

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 across.
- 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

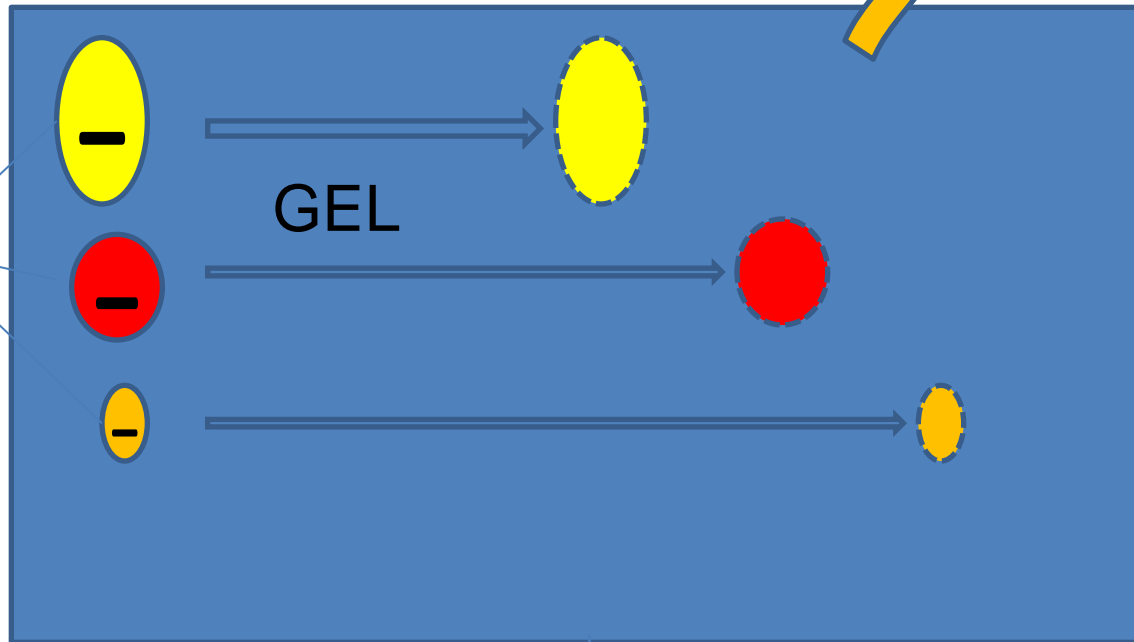
Cathode

Buffer

Anode



Sample



Electric Field

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

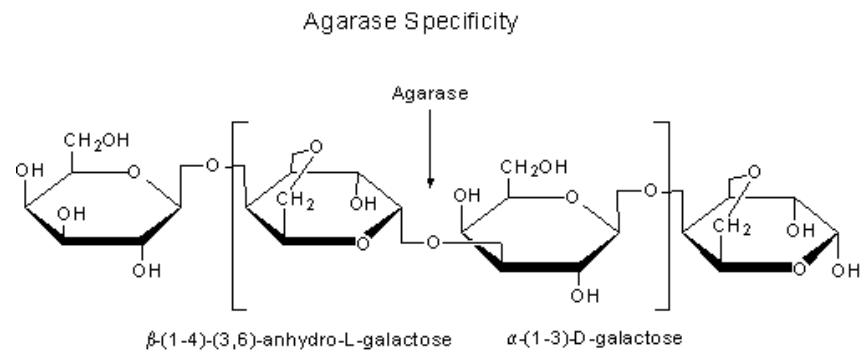
- Use of a gelatinous material.
- The gel acts as a support medium
- Used to separate proteins or nucleic acids.

