Electrophoresis

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Questions

- What is electrophoresis?
- History of electrophoresis?
- Types of electrophoresis
- What are the main principles of electrophoresis?
- What are the equipments and reagents of electrophoresis?
- How can we pour an agarose gel?
- What is the meaning of the bands after electrophoresis?
- Is there an automated type of electrophoresis?

Electrophoresis-**Definition**

- Electro=Electric; phoresis= Migration;Carry accross.
- A kind of separation technique based on the differential migration features of charged molecules in an electric field.
- An analytical method frequently used in molecular biology, biochemistry and medicine.

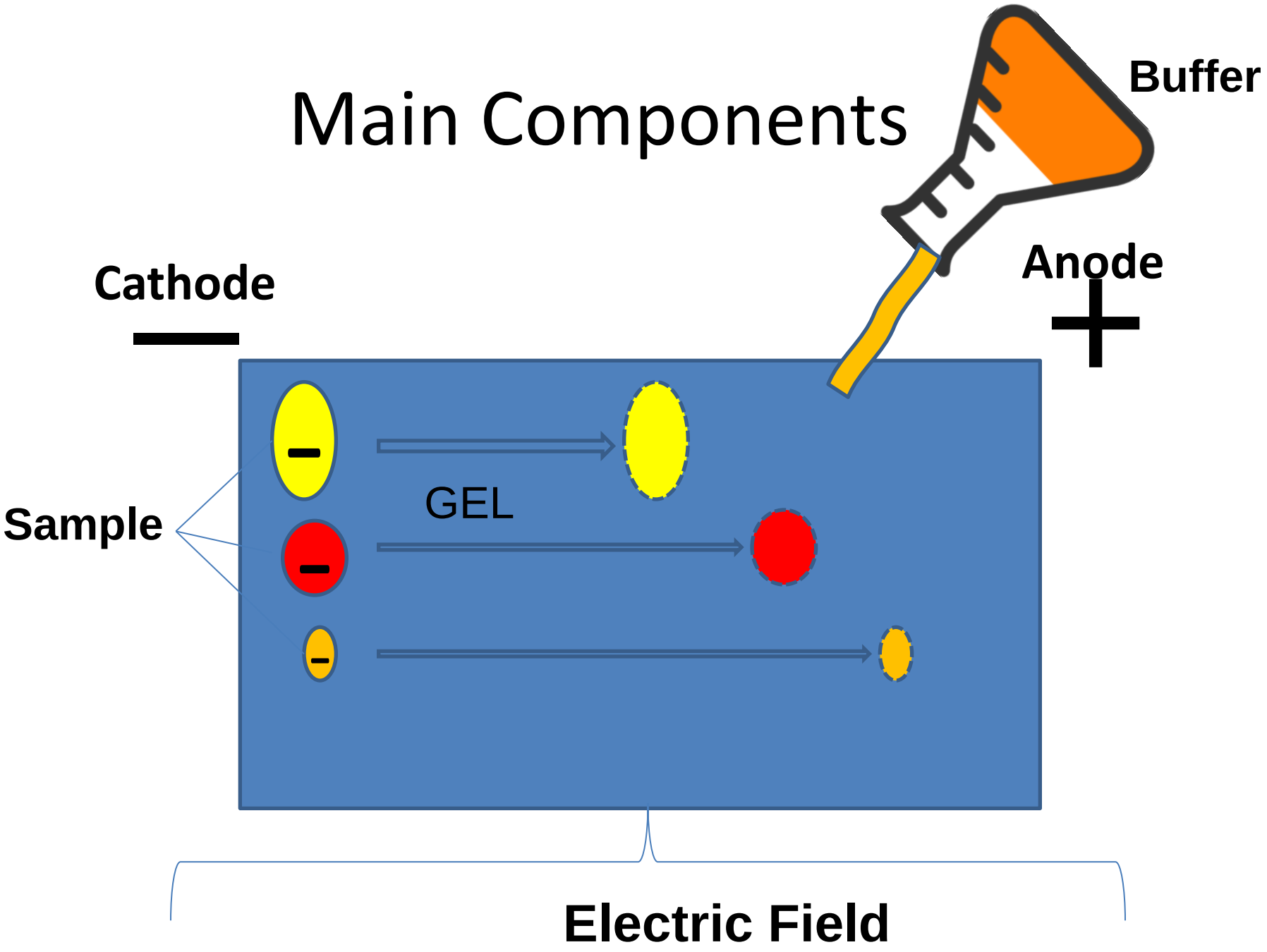
History of electrophoresis

- 1834-Michael Faraday –“Faraday's laws of electrolysis”
- 1882-Robert Koch- Agarose culture medium
- 1937- Arne Tiselius “A New Apparatus for Electrophoretic Analysis of Colloidal Mixtures"- Tiselius apparatus.
- 1946-Agarose gel
- 1955-Oliver Smithies-starch gels
- 1957-Joachim Kohn-Cellulose acetate electrophoresis
- 1959-Acrilamide gels
- 1969-Weber K and Osborn M. SDS gel electrophoresis
- 1971-Danna and Nathans-The separation of DNA by gel electrophoresis
- 1977-Sequencing gels.
- 1983-Pulsed field Electrohoresis
- 1983-Capillar electrophoresis

Electrophoresis

- Principle:
  - In an electrical field charged molecules and particles migrate to the opposite charge.
  - Usually in aqueous solution(Buffer).
  - Due to their varying charges and masses, different molecules and particles in the mixture are migrate at different speeds.
  - As a result; separated into single fractions(bands).

Main Components



Migration Depends on

- Strength of electric fields.
- Temperature
- Features of the molecule
  - Net charge of molecule
  - Size of molecule
  - Shape of molecule
- Features of the Gel
  - Gel type
  - Gel concentration
- Buffer Type/pH.

Electrophoresis

- Separates
  - Nucleic acids
  - Proteins
  - Peptides
  - Amino acids
  - Organic acids/bases
  - Drugs
  - Pesticides
  - Inorganic anions/cations.
- Everything that can carry a charge.!

Molecular Pathology

- Nucleic acids.
  - Determining quality of DNA/RNA
  - Analyses of PCR products
  - Mutation detection
  - Southern and Northern blotting
  - Sequencing
- Proteins
  - Western blotting
  - Protein purification

Electrophoresis Types

- Gel electrophoresis
  - Agarose gel
  - Polyacrylamide gel
  - Others.
- Pulsed Field Gel Electrophoresis
- Capillary Electrophoresis
- Isoelectric focusing
- 2D electrophoresis

Gel Electrophoresis

- Use of a gelatinous material.
- The gel acts as a support medium
- Used to separate proteins or nucleic acids.

