойства буфера для электрофореза белков в нативных условиях

1. Важна емкость буфера, т.е. концентрация буфера > 100 мМ.
2. pH буфера должно обеспечивать максимальное различие зарядов разделяемых белков.
3. pH буфера выбирают отличающимся на 3-4 единицы от среднего значения pI разделяемых белков.
4. Предпочтительны буферы на основе однозарядных ионов, обеспечивающие однородную ионную силу.

Концентрация ПААГ для белков разного размера
(Стручкова, Кальясова, 2012)

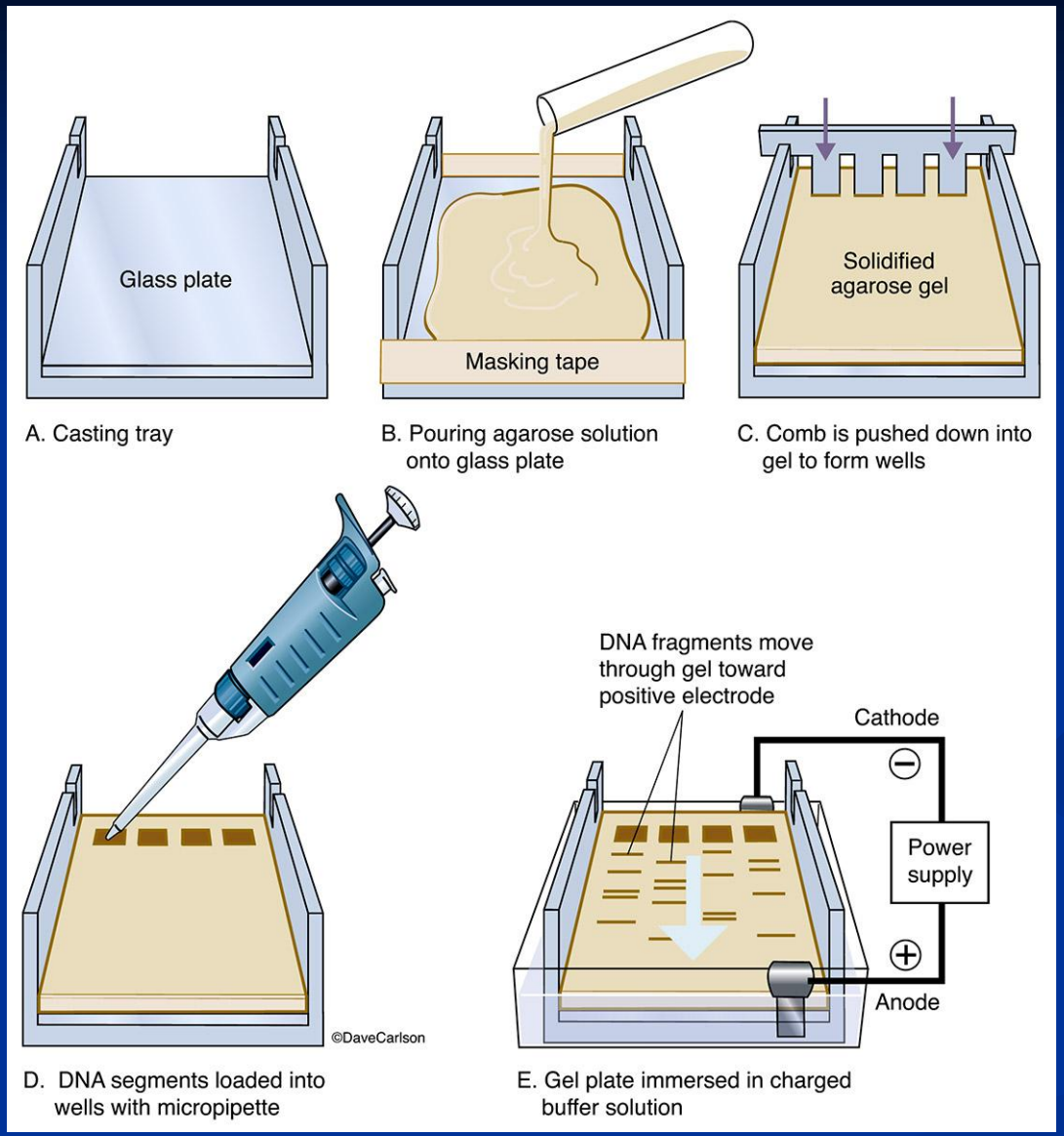
Белки, кДа	% ПААГ
36-205	5
24-205	7,5
14-205	10
14-66	12,5
10-45	15



Following electrophoresis, the gel may be stained
for proteins: Coomassie Brilliant Blue or autoradiography;
for nucleic acids: ethidium bromide;
proteins or nucleic acids: silver stain. Silver staining is used when more sensitive method for detection is needed.