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

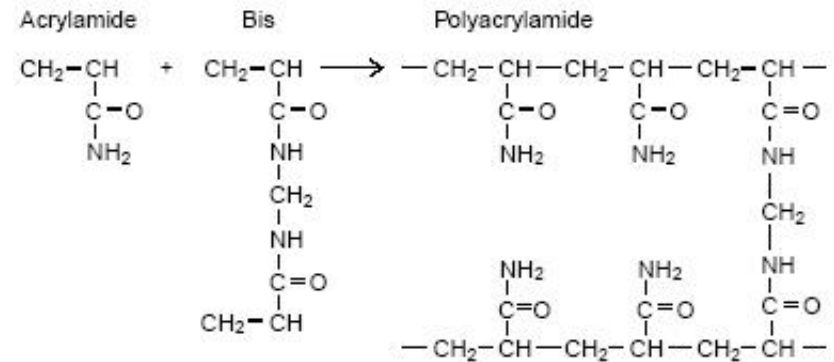
Agarose Gel Concentration/DNA-Size

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/>

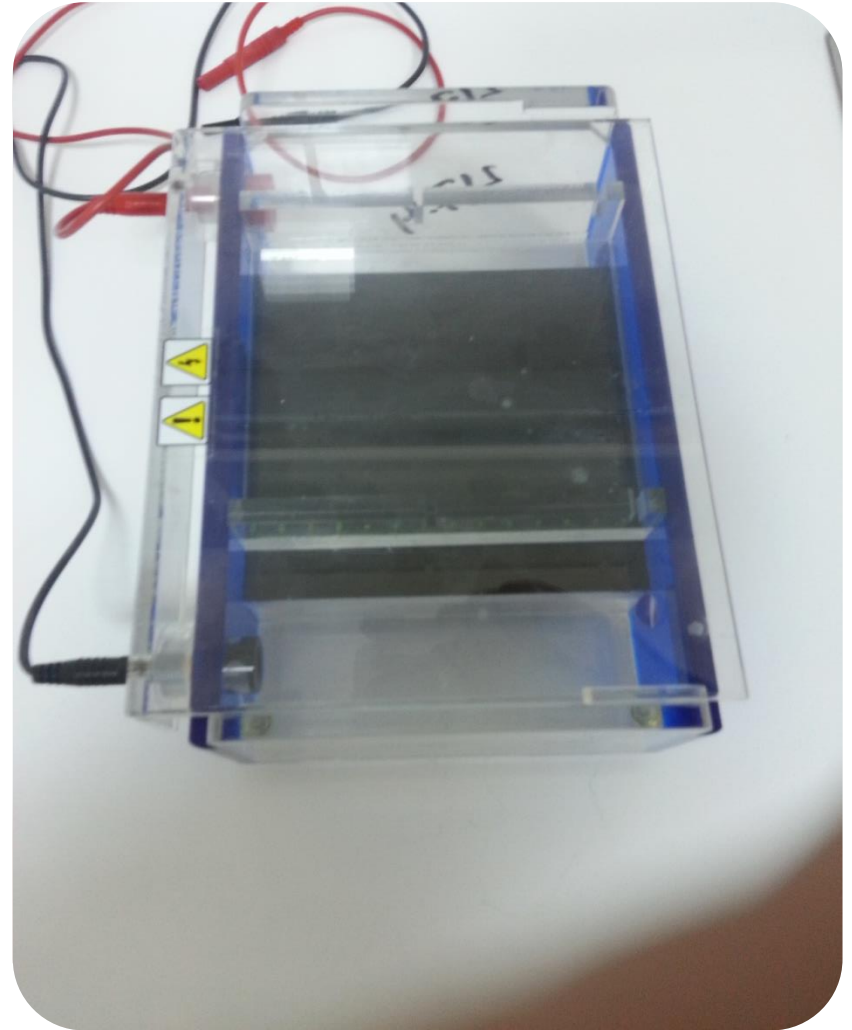
Equipment

- 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 laboratory equipments:
 - pH meter
 - Pipettors
 - Lab. Scale
 - Stir plates

Electrophoresis Gel Apparatus

Horizontal (Flat bed)

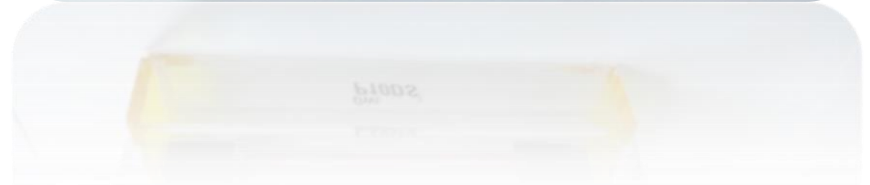
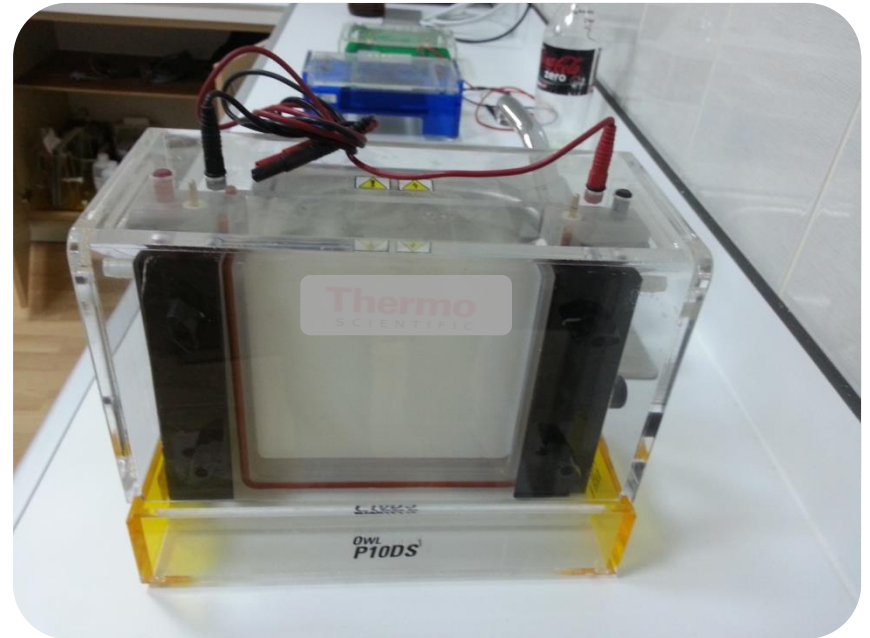
- Gel thickness limited.
- Only one gel per apparatus
- Easily adapts different techniques.
- Technician friendly
- More safe for electricity accidents.
- Gnl. used for agarose gel electrophoresis