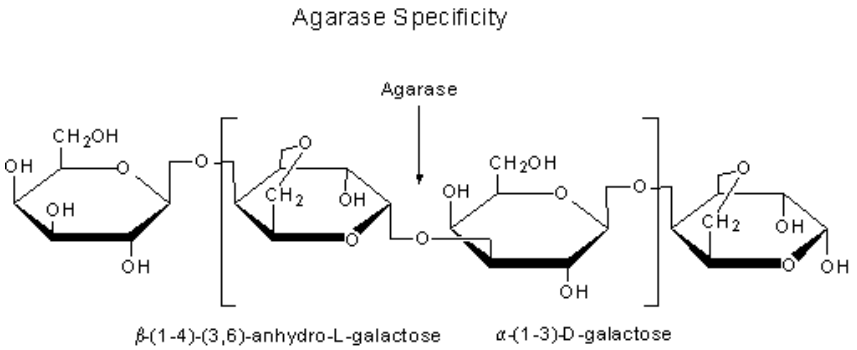
Gel Types

- Starch-Rarely used
- Polyacrylamide-Protein, small nucleic acid fragments
- Agarose-Nucleic acids, large proteins
- Cellulose acetate-Proteins

Commonly used

Agarose Gel Electrophoresis

- Easy, fast, well established method for separating DNA fragments.
- Agarose, a polysaccharide derived from seaweed.
- β-1,3-D-galactose/3,6-anhydro-α-1,4-galactose.
- Dissolves in boiling water, and hardens, becomes gel when cooling.
- Bigger pore size than polyacrylamide



*Sigma MSDS

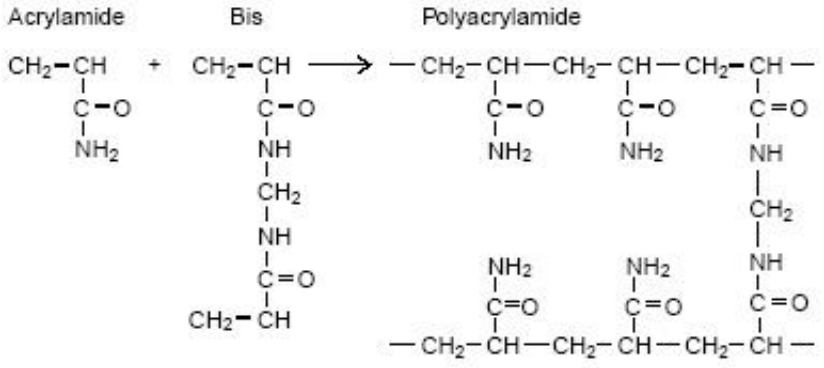
Agarose Gel Concentration/DNA-Size

Concentration	Size
0,3	5000-60000 base
0,6	1000-20000 base
0,7	800-10000 base
0.9	500-7000 base
1.2	400-6000 base
1.5	200-3000 base
2.0	100-200 base

Temizkan G, Arda N. Moleküler biyolojide kullanılan yöntemler. Nobel tıp.

Polyacrylamide Gel Electrophoresis (PAGE)

- Synthetic polymer
- Formed from acrylamide subunits.
- Acrylamide with a cross linker, methylene bis-acrylamide .
- Polymerization catalysts:
  - Ammonium persulfate (APS) +Tetramethylethylenediamine (TEMED)
  - Light
- 3.5–20% concentration.
- High resolution.
- Acrylamide is a dangerous neurotoxin



http://www.biocompare.com/Application-Notes/42631-Acrylamide-Polymerization-A-Practical-Approach/

Acrylamide/Bis Ratio	Gel %	Native DNA/RNA (bp)	Denatured DNA/RNA (bp)
19:1	4	100-1500	70-500
	6	60-600	40-400
	8	40-500	20-200
	10	30-300	15-150
	12	20-150	10-100
29:1	5	200-2000	70-800
	6	80-800	50-500
	8	60-400	30-300
	10	50-300	20-200
	12	40-200	15-150
	20	<40	<40

Introduction to Agarose and Polyacrylamide Gel Electrophoresis Matrices with Respect to Their Detection Sensitivities

Patricia Barril and Silvia Nates

www.intechopen.com

Buffer

- Provides ions in solution for electrical conductivity.
- Prevents the pH changing.
- Common using buffers:
    - Tris Borate EDTA (TBE)-Stable, expensive,PAGE, long separation time.
    - Tris Acetate EDTA (TAE)-Inexpensive,short separation time.
    - Tris Phosphate EDTA (TPE)
- RNA
  - Sodium phosphate Buffer
  - MOPS Buffer (-3-(N-morpholino) propanesulfonic acid)

  *Buffer formulation

  http://www.elabprotocols.com/

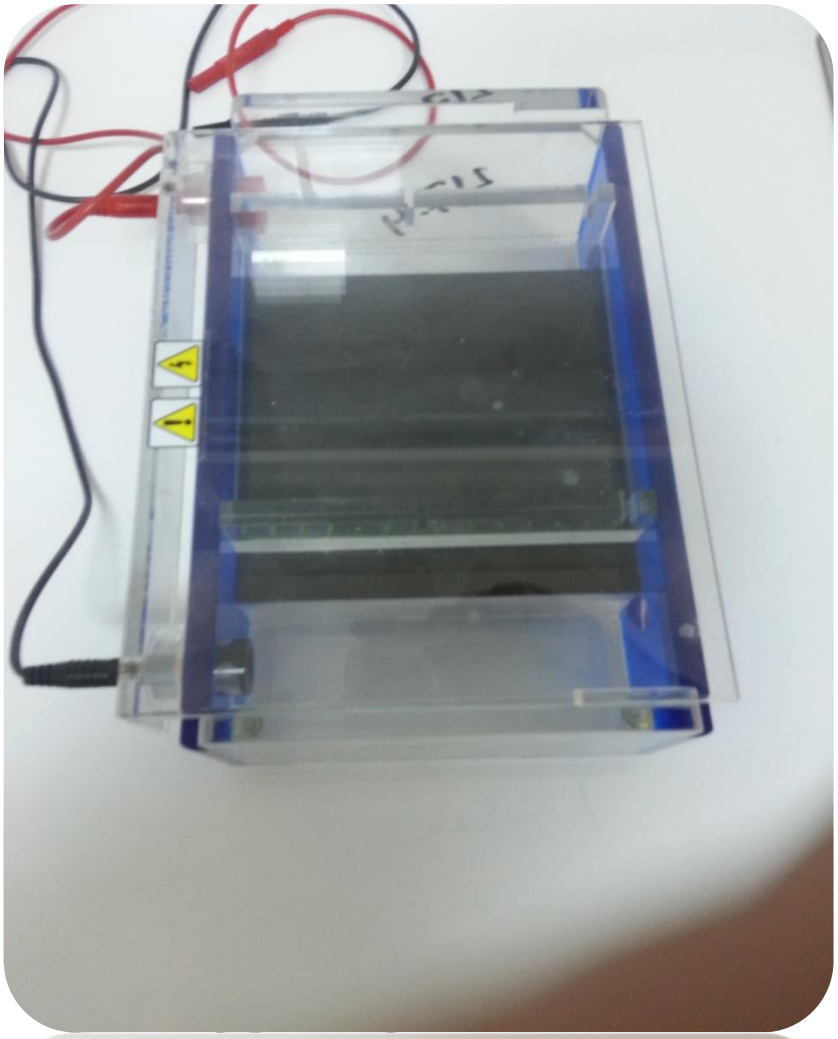
Equipment

- Power supply
- Cooling Apparatus
- Electrophoresis gel apparatus-Vertical or Horizontal
- White Light/UV Light Box/Digital Camera/Gel Documentation System
- Reagents:
    - Gel staining chemicals(eg.EtBr)
    - Prepared gels or gel chemicals
    - Buffers
    - Loading dyes
- Other laboratuary equipments:
    - pH meter
    - Pipettors
    - Lab. Scale
    - Stir plates

Electrophoresis Gel Apparatus

Horizontal (Flat bed)