It is common to run molecular weight size markers of known molecular weight in a separate lane in the gel to calibrate the gel and determine the approximate molecular mass of unknown biomolecules

Horizontal agarose gel electrophoresis



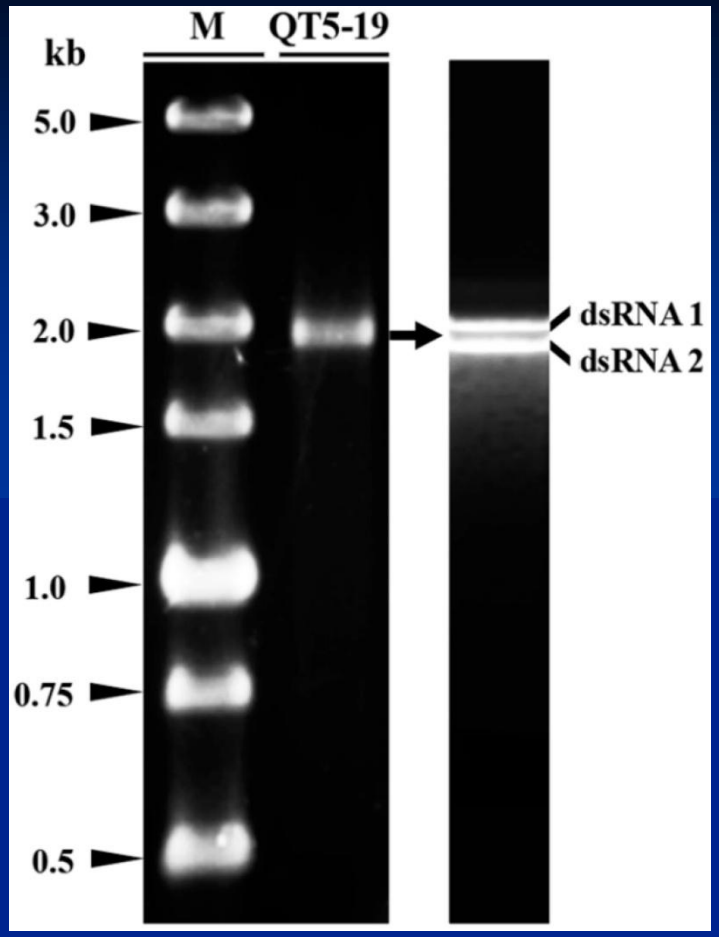
The gel is prepared by dissolving the agarose powder (0.7–2%) in an appropriate buffer (TAE or TBE), melted at 95-98 °C, cooled to >45 °C (avoiding gelling) and pouring the solution into a cast is performed.

TAE: Tris/Acetate/EDTA buffer; TBE: Tris/Borate/EDTA buffer.

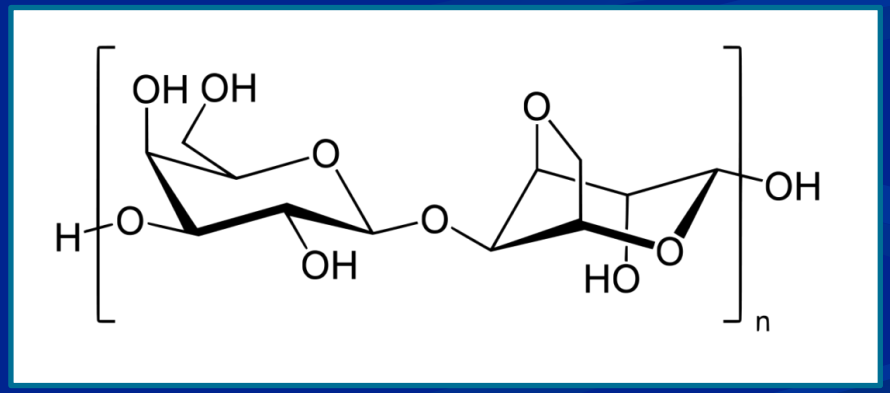
The pore size of a 1% gel varies from 100 to 500 nm.

Smaller molecules travel faster than larger molecules in gel.

Larger molecules are resolved better using a low concentration gel while smaller molecules separate better at high concentration of gel.



Размер ДНК, п.н.	Концентрация агарозы, %
50-1500	2
300-3000	1,5
400-6000	1,2
500-10000	1
800-10000	0,7
1000-20000	0,5



Агароза —линейный полисахарид

Features of DNA electrophoresis

Agarose gel electrophoresis is most commonly done horizontally in a submarine mode: the slab gel is completely submerged in buffer during electrophoresis.