Electrophoresis Gel Apparatus

Vertical

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



Gel Documentation System



Gel Documentation System

UV/White Lamp Box



Power Supply



Technique

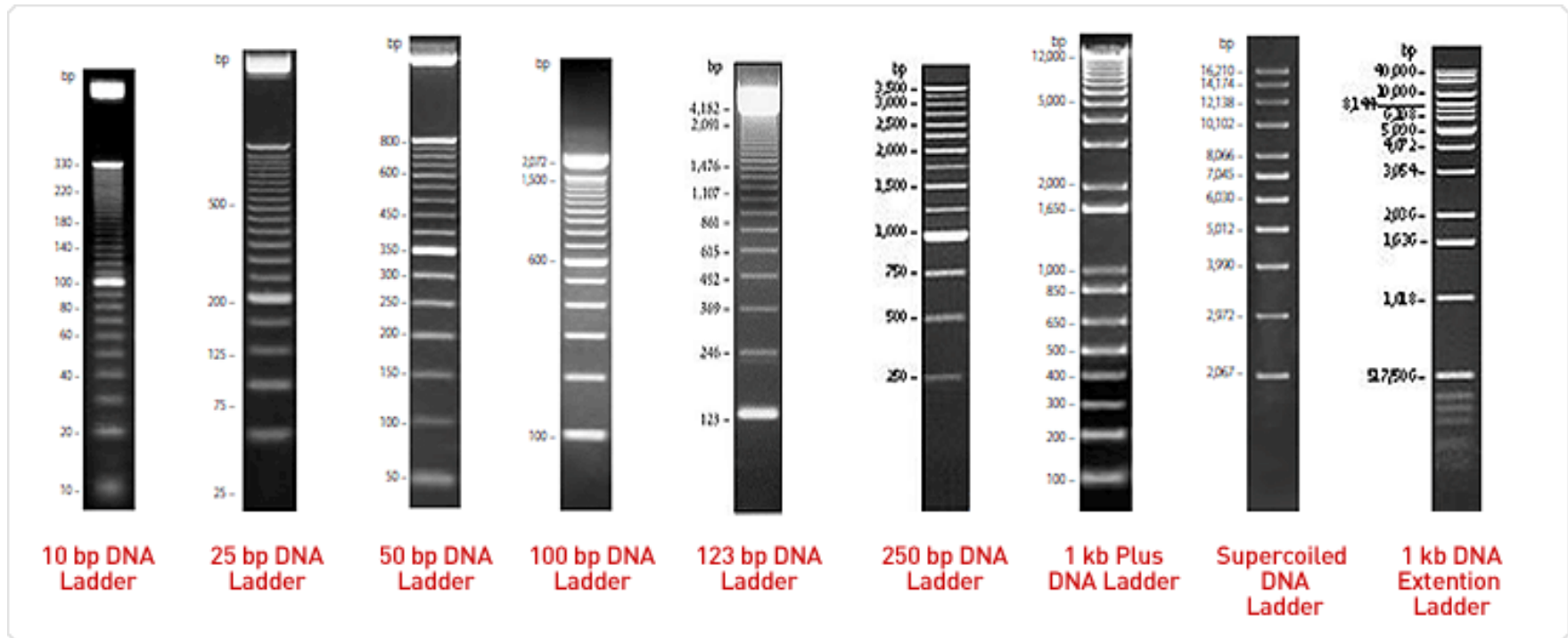
- Steps
 - Sample preparation
 - Gel, buffers, etc. preparation.
 - Load markers
 - Load samples
 - Running of the gel
 - Staining of the gel
 - Photography, gel documentation
 - Interpret/analysis of gel



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

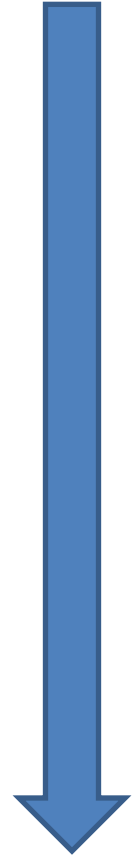
- Sybr stains.
 - Silver stains
 - Methylene Blue
 - Commercial stains.
-
- *More safe, less sensitive.*
 - *Syb stains also mutagenic.??*

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 (Empirical approach)

Technique

- Sample preparation
- Gel, buffers, etc. preparation.
- Load markers
- Load samples
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Technique