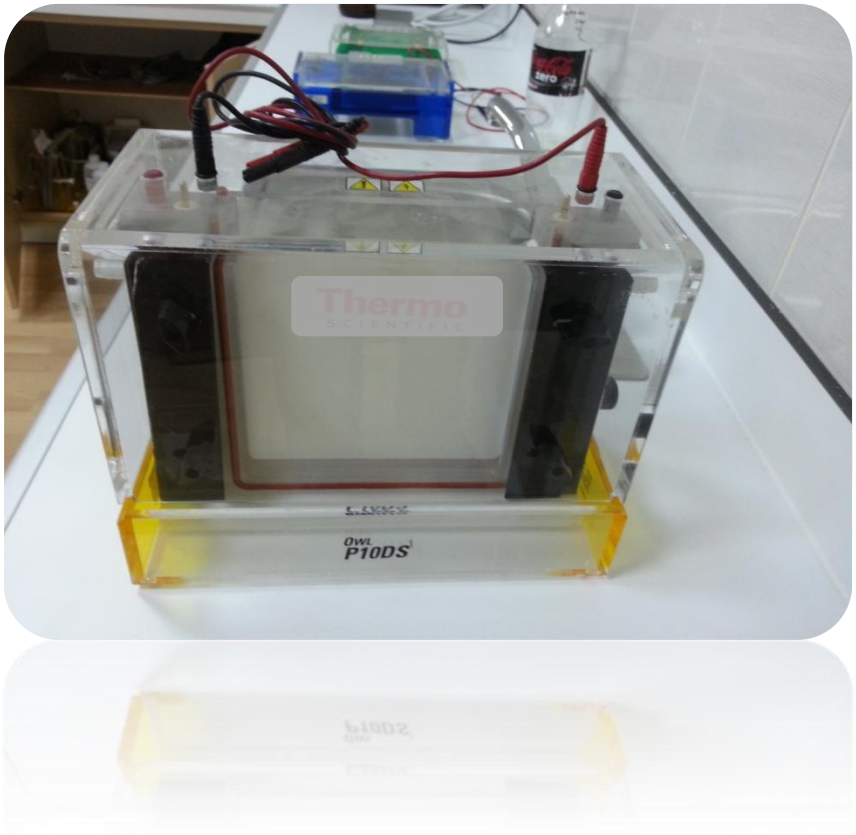
- Gel thickness limited.
- Only one gel per aparatus
- Easily adapts different techniques.
- Technician friendly
- More safe for electricity accidents.
- Gnll. used for agarose gel electrophoresis



Electrophoresis Gel Apparatus

Vertical

- Different gels thicknes can be used.
- More than one gel per apparatus
- Not easily adapted for different techniques.
- No technician friendly.
- Gnll. used for polyacrylamide gel electrophoresis



Gel Documentation System

Gel Documentation System UV/White Lamp Box

Power Supply

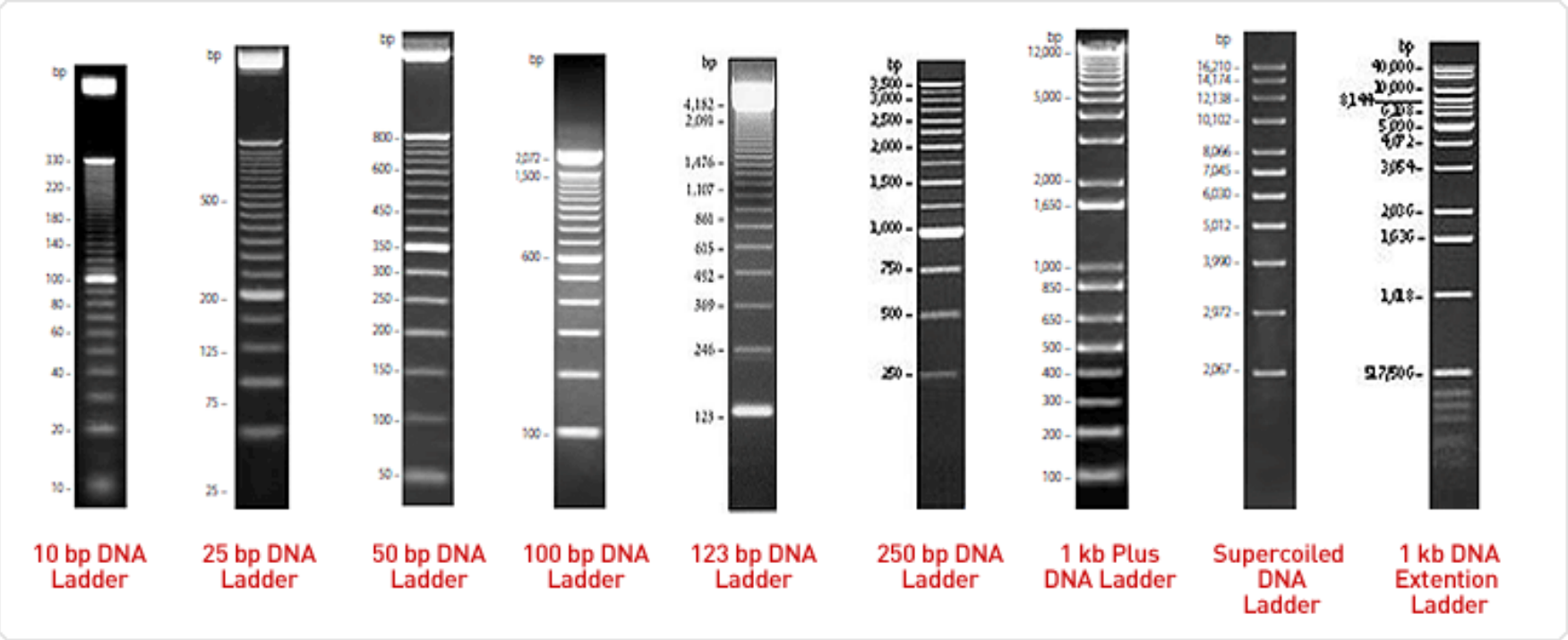
Technique

- Steps
  - Sample preparation
  - Gel,buffers, etc. preparation.
  - Load markers
  - Load samples
  - Running of the gel
  - Staining of the gel
  - Photography, gel documentation
  - Interpret/analysis of gel

Do not forget!

- DNA molecule is an organic acid.
- Negatively charged.
- Migrate toward the positive electrode(Anode) in an electromagnetic field.
- Small fragments go further than large fragments of DNA.
- Do not forget “Running of the gel “
- Cut off electricity before taking gel from apparatus.

Markers



Selection of the suitable marker for the expected fragment size is very important.!

Ethidium Bromide

- Powerful mutagen but it works well.
- Cheap, sensitive, easy to use, fast.
- Binds to DNA .
- Fluorescens under UV lamp and visualizes of DNA on the Gel.
- Can be added directly into the gel and/or buffer

  or

- Gel can be stained after run.
- Concentration 0.5-1ug/ml for staining gels.

EtBr Alternatives

- Sybr stains.
- Silver stains
- Methylen Blue
- Commercial stains.

