MOLECULAR BIOLOGY RESEARCH UNIT

AGAROSE GEL ELECTROPHORESIS

By

Prof. Dr. Asmaa Hussein

Prof. of Zoonoses & Director of the MBRU

Assiut University

AGAROSE GEL ELECTROPHORESIS

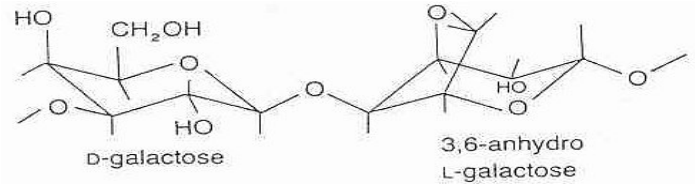
The standard **method used to separate, identify and purify DNA fragments is electrophoresis through agarose gels.**

The technique **is**

- ⇒ **Simple**
- ⇒ **Rapid to perform**
- ⇒ **Capable of resolving mixtures of DNA fragments**
- ⇒ **The location of DNA within the gel can be determined directly**

(AGAROSE)

Is extracted **from seaweed and is a linear polymer whose basic structure is**



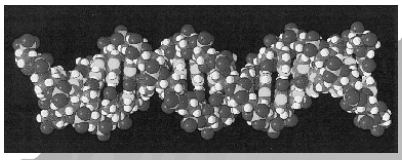
Commercially **available agarose is not completely pure; it is contaminated with other polysaccharides, salts, and proteins.**

The amount **of contamination varies from batch to batch and from manufacture to manufacture.**