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

TBE Buffer X10

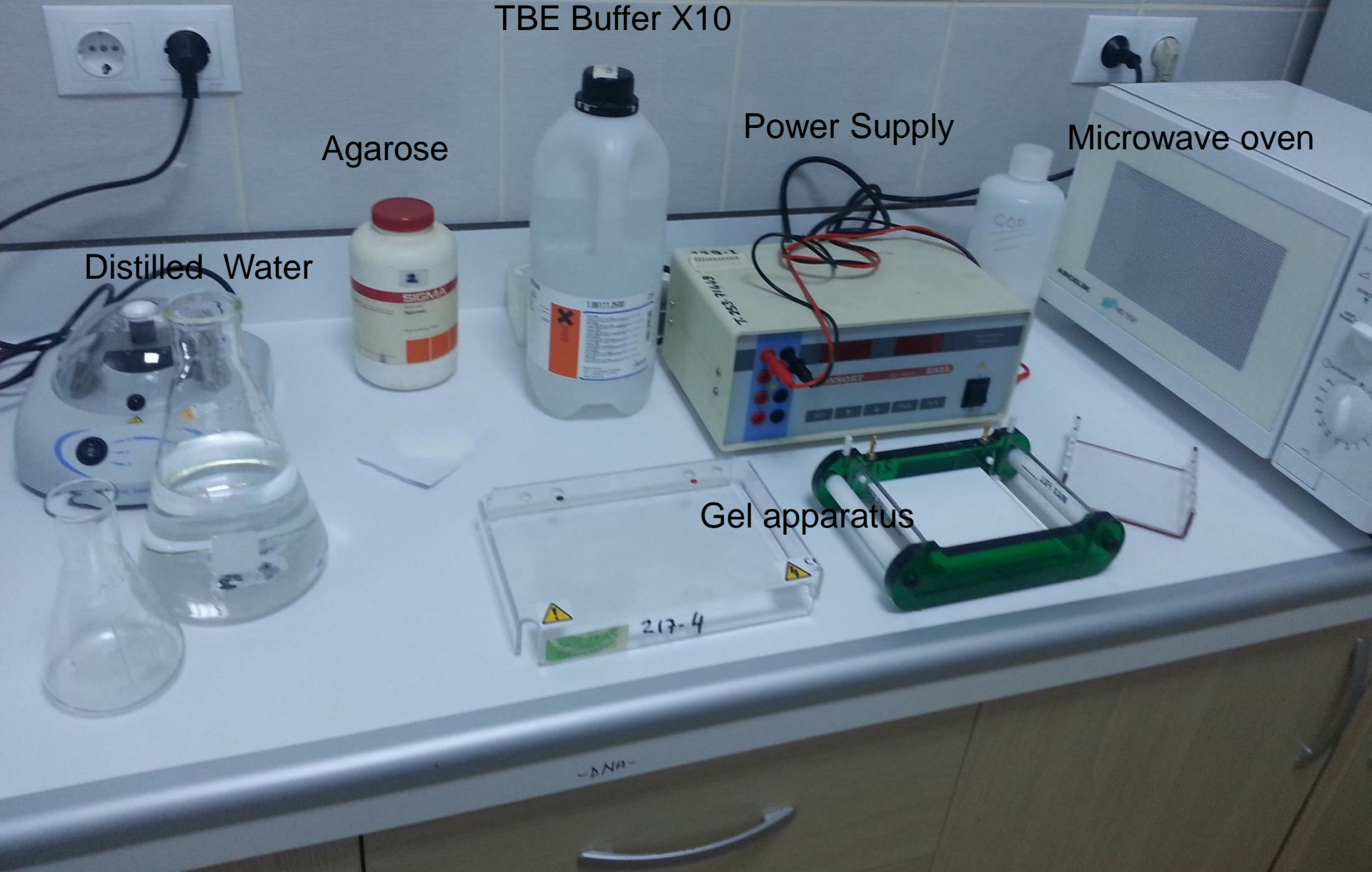
Agarose

Power Supply

Microwave oven

Distilled Water

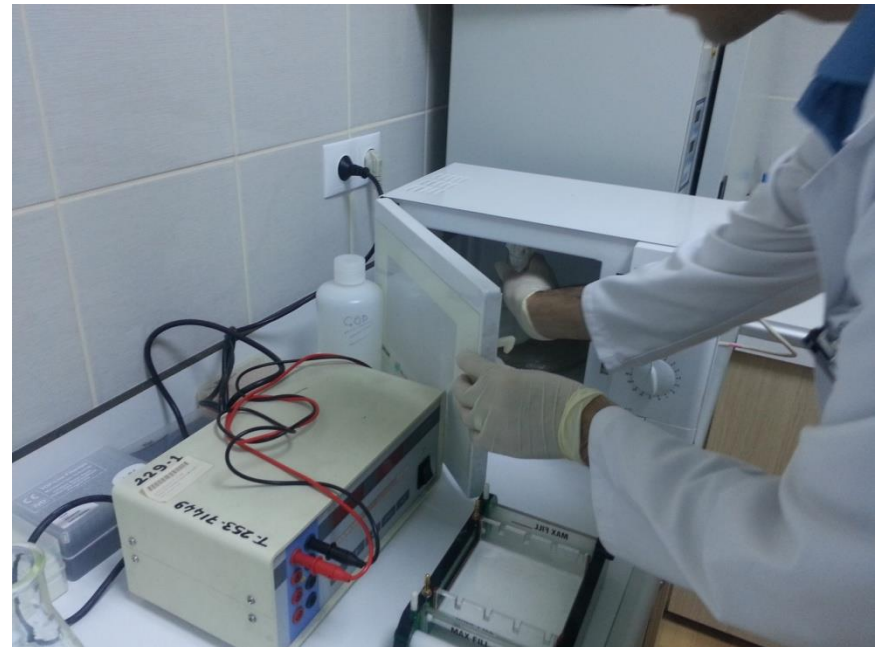
Gel apparatus



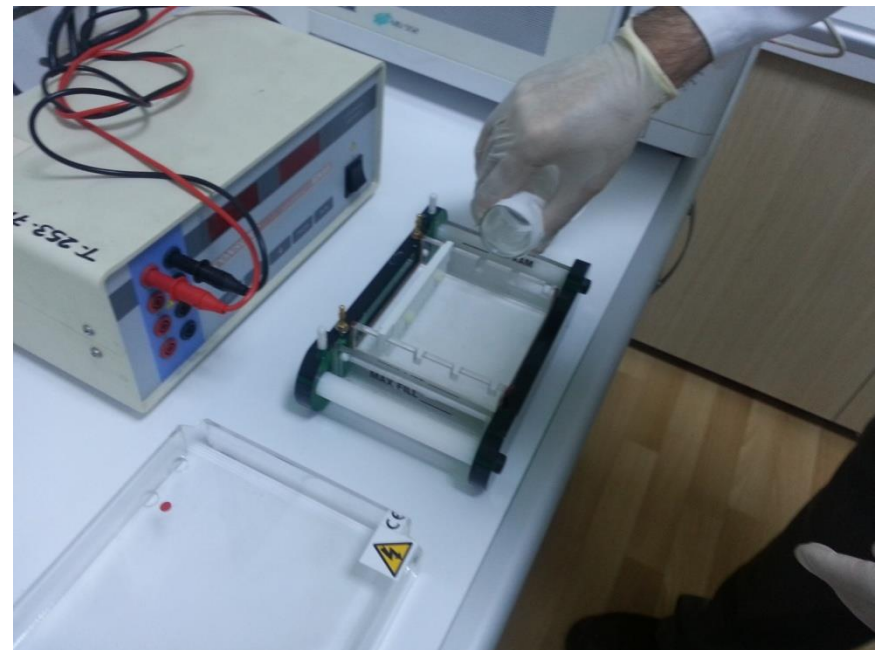
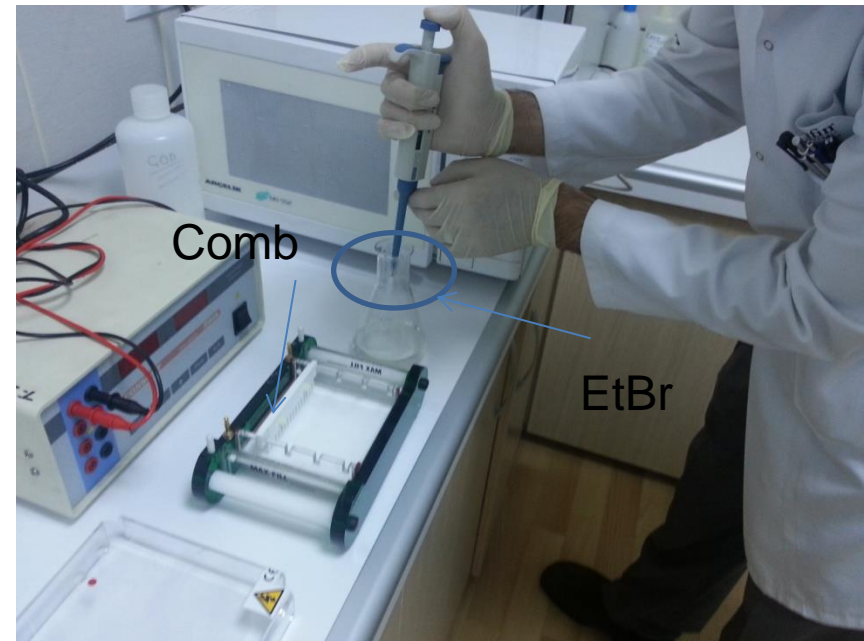
Gel, buffers, etc. preparation.

- Agarose, desired concentration.
 - 100 mlgr.
- 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 mlgr.