The buffer used in the gel is the same as the running buffer in the electrophoresis tank.

The loading buffer contains a dense compound (glycerol, sucrose, or Ficoll) that raises the density of the sample so that DNA may sink to the bottom.

The rate of migration of the DNA is proportional to the voltage applied, i.e. the higher the voltage, the faster the DNA moves.

The resolution of large DNA fragments however is lower at high voltage.

dsDNA moves at a rate that is inversely proportional to the logarithm of the number of base pairs.

$$L=K/\lg(b.p.)$$

Features of DNA electrophoresis

Agarose gel electrophoresis can be used to

- resolve DNA by length
- resolve the conformation of DNA. Supercoiled DNA moves faster than relaxed DNA because it is more compact.
- resolve circular DNA with different supercoiling topology;
- resolve different forms of a plasmid. The plasmids normally show the negatively supercoiled form as the main band, while nicked DNA (open circular form) and the relaxed closed circular form appears as minor bands.
- reveal DNA damage (increased cross-linking reduces electrophoretic DNA migration)

Agarose gel has lower resolving power than polyacrylamide gel for DNA but has a greater range of separation, and is therefore used for DNA fragments of usually **50–20,000 bp** in size.

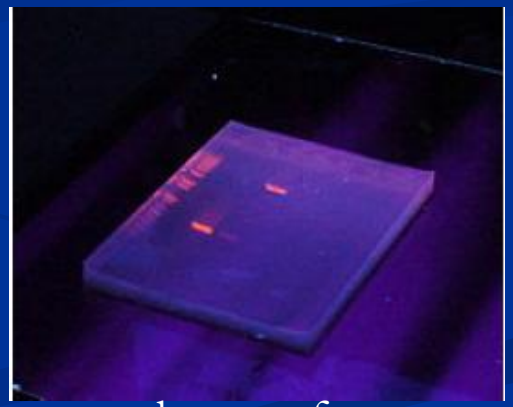
The limit of resolution for standard agarose gel electrophoresis is around **750 kb**

DNA is not visible in natural light, and the progress of the electrophoresis is monitored using colored dyes (Xylene cyanol or Bromophenol blue).

The separated DNA may be viewed **with stain**, most commonly under UV light, and the DNA fragments can be extracted from the gel with relative ease.

DNA stains: ethidium bromide, SYBR Green, crystal violet, methylene blue, brilliant cresyl blue, and Nile blue sulphate.

Stains may be added to the agarose solution before it gels, or the DNA gel may be stained later after electrophoresis.



the use of a
UV transilluminator

Horizontal agarose gel electrophoresis can also be used:

- to separate **large** proteins
- for electrophoresis of particles with effective radii larger than 5–10 nm.
- 0.9% agarose gel has pores large enough for the entry of bacteriophage T4

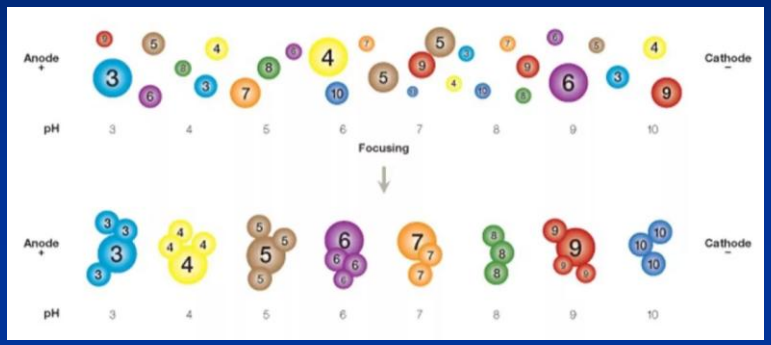
For gel electrophoresis of protein, the bands may be visualized with Coomassie or silver stains.

Applications of agarose gel electrophoresis

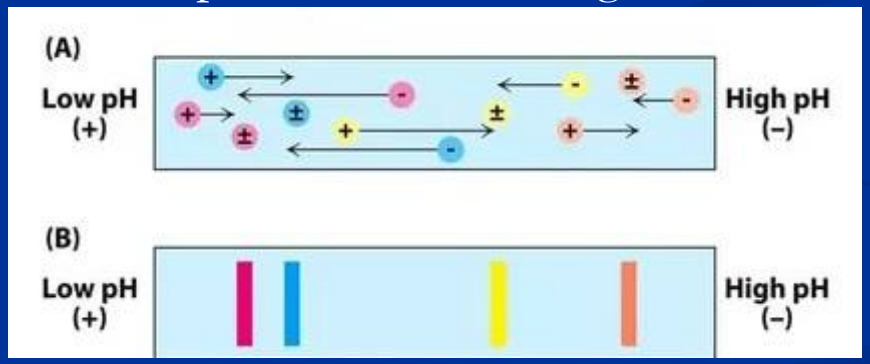
- Estimation of the size of DNA molecules following digestion with restriction enzymes, e.g. in restriction mapping of cloned DNA.
- Analysis of products of a polymerase chain reaction (PCR), e.g. in molecular genetic diagnosis or genetic fingerprinting.
- Separation of DNA fragments for extraction and purification.
- Separation of restricted genomic DNA prior to Southern transfer, or of RNA prior to Northern transfer.
- Separation of proteins, for example, screening of protein abnormalities in clinical chemistry