- *More safe, less sensitive.*
- *Syb stains also mutagenic.??*

Voltage

- More voltage, more quick gel runs.
- But,
  - Low resolution.
  - Increase temperature
- As a result, low quality separation.
- <5-8 V/cm of gel length 75mA.(100mA for minigels)
- By trial and error (Emprical approach)

Technique

- Sample preparation
- Gel,buffers, etc. preparation.
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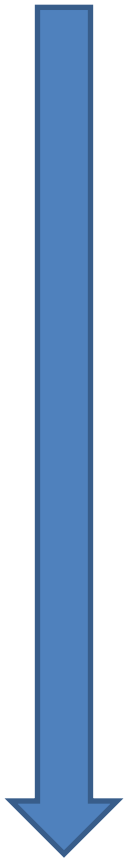


Technique

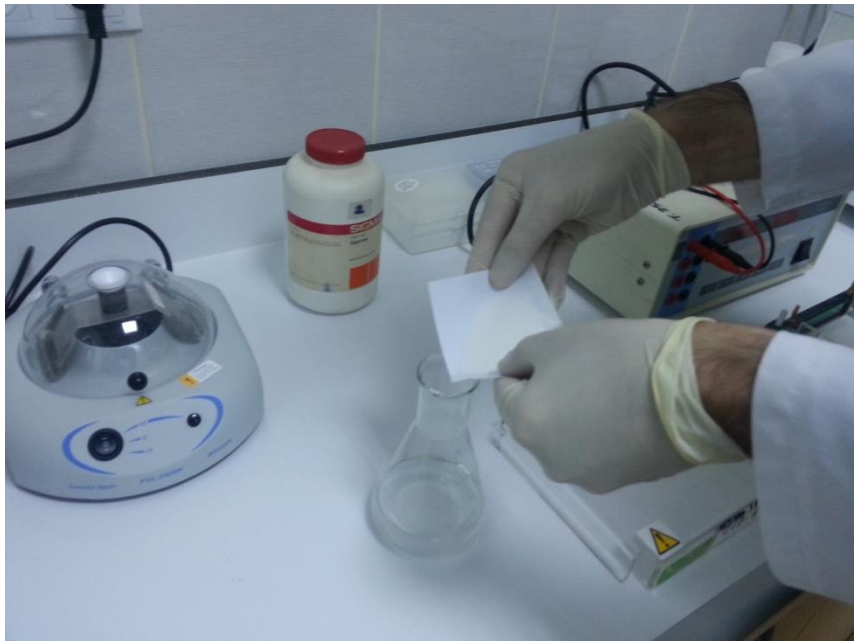
- Sample preparation
  - PCR products
  - DNA, cut with restriction enzymes
  - Others

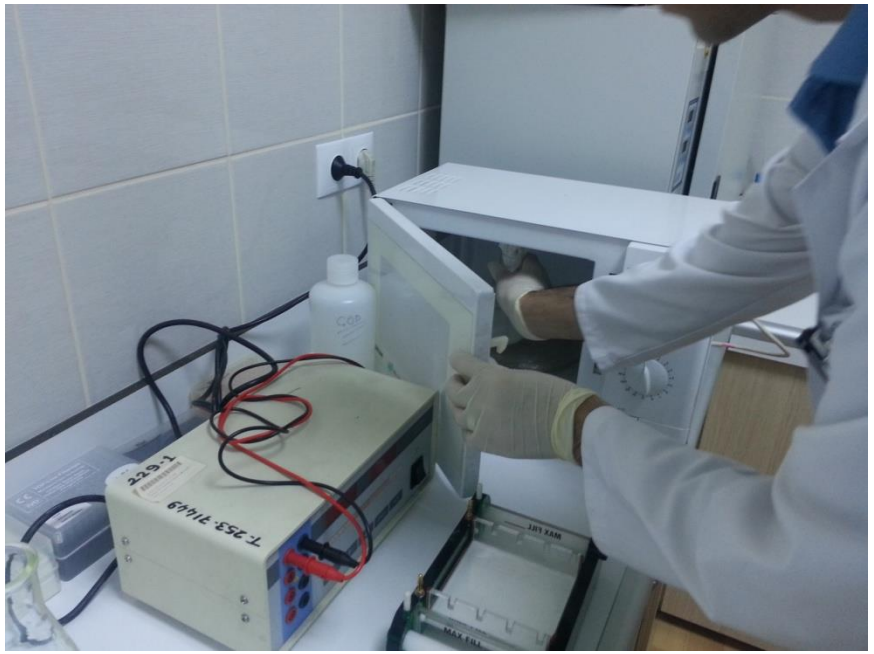

TBE Buffer X10
Agarose
Power Supply
Microwave oven
Distilled Water
Gel apparatus
SIGMA
217-4

Gel,buffers, etc. preparation.

- Agarose, desired concentration.
  - 100 ml .....gr.
- Solved in buffer.
- Melt in the microvave.
  - Clear solution when melt.
- Wait a few minutes and add EtBr.
- Insert the" comb"
- Prepare apparratus, pour gel.
- Wait until the gel hardens
- Pull out the comb carefully.

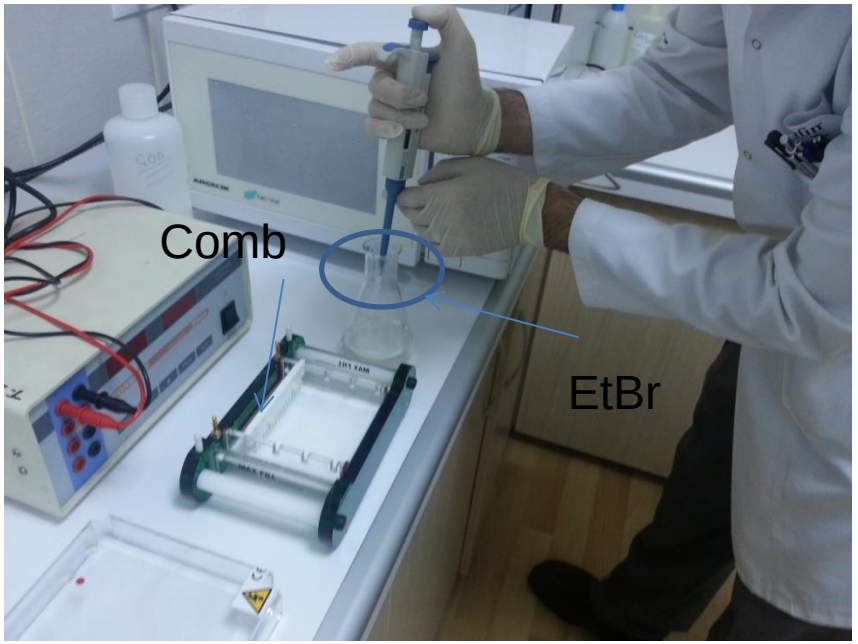
- Agarose, desired concentration.
  - 100 ml .....gr.
- Dissolve in buffer.
- Swirl the solution periodically.
- Melt in the microvave.
  - Clear solution when melt.
  - Be careful. The boiling agarose solution may be so hot.

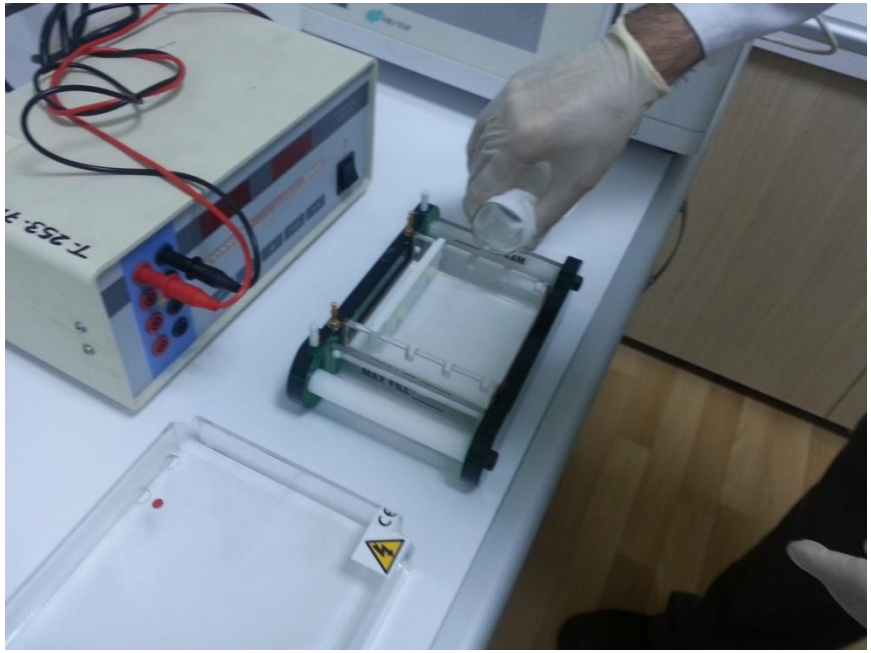




- Wait a few minutes for cooling and add EtBr.
- Insert the” comb”
- Prepare apparratus, pour gel.
- Wait until the gel hardens(15-20min.) control the gel
- Pull out the comb carefully.