- 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.

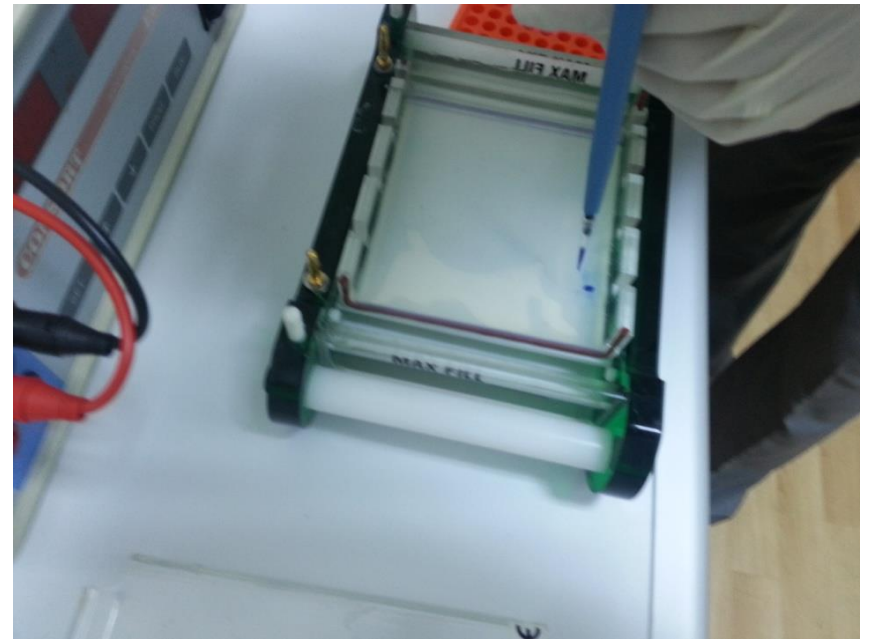
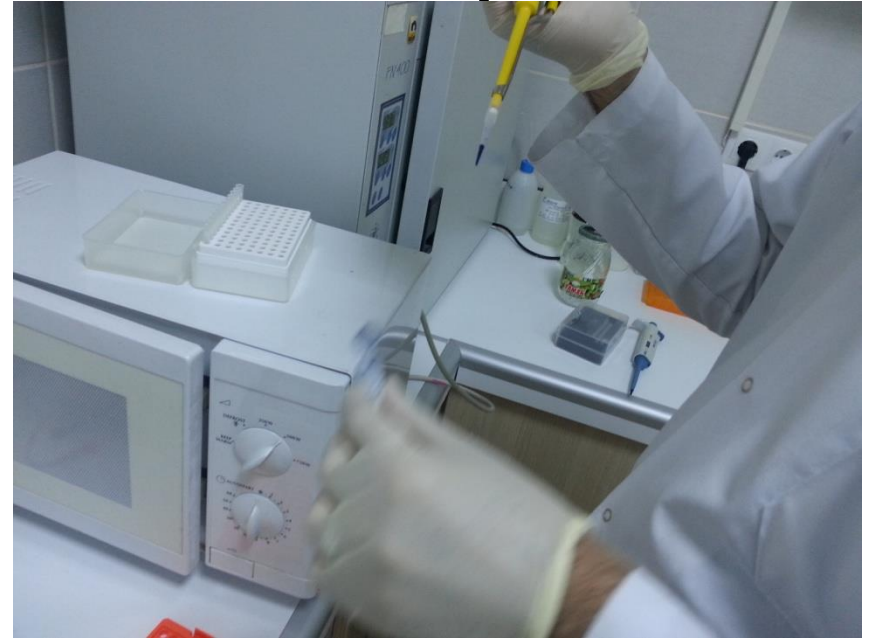


- Wait a few minutes for cooling and add EtBr.
- Insert the “comb”
- Prepare apparatus, pour gel.
- Wait until the gel hardens (15-20 min.)
control the gel
- Pull out the comb carefully.
- Do not forget ! EtBr is a powerful mutagen wear gloves.