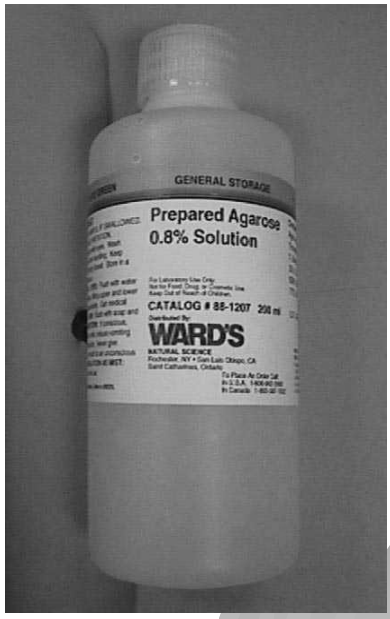
These difference can affect on

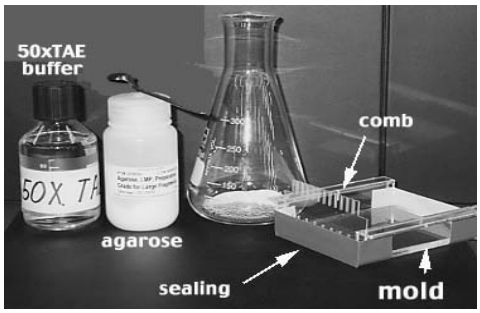
⇨ **Migration of the DNA**

⇨ **Ability of the DNA recovered from gel**

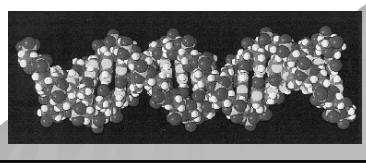


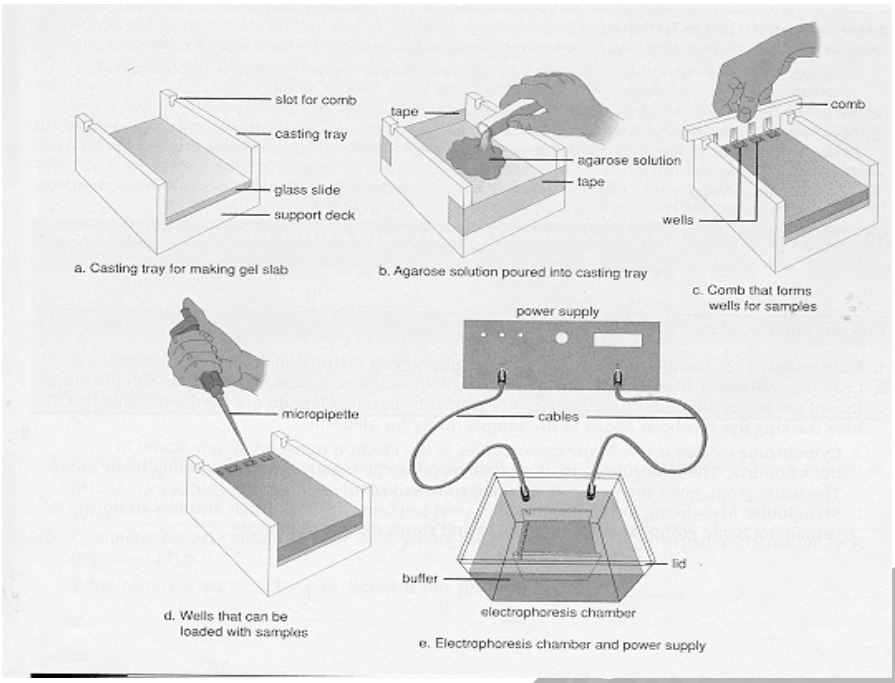
Now, **most manufacturers prepare special grades of agarose that are screened for the presence of inhibitors and nucleases and for minimal background fluorescence after staining with ethidium bromide.**





Agarose gels **are cast by melting the agarose in the presence of the desired buffer until a clear, transparent solution is achieved.** ***The melted solution*** **is then poured into a mold and allow to harden. Upon hardening, the agarose forms matrix and when an electric field is applied across the gel, DNA which is negatively charged at neutral PH, migrates toward the anode.**





Factors Affecting the Rate of DNA Migration in Agarose Gels:

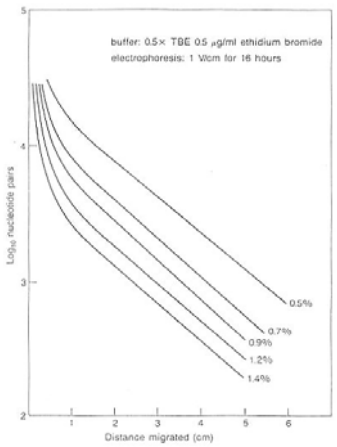
1) Molecular size of the DNA:

Larger molecules **migrate more slowly than smaller molecules.**

Because of

⇨ **Greater frictional drag.**

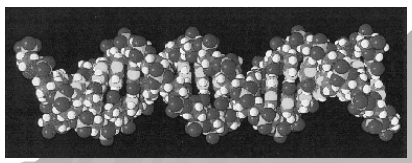
⇨ **They worm their way through the pores of the gel less efficiently than smaller molecules.**



2) Agarose Concentration:

A linear DNA **fragment of a given size migrate at different rates through gels containing different concentrations of agarose.**

By using gels **of different concentrations, it is possible to resolve a wide size of DNA molecules.**



Range of separation in gels containing different amounts of agarose

Amount of agarose in gel (%[w/v])	Efficient range of separation of linear DNA molecules (kb)
0.3	5-60
0.6	1-20
0.7	0.8-10
0.9	0.5-7
1.2	0.4-6
1.5	0.2-3
2.0	0.2-3

3) DNA Conformation:

Superhelical circular DNAs

Niked circular DNAs

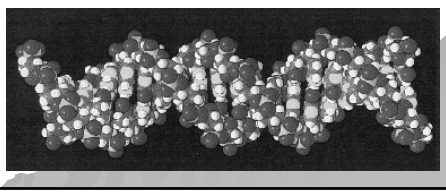
Linear DNAs

Of the same molecular weight

Migrate through agarose gels at different rates

The relative mobilities of the 3 forms depend on:

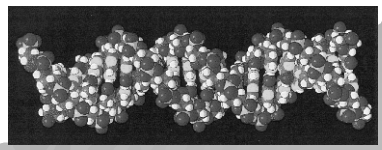
- **Agarose concentration in the gel (Primarily)**
- **Strength of the applied current**
- **Ionic strength of the buffer**



4) Applied Voltage:

At low voltages, **the rate of migration of linear DNA fragments is proportional to the voltage applied.**