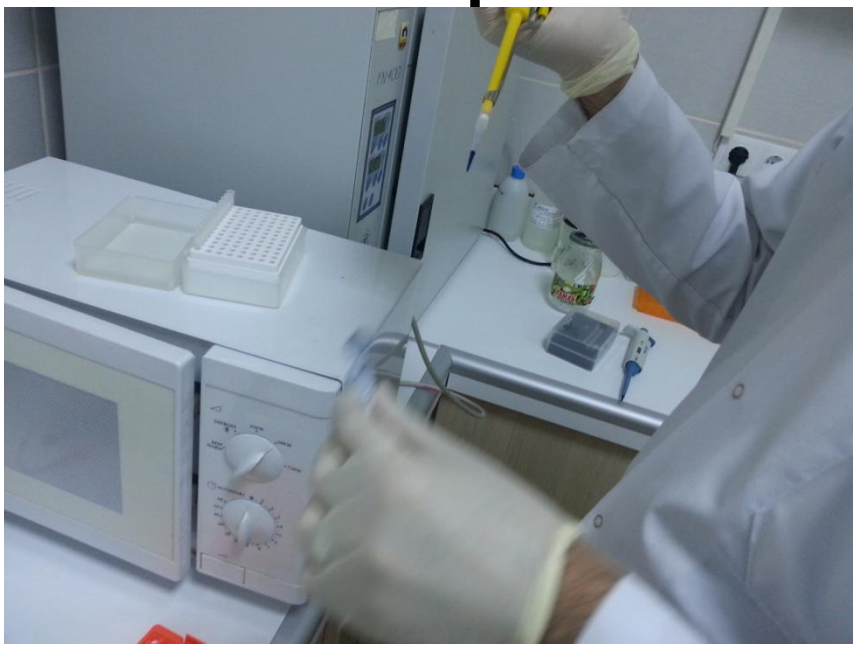
- Do not forget ! EtBr is a powerful mutagen wear gloves.

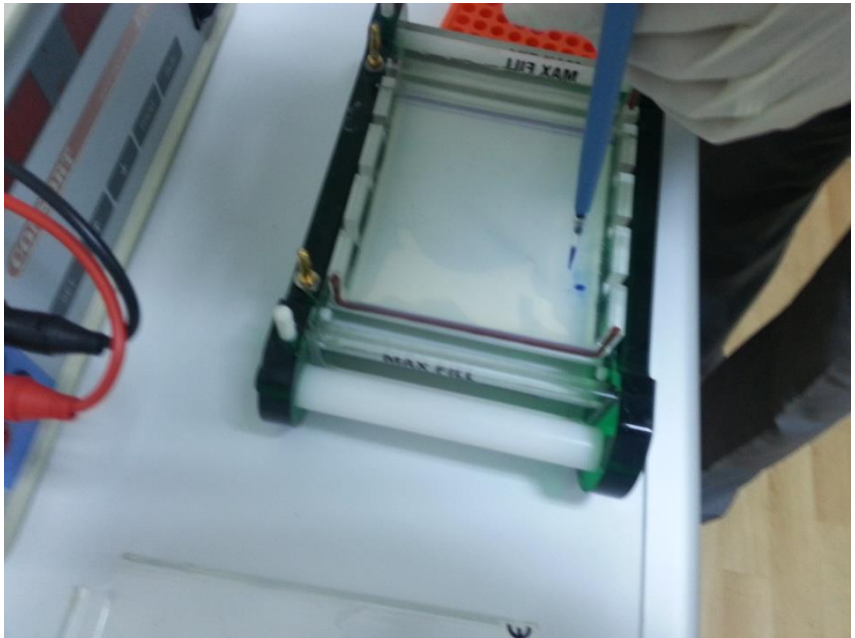




Load markers/Load samples

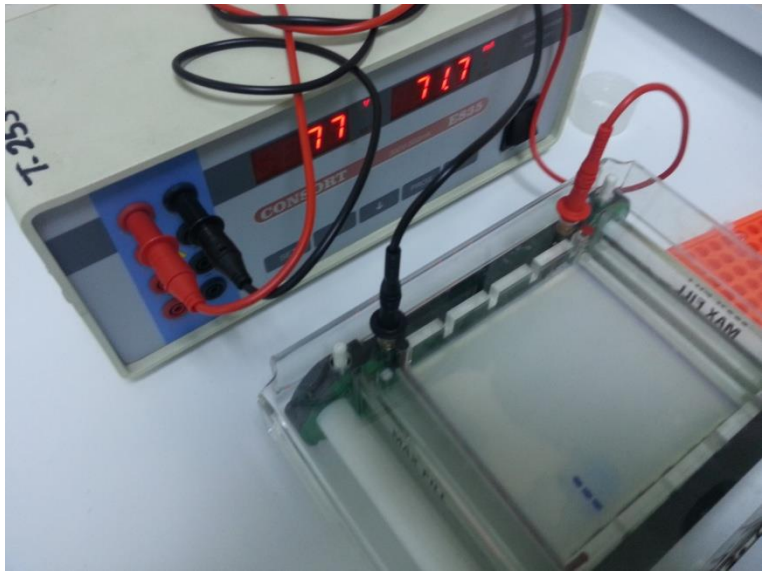
- Load markers to first well.
- Load the samples mixed with a dense loading dye.
- Be careful ! Not to contaminate other wells
- Be careful! Not to perforate the well.
- Loading dye includes:
  - Bromophenol Blue
  - Glycerol
  - Other:Xylene Cyanol FF ,EDTA