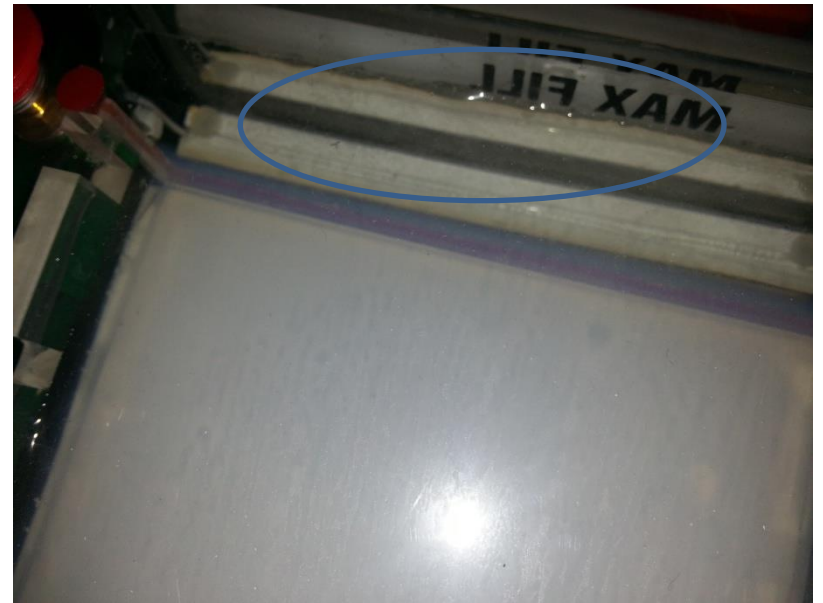
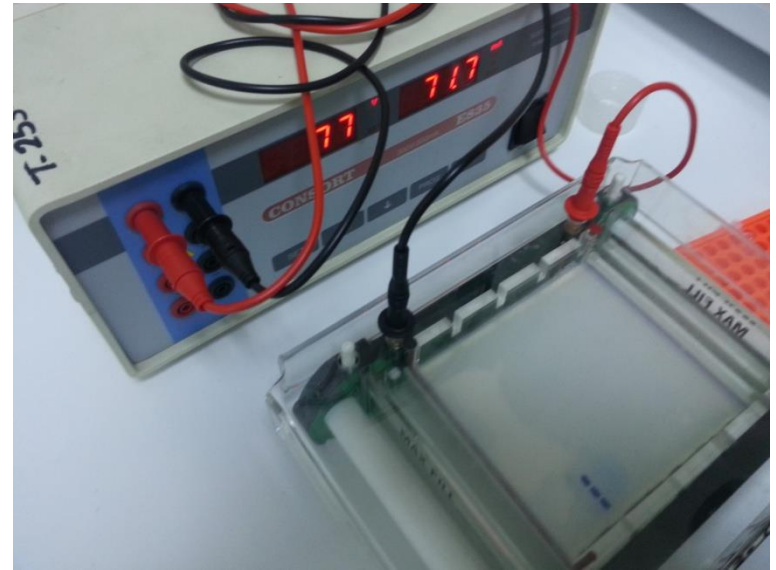
Load markers/Load samples

- 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



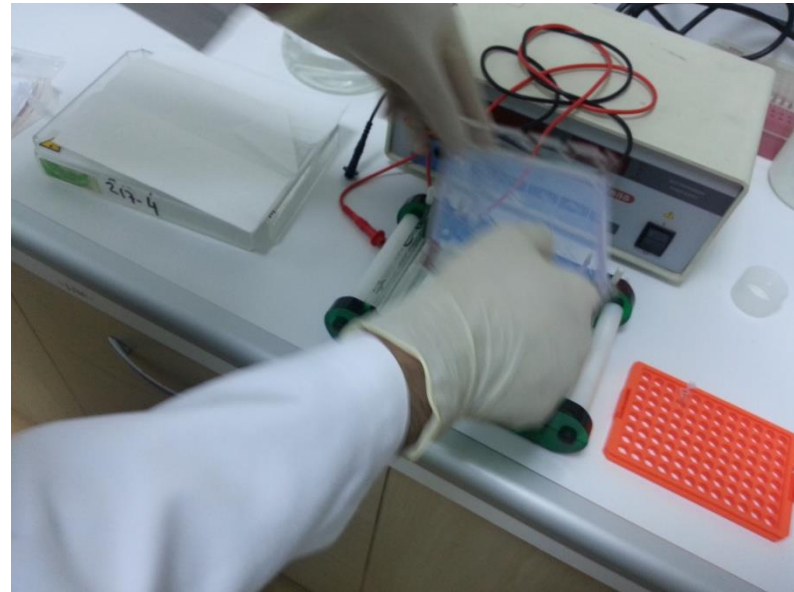
Running of the gel

- 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)