However, **as the electric field strength is raised, the mobility of high-molecular-weight fragments of DNA increase differentially.**



5) Direction of the electric field:

DNA molecules **larger than 50-100 kb in length migrate through agarose at the same rate if the direction of the electric field remains constant.**

6) Base composition & Temperature:

The electrophoretic **behavior of DNA in agarose gels is not significantly affected by either the base composition of the DNA or the temperature at which the gel is run.**

In general, agarose gels are run at room temperature

7) Presence of Intercalating Dyes:

- **The central dye in agarose gel electrophoresis is *ethidium bromide*.**
- **It has the unique property of fluorescing under UV light when intercalated with DNA.**
- **By running DNA through an EtBr-treated gel and exposing it to UV light, distinct bands of DNA become visible.**

Ethidium Bromide is a carcinogen and should be handled with care

Other dyes **are sometimes used including SYBER green or SYBER safe. SYBER dyes are thought to be less carcinogenic than EtBr and to give cleaner, higher powered staining.**

8) Electrophoresis Buffer:

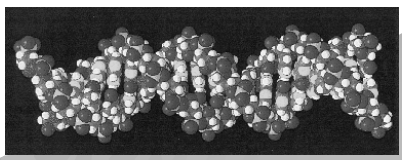
The electrophoretic **mobility of DNA is affected by the composition and ionic strength of the electrophoresis buffer.**

In the absence of ions **➔ electrical conductance is minimal and DNA migrates very slowly.**

In buffers **of high ionic strength ➔ electrical conductance is very efficient and significant mount of heat are generated.**

Several different **buffers are available for electrophoresis (TAE, TPE & TBE).**

Electrophoresis buffers **are usually made up as concentrated solutions and stored at room temperature.**



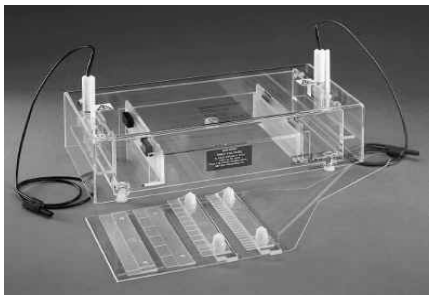
Commonly used Electrophoresis Buffers

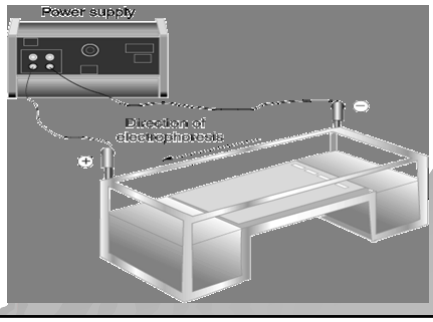
Buffer	Working solution	Conc. Stock solution (Per Liter)
Tris-acetate (TAE)	1X: 0.04 M Tris-acetate 0.001 M EDTA	50X: 243 g Tris base 57.1 ml glacial acetic acid 100 ml 0.5M ETDA (PH 8.0)
Tris-phosphate (TPE)	1X: 0.09 M Tris-phosphate 0.002 M EDTA	10X: 108 g Tris base 15.5 ml 85% phosphoric acid (1.679g/ml) 40 ml 0.5M ETDA (PH 8.0)
Tris-borate (TBE)	0.5X: 0.045 M Tris-borate 0.001 M EDTA	5X: 54 g Tris base 27.5 g boric acid 20 ml 0.5M ETDA (PH 8.0)
Alkaline	1X: 50 mN NaOH 1 mM EDTA	1X: 5 ml 10 N NaOH 2 ml 0.5M ETDA (PH 8.0)

Apparatuses Used For Agarose Gel Electrophoresis

The most **commonly used configuration is the horizontal slab gel.**

Horizontal **slab gels are usually poured on a glass plate or plastic tray that can be installed on a platform in the electrophoresis tank.**