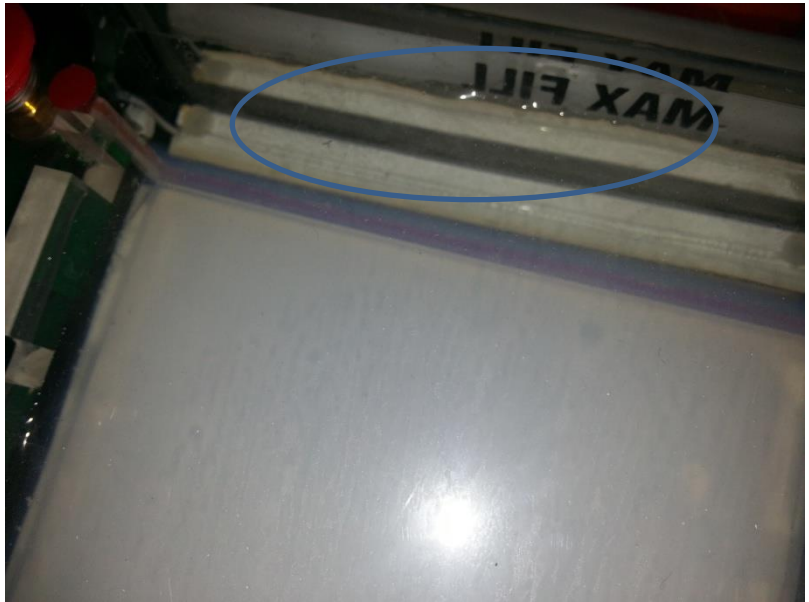




Running of the gel

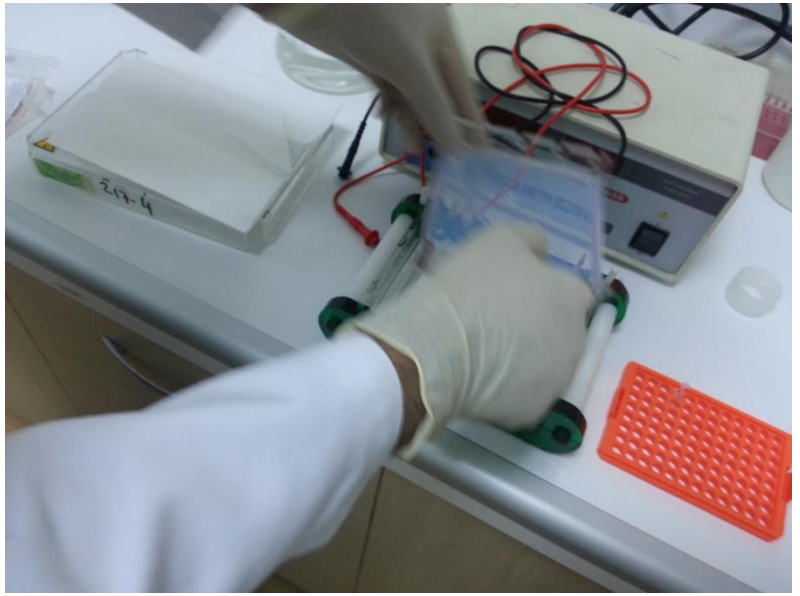
- Control the anode and cathode
- The power source is turned on.
- Air bubbles!
- The gel is run.
- The time depends upon the amount of current and % gel.
- Control the gel several times
- Track bromophenol blue. (migrate near 300bp/%1,5 agarose)