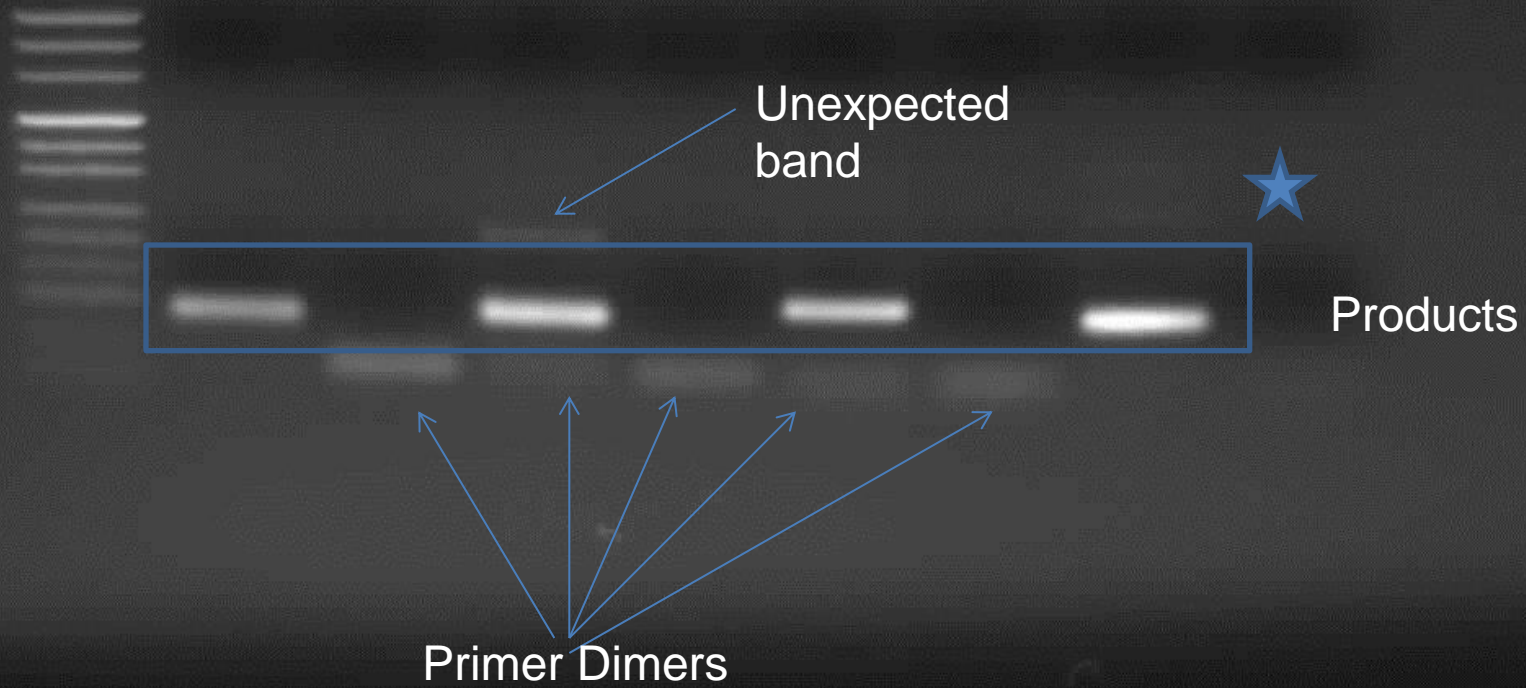


Photography, gel documentation

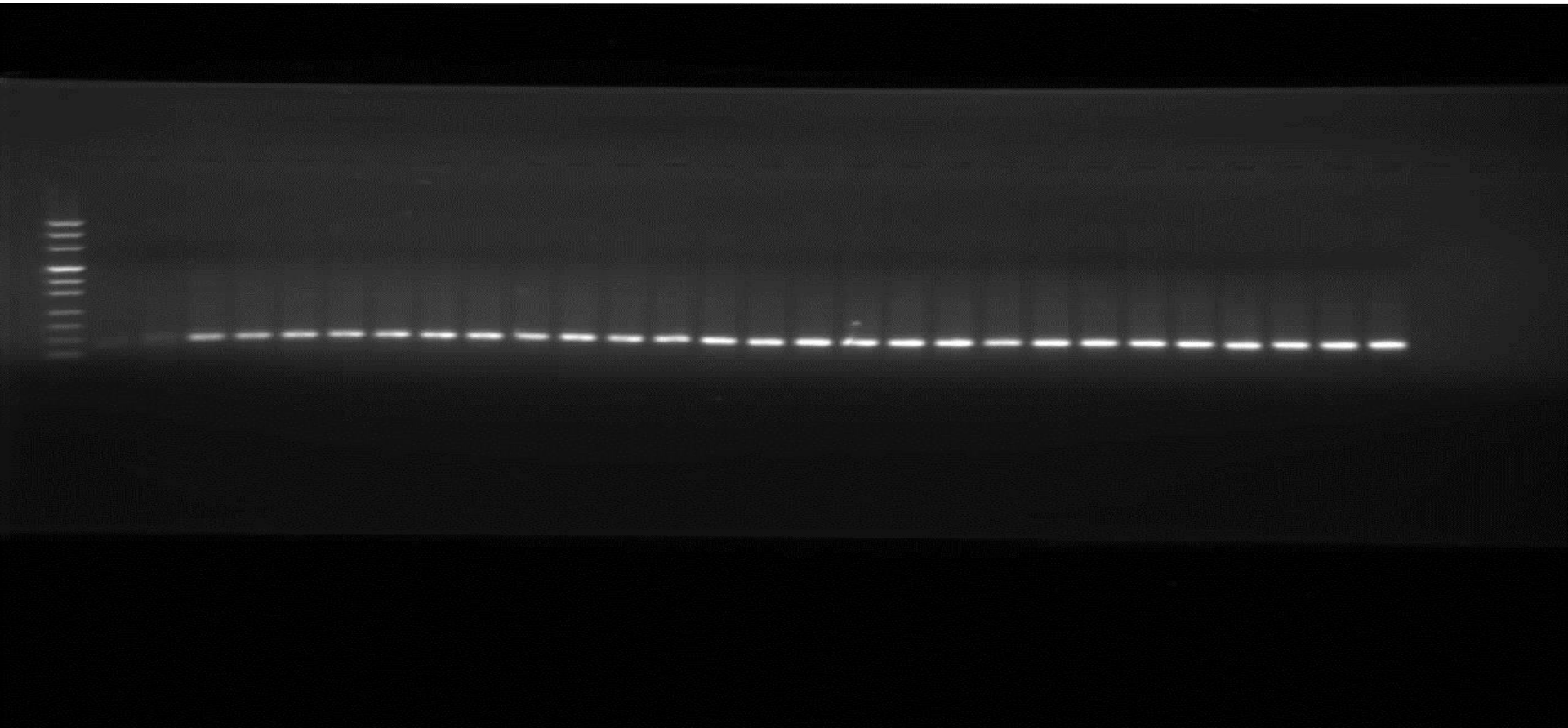
- Take off the gel.
- Stain if you do not add EtBr before gel casting.
- The gel is then visualized by UV light.
- Analyse the gel.
- You can also
 - Cut the band on the gel(DNA) for further techniques.(e.g. Plasmid studies)
 - Remove from the gel(e.g. Southern Blotting)