Isoelectric focusing agarose electrophoresis

Isoelectric focusing is a technique for separation of different molecules by differences in their isoelectric point (pI). It is size independent
It is a type of zone electrophoresis, usually performed on proteins in a gel.

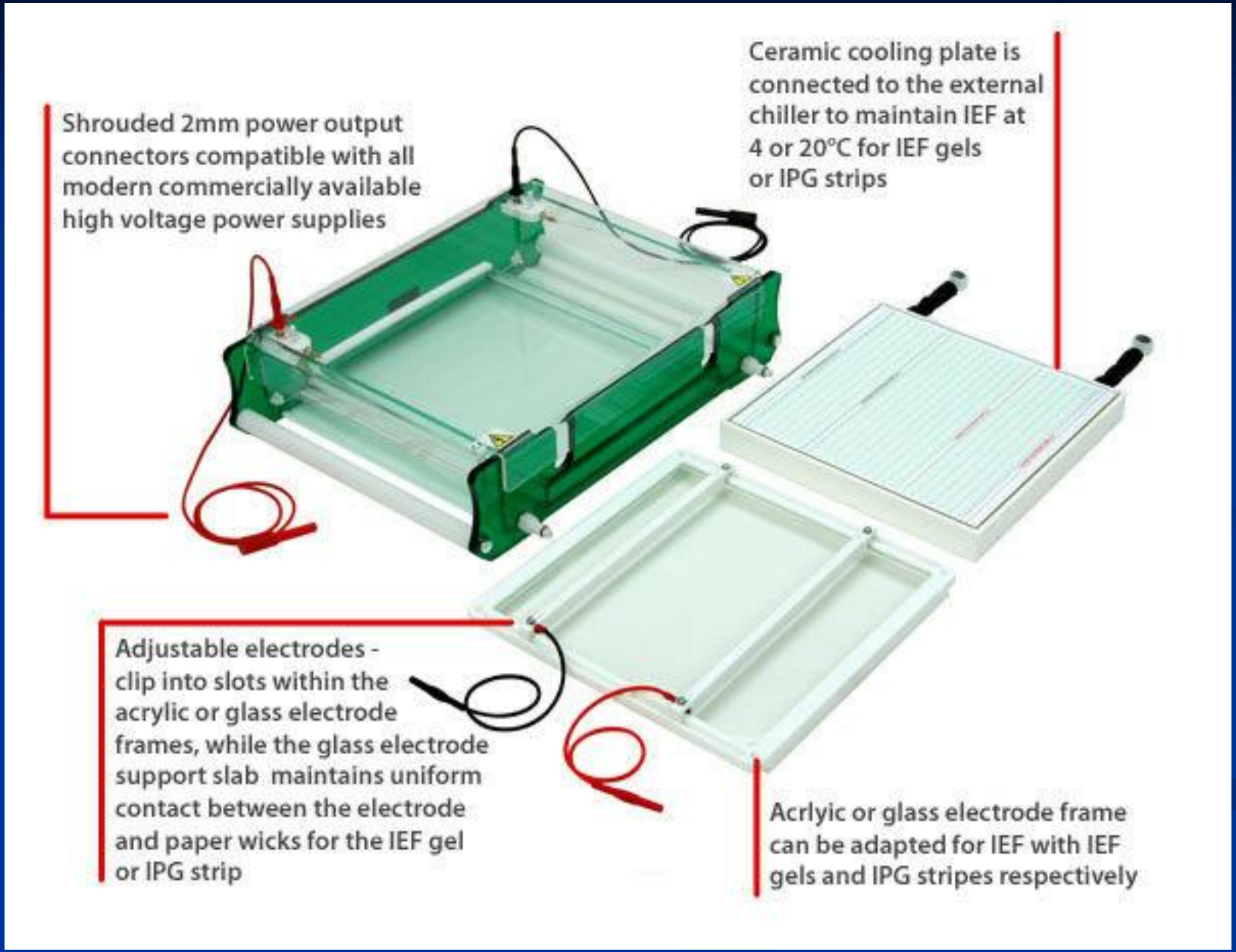


The pH gradient is established before adding the proteins by first subjecting a solution of small molecules-polyampholytes with varying pI values to electrophoresis. Gel is composed of polyacrylamide, starch, or agarose.
Gels with large pores are usually used to eliminate any "sieving" effects (artifacts) caused by differing migration rates for proteins of differing sizes.