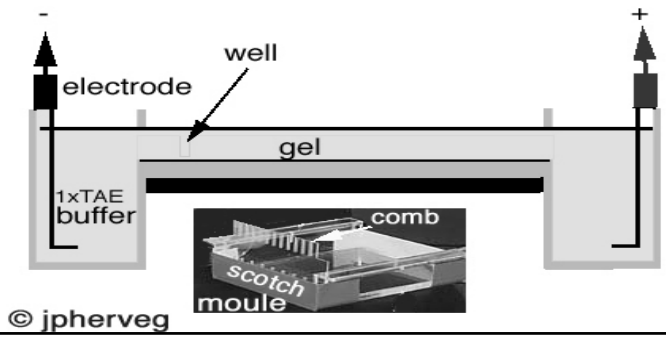




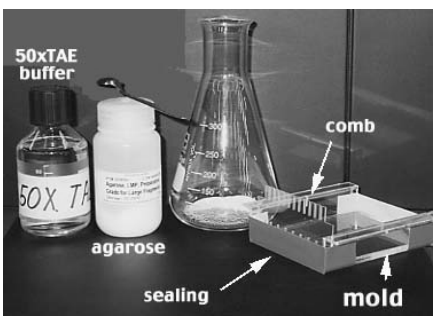
Preparation & Examination of Agarose gels

1. Seal the edges of a clean, dry, glass plate (plastic) with tape so as to form a mold. Set the mold on a horizontal section of bench.

2. Prepare the materials you will need.

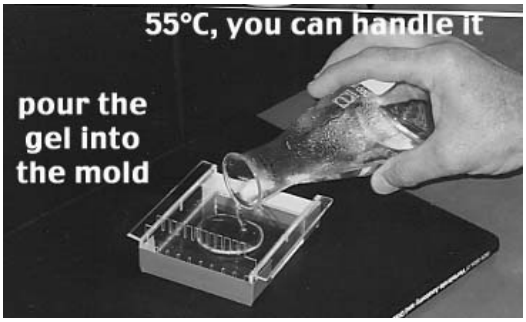


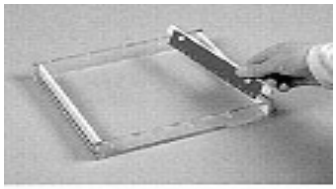
3. Prepare the mixture.