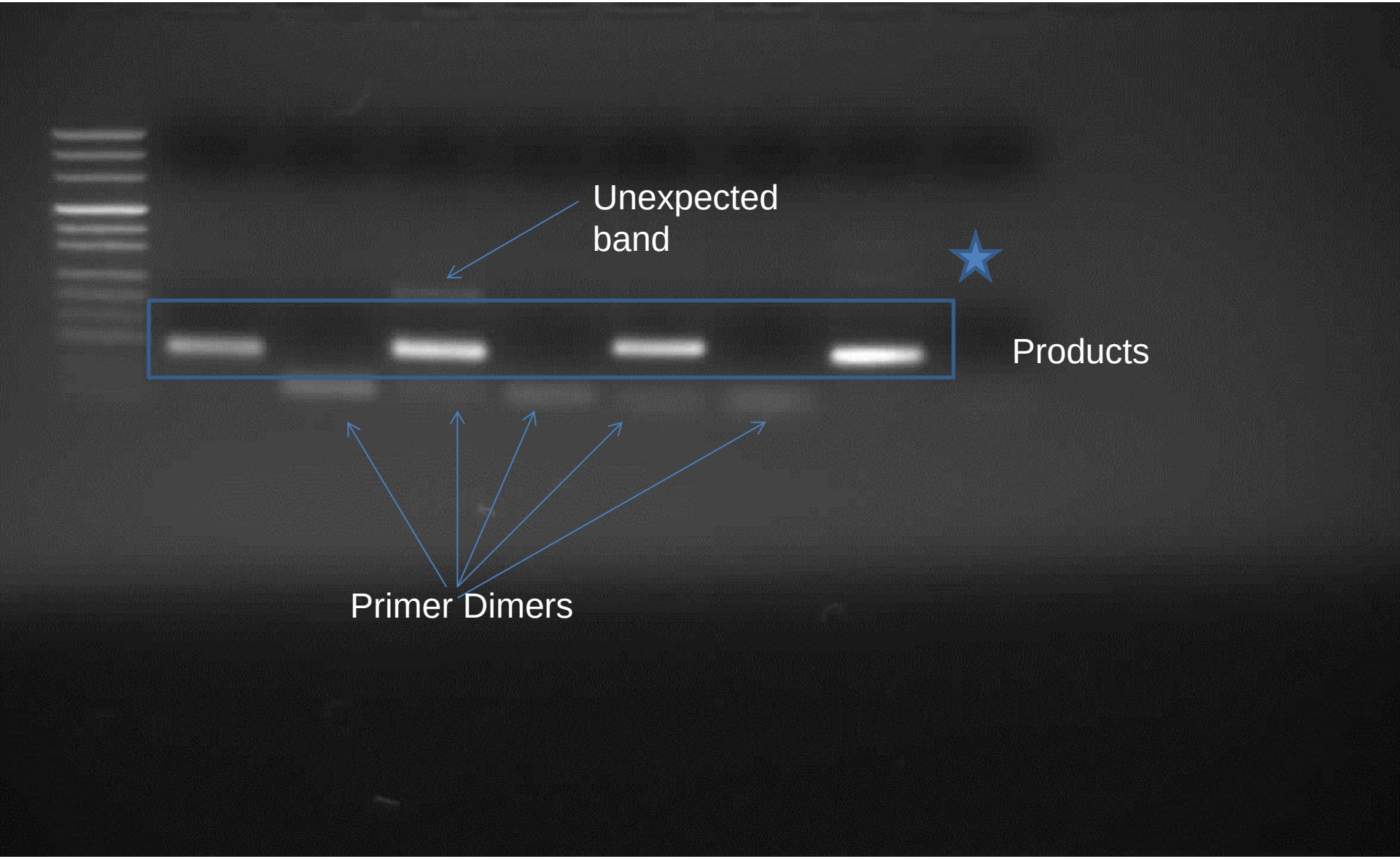


Photography, gel documentation

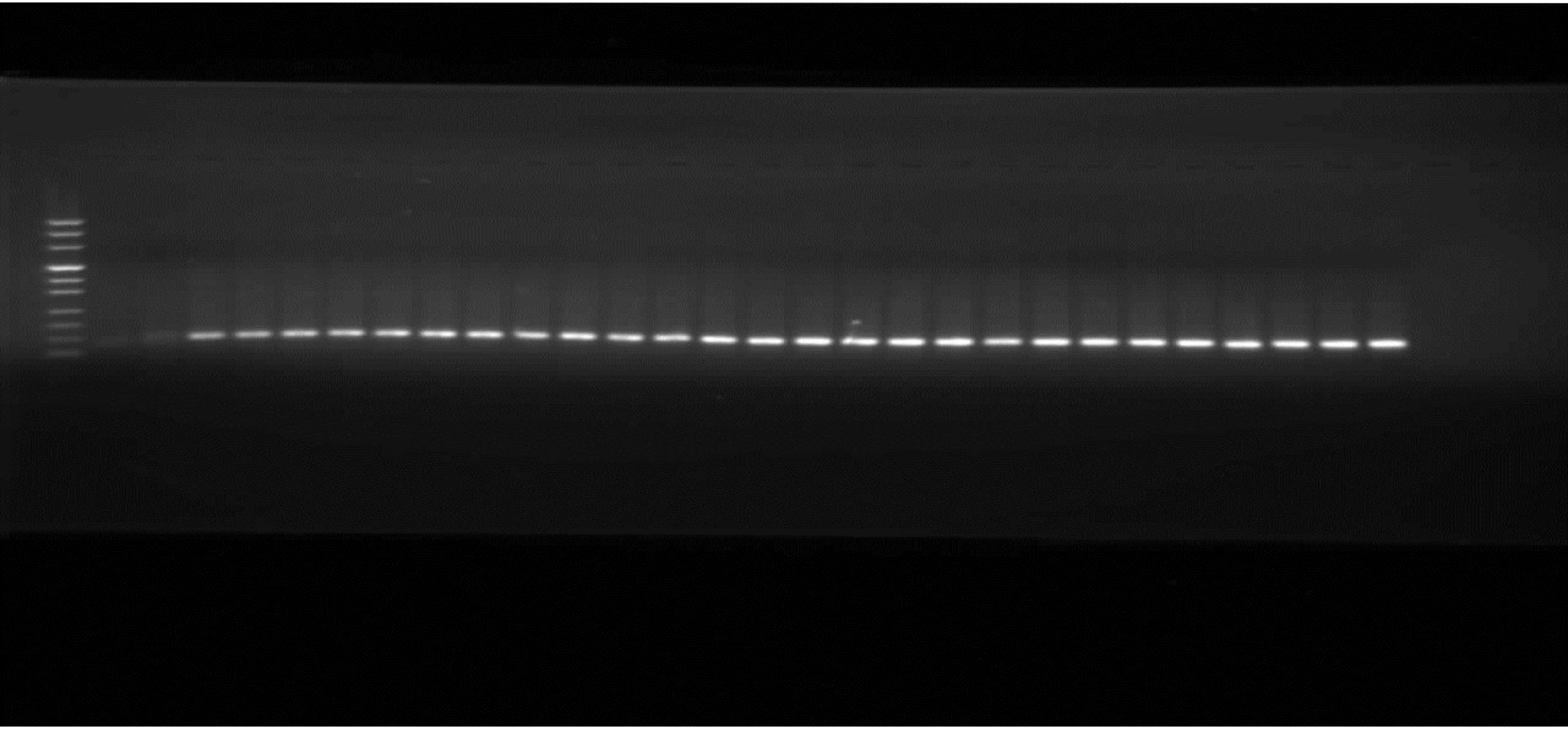
- Take off the gel.
- Stain if you do not add EtBr before gel casting.
- The gel is then visualized by UV light.
- Analyse the gel.
- You can also
  - Cut the band on the gel(DNA) for further techniques.(e.g. Plasmid studies)
  - Remove from the gel(e.g. Southern Blotting)



TXNIP Primer selection.



Kolon cell lines GAPDH



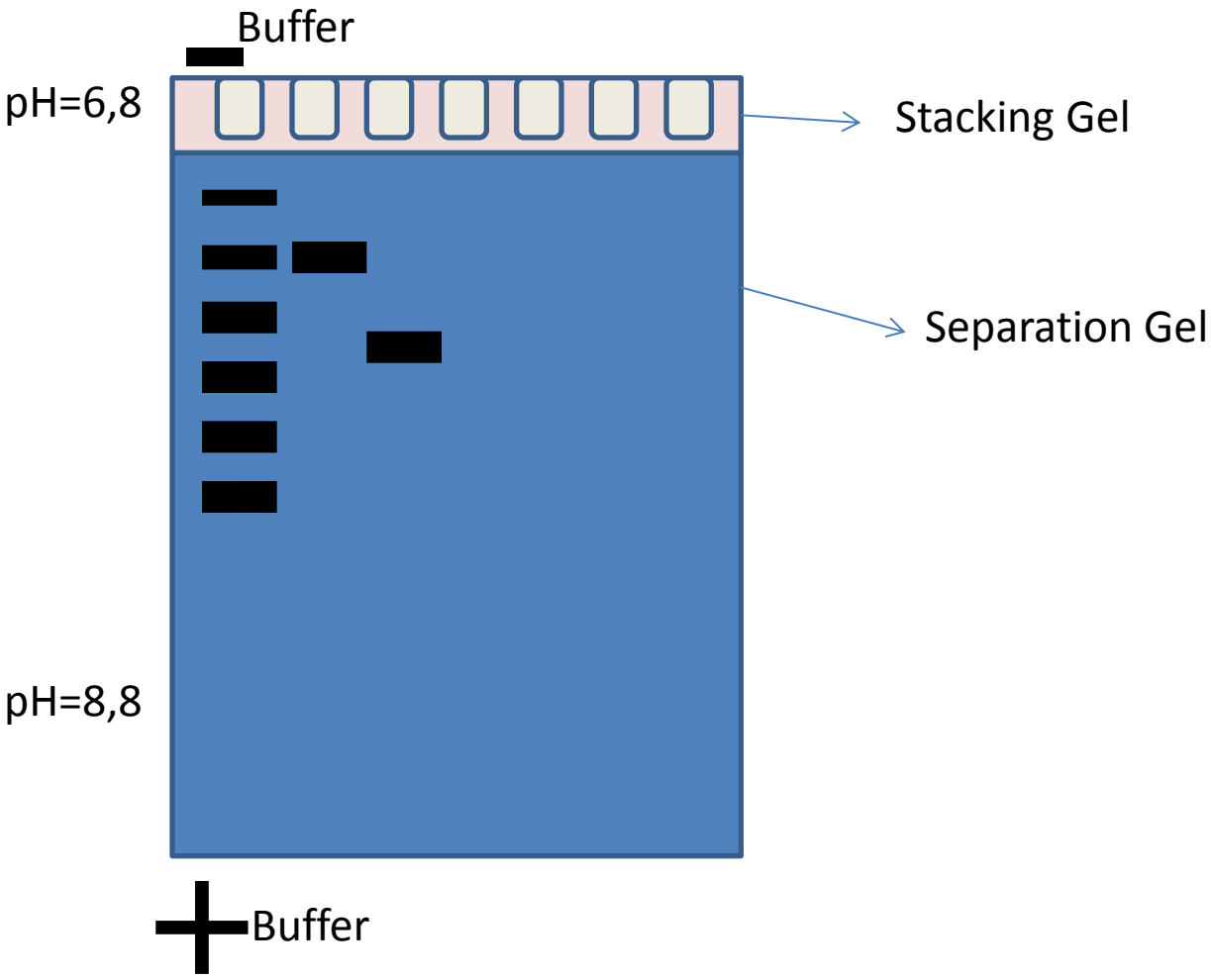
Protein Electrophoresis

- Simple to use and highly reproducible technique.
- Provide information of the molecular weight, charged, subunits, purity of protein mixture.
- SDS-Page most common used technique.
  - Native PAGE:
    - Separates folded proteins by charge, size, and shape.
  - Denaturing gel electrophoresis
    - Separates folded proteins by size.

Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

- SDS PAGE:
  - Denaturing gel electrophoresis
  - Give information of the size of polypeptide chains.
  - Separated by length of their polypeptide chains not by its charge.
  - SDS binds to and unfolds the protein established a negative charge.
  - Without SDS proteins migrate charge mass ratio.