The analyte (proteins) is loaded into a gel with immobilized pH gradient. An electric current passes through the medium, and the proteins become focused into sharp stationary bands with each protein positioned at a point in the pH gradient corresponding to its pI. Isoelectric focusing can resolve proteins that differ in pI value by as little as 0.01.

Isoelectric focusing agarose electrophoresis



Среды для изоэлектрического фокусирования

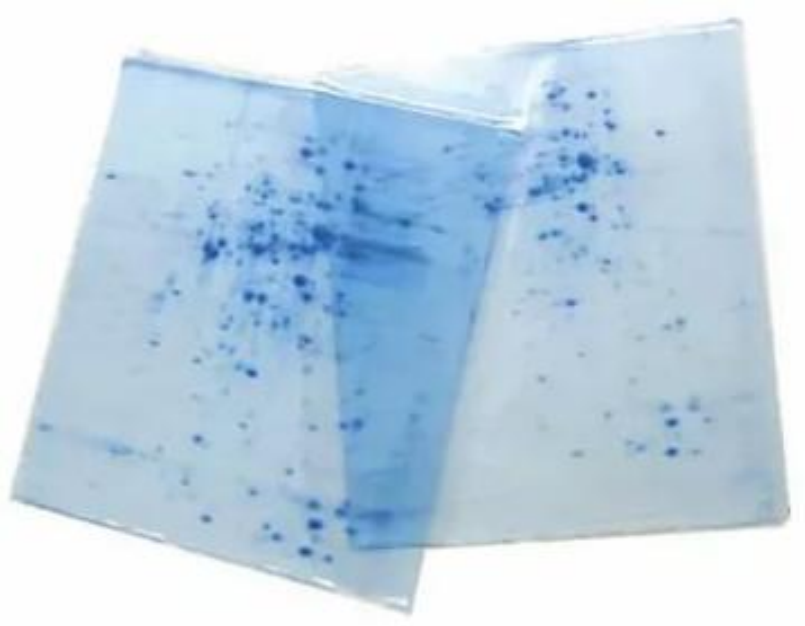
1. Иммобилизованные градиенты pH (IPG): полиакриламидные полоски геля сополимеризованные с т.н. иммобилинами. Иммобилин - это производные акриламида с функциональными группами для слабых кислот или оснований с определенным значением pK . Сополимеризация нейтрального акриламида с кислотными и основными иммобилинами создает неизменный, неподвижный градиент pH.

2. Градиенты pH на основе амфолитов-носителей - синтетических аминокислот, свободно перемещающихся в геле и образующих градиент pH в электрическом поле.

2D electrophoresis

2D electrophoresis is a powerful and widely used method for the analysis of complex protein mixtures extracted from cells, tissues, or other biological samples. It separates proteins according to two independent properties in two discrete steps.