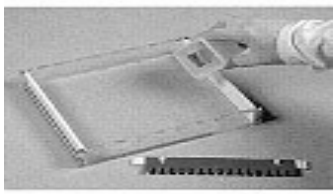


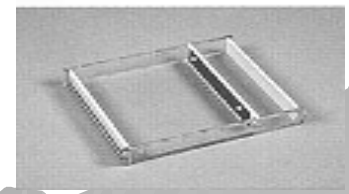
4. Gel the mixture

5. Cast the gel (add ethidium bromide to the cooled gel)

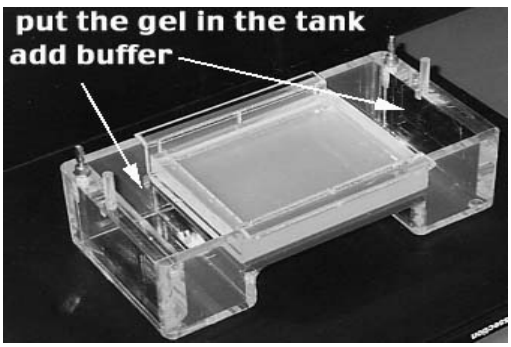


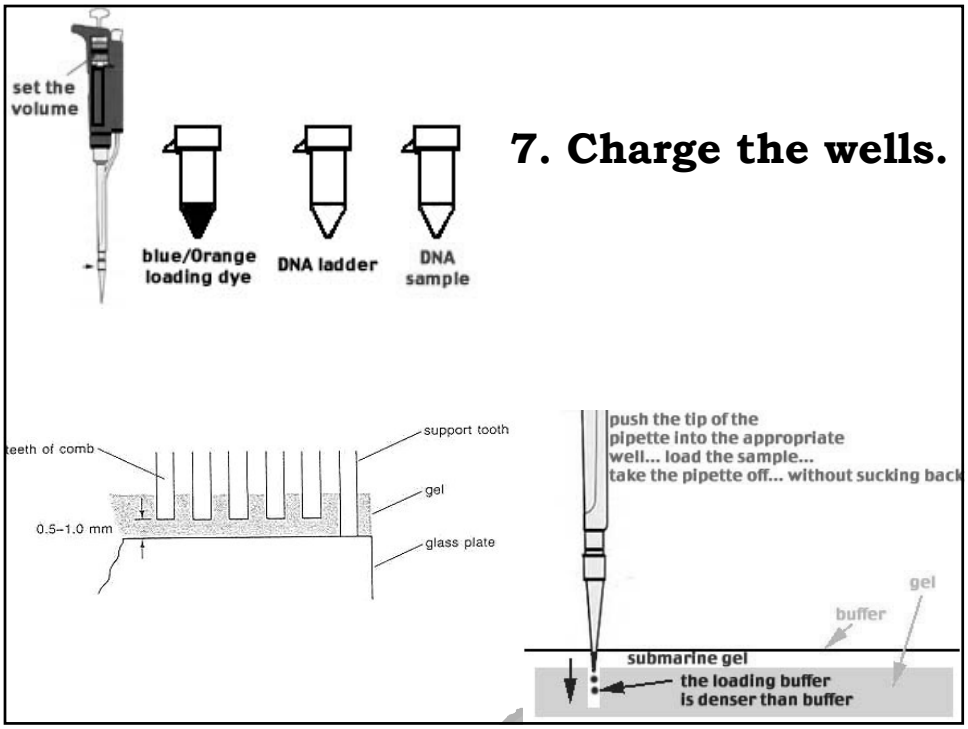


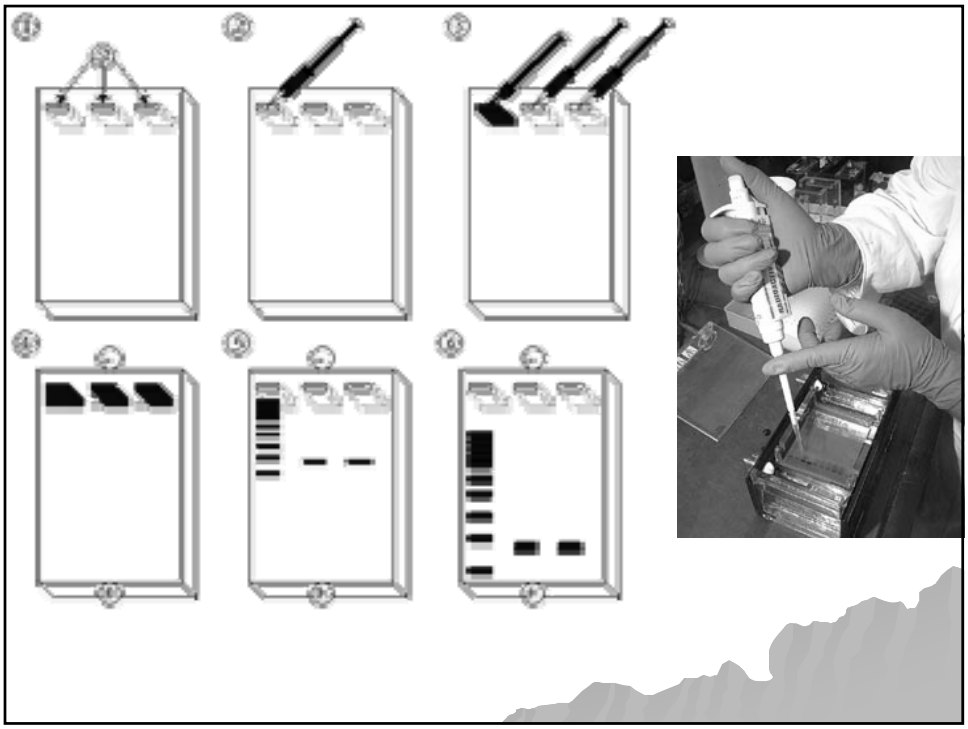


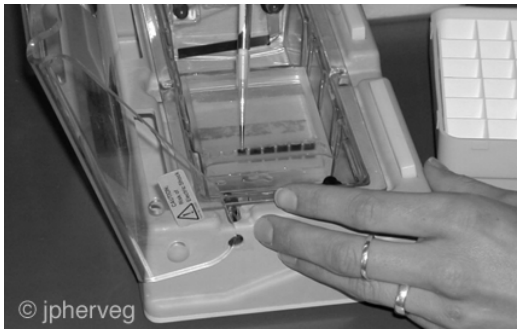


6. Place the mold in the tank




set the volume
blue/Orange loading dye
DNA ladder
DNA sample
7. Charge the wells.
teeth of comb
support tooth
gel
0.5–1.0 mm
glass plate
push the tip of the pipette into the appropriate well... load the sample... take the pipette off... without sucking back
gel
buffer
submarine gel
the loading buffer is denser than buffer