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.

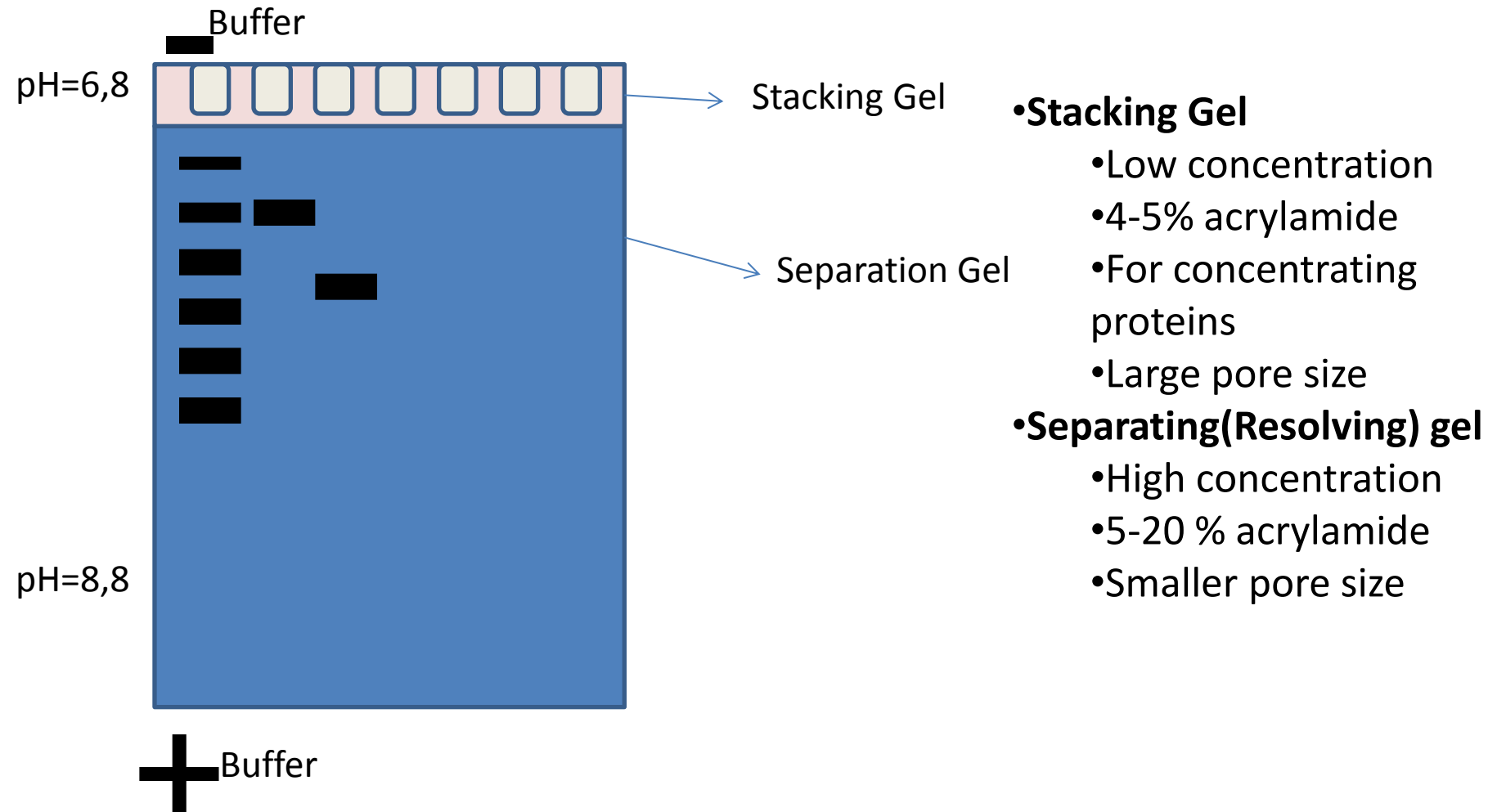
Sodium Dodecyl Sulfate Polyacrylamide Gel Electrophoresis

– 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)



Acrilamide concentration/Molecular weight

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

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Protein Electrophoresis

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