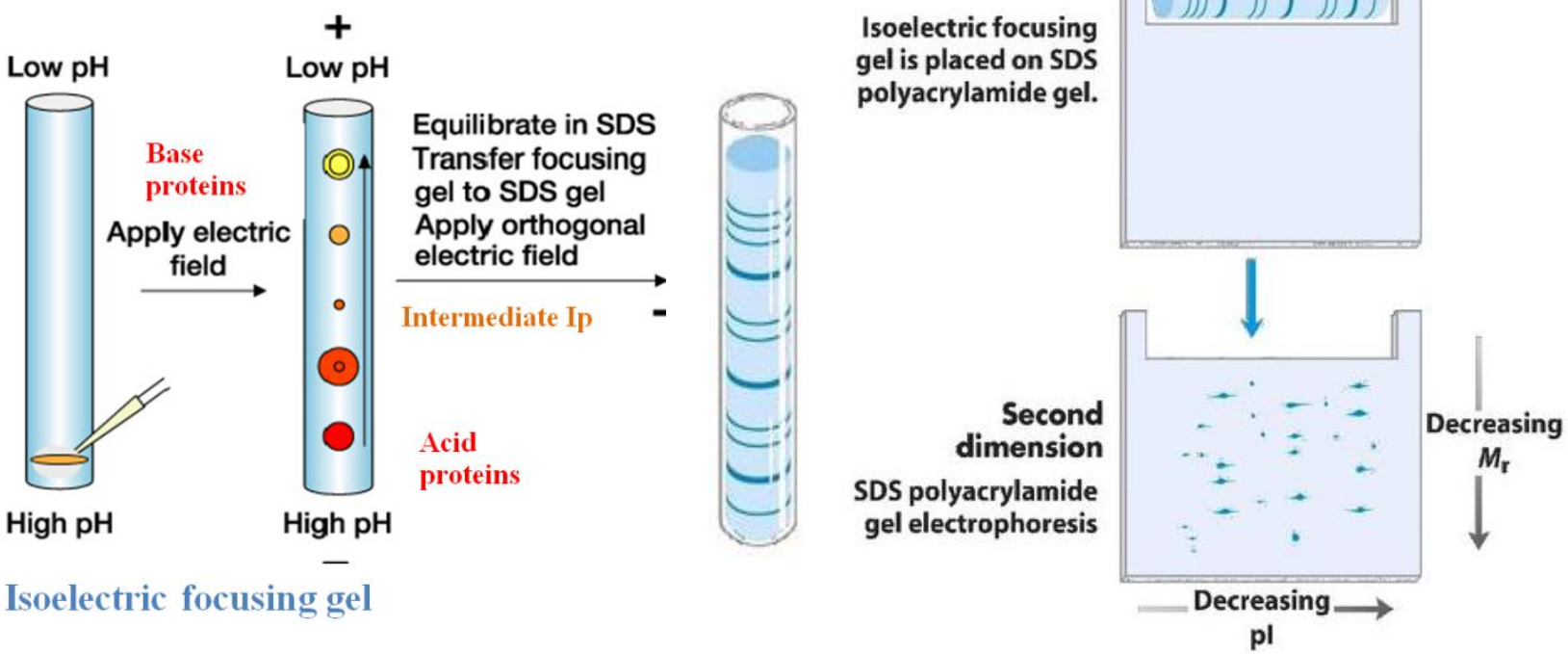
The first stage is isoelectric focusing that separates proteins according to their isoelectric points (pI);
The second stage is sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) that separates proteins according to their molecular weights.



Each spot on the 2D-gel potentially corresponds to a single protein species in the sample. Thousands of proteins can be separated providing information on:

- protein pI,
- apparent molecular weight,
- amount of each protein.

2D electrophoresis



The protein spots can be stained to detect them in the second-dimension gel matrix.

If the proteins were prelabeled, the spots can be imaged by autoradiography, by illumination of the gel with UV light, or by using a fluorescence imager.

In the currently employed 2-D technique:

- carrier-ampholyte-generated pH gradients were replaced with immobilized pH gradients,
- tube gels were replaced with gels supported by a plastic backing (strips).

Isoelectric focusing of proteins in strips

Voltage, current, temperature, and time controls are programmable.

vertical electrophoresis systems

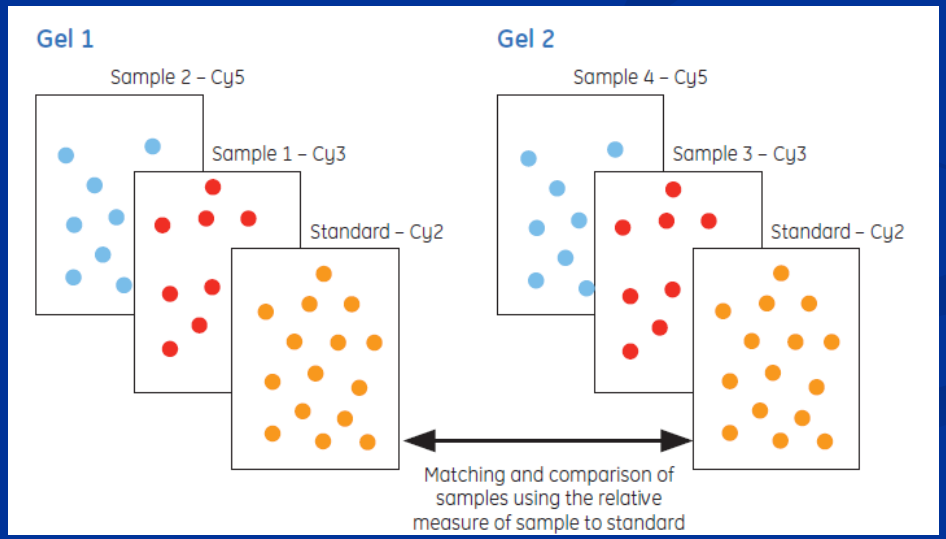
2-D Fluorescence Difference Gel Electrophoresis (2D DIGE)

2D DIGE is a method that labels protein samples prior to 2-D electrophoresis, enabling accurate analysis of differences in protein abundance between samples.

The technology is based on the specific properties of spectrally resolvable dyes.