Polyacrylamide Gel Electrophoresis (PAGE)



- **Stacking Gel**
  - Low concentration
  - 4-5% acrylamide
  - For concentrating proteins
  - Large pore size
- **Separating(Resolving) gel**
  - High concentration
  - 5-20 % acrylamide
  - Smaller pore size

Acrilamide concentration/Molecular weight

Concentration	Molecular weight
15	12-43 kD
10	16-68
7,5	36-94
5	57-212

Temizkan G, Arda N. Moleküler biyolojide kullanılan yöntemler. Nobel tıp.

Protein Electrophoresis

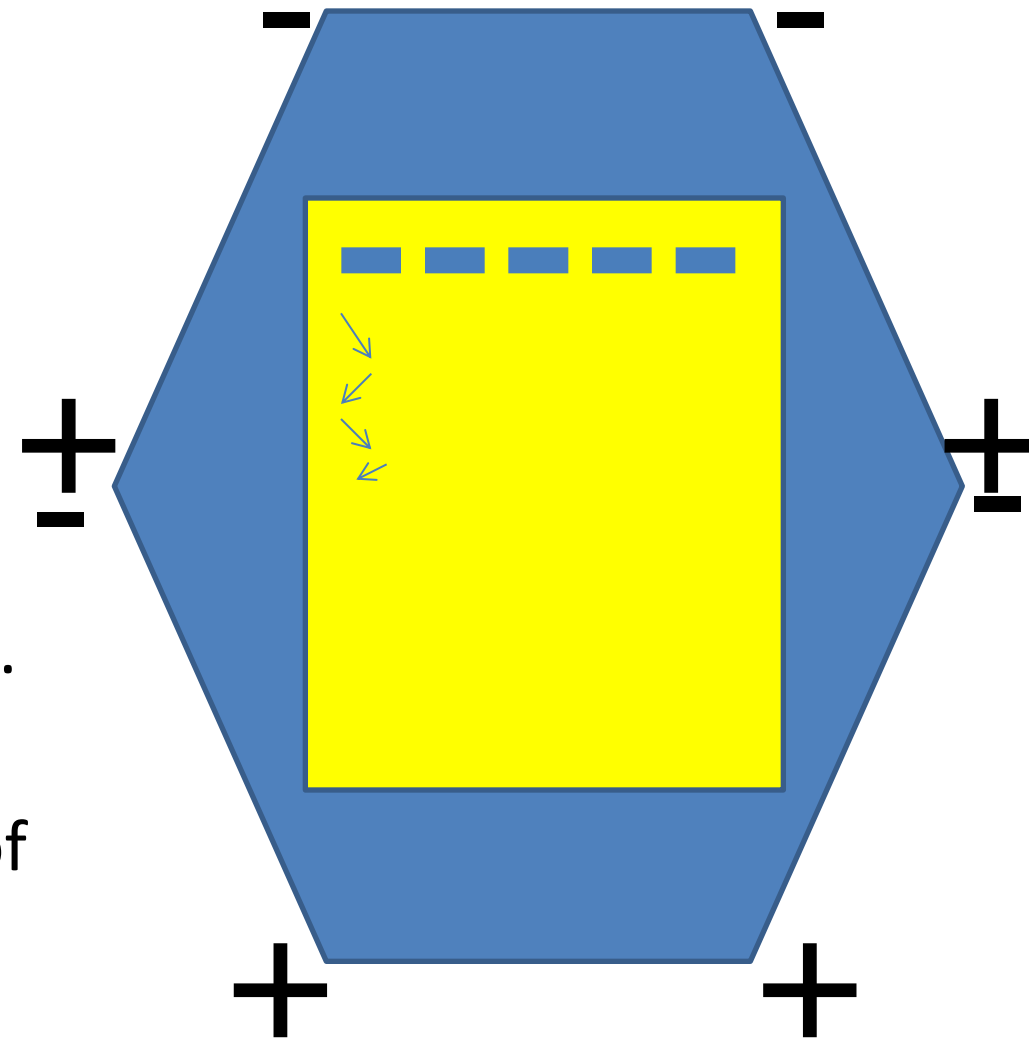
- Proteins in the gel stained by:
  - Coomassie Blue dye
  - Silver staining
  - Others(Flourescence, commercial dyes)

Other Protein Electrophoresis Techniques

- IEF(Isoelectric focusing)
  - Separates proteins by their isoelectric points (pI) by using pH gradient of the gel.
- 2D PAGE(Two dimensional gel electrophoresis)
  - Separates proteins are by two properties (eg: pI and size) in a mixture.
- Western blotting:
  - Separating proteins first by size then staining with specific antibody-antigen reactions.
  - Technique gives molecular weight and identifies specific protein.

Pulsed Field Gel Electrophoresis (PFGE)

- Used for separating very large DNA molecules. (1Mb<)
- Based on the periodically changes of directions in the electric field.
- Gnll. used for genotyping.
- Gold standard in epidemiological studies of (Subtypes)pathogenic organism

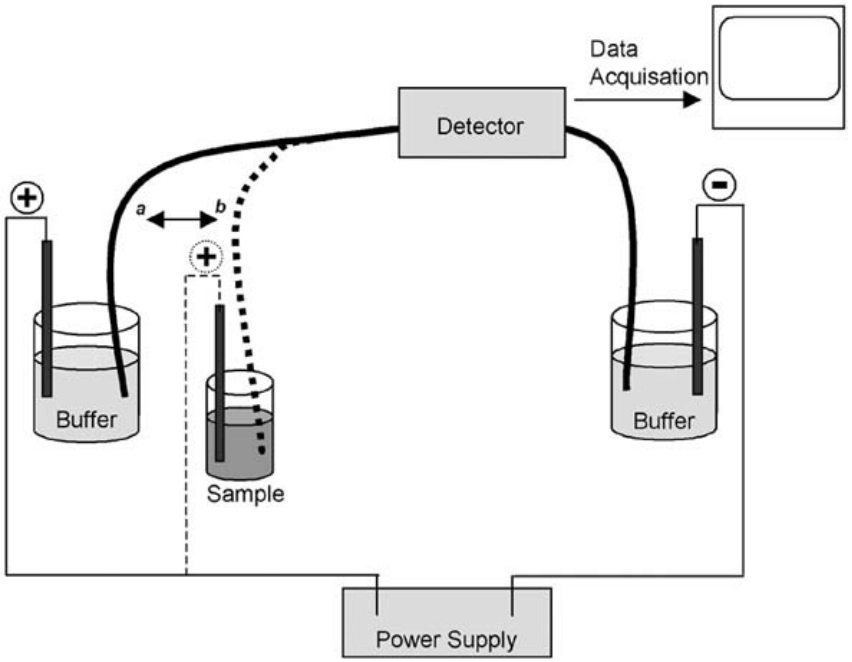


Capillary Electrophoresis

- Process large number of samples than classical techniques.
- Main technique first described by Hjerten in 1967.
- The first commercial CE instrument in 1988.

Principle

- Power supply.
- The anode and cathode buffer reservoirs with corresponding electrodes.
- The separation chamber(capillary tube).
- The injection system.
- The detector



*GEORGE P. PATRINOS , WILHELM ANSORGE ; Molecular Diagnosis

Capillary Electrophoresis

- Applications
  - Analyzing proteins in physiological matrices (eg.Serum, urine)
  - DNA analysis
  - Drug screening.
  - Analysis of pesticides, food content, pollutants.

Specific Applications

- Neoplastic disorders
  - Detection of tumor-related mutation.
  - Microsatellite instability
  - Analysis of monoclonality.
- Diagnosis of hereditary diseases and prenatal testing
- Diagnosis of infectious diseases
- Identity testing

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