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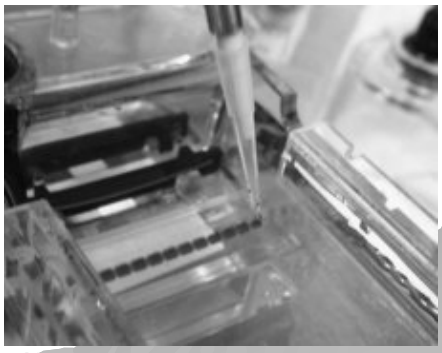

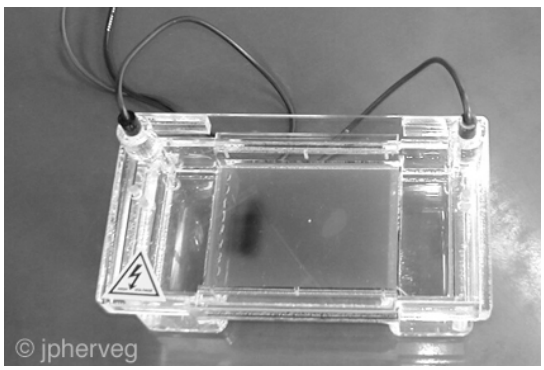
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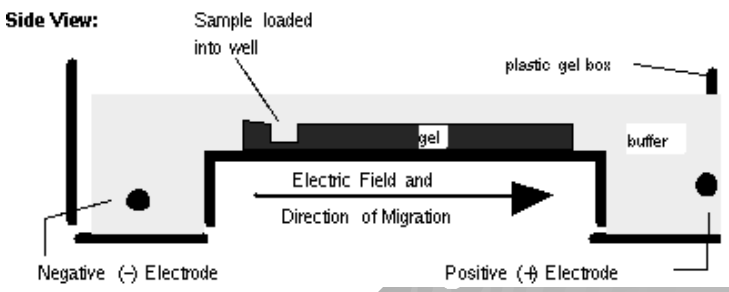
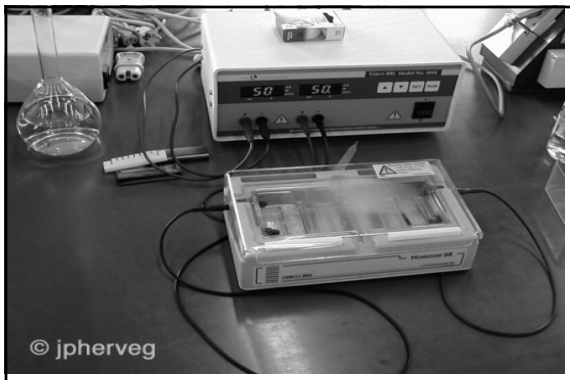
8. Plug in


Side View:
Sample loaded into well
plastic gel box
gel
buffer
Electric Field and
Direction of Migration
Negative (–) Electrode
Positive (+) Electrode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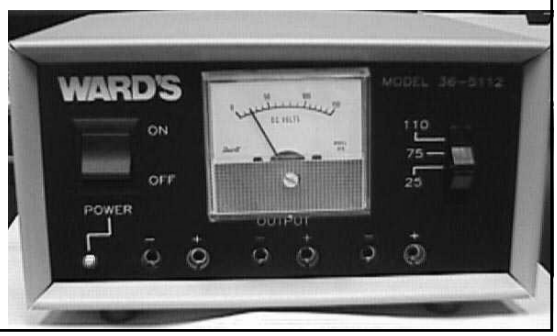