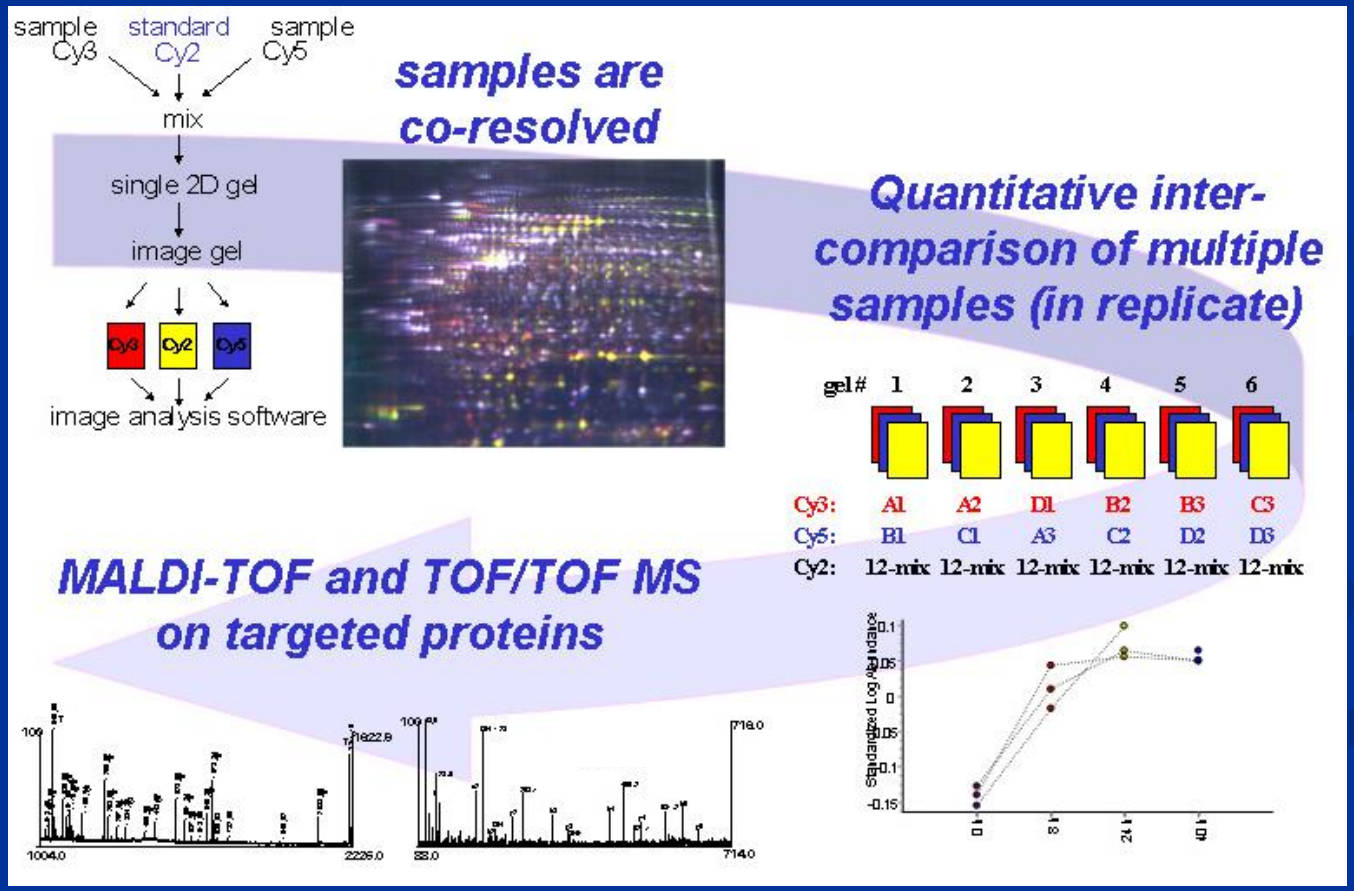
Identical proteins labeled with different dyes will migrate to the same position on a 2D gel, and up to two samples and an internal standard (internal reference)can be loaded and studied in every gel.

The internal standard is prepared by mixing together equal amounts of each sample in the experiment and including this mixture on each gel.



The advantages of linking every sample in-gel to a common standard are:

- Accurate quantitation and accurate spot statistics between gels
- Increased confidence in matching between gels
- Flexibility of statistical analysis depending on the relationship between samples
- Separation of system variation from biological variation



Electrophoresis

Slab gel electrophoresis generally suffers from:

- long analysis times,
- low efficiencies,
- difficulties in detection
- difficulties in automation.

Applications of 2-D electrophoresis include:
proteome analysis, cell differentiation, detection of disease markers, therapy monitoring, drug discovery, cancer research, purity checks, and microscale protein purification.

The technique is also unique in its ability to detect post- and co-translational modifications, which cannot be predicted from the genome sequence.

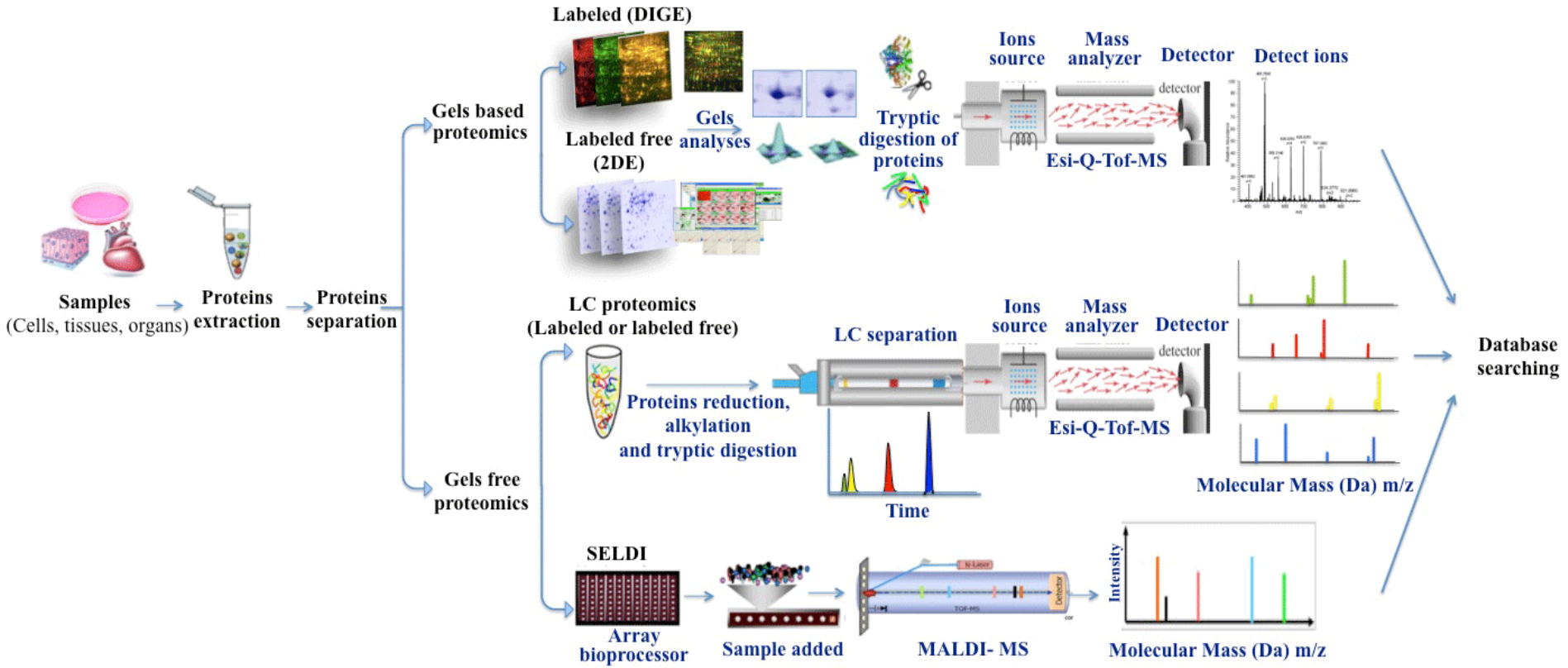
Chromatography and clinical analysis

Accurate clinical diagnosis often requires detection and measurement of biological analytes in body fluids such as:

- serum/plasma,
- urine,
- cerebrospinal fluid,
- saliva.

Chromatography methods used in clinical analysis:

- standard liquid chromatography
- liquid chromatography-mass spectrometry
- HPLC
- gas chromatography
- gas chromatography-mass spectrometry
- one- or two-dimensional electrophoresis
- high performance capillary electrophoresis


Samples
(Cells, tissues, organs)
Proteins extraction
Proteins separation
Gels based proteomics
Gels free proteomics
Labeled (DIGE)
Labeled free (2DE)
Gels analyses
Tryptic digestion of proteins
Ions source
Mass analyzer
Detector
Detect ions
detector
Esi-Q-Tof-MS
LC proteomics
(Labeled or labeled free)
Proteins reduction, alkylation and tryptic digestion
LC separation
Time
Molecular Mass (Da) m/z
SELDI
Array bioprocessor
Sample added
MALDI- MS
Intensity
Molecular Mass (Da) m/z
Database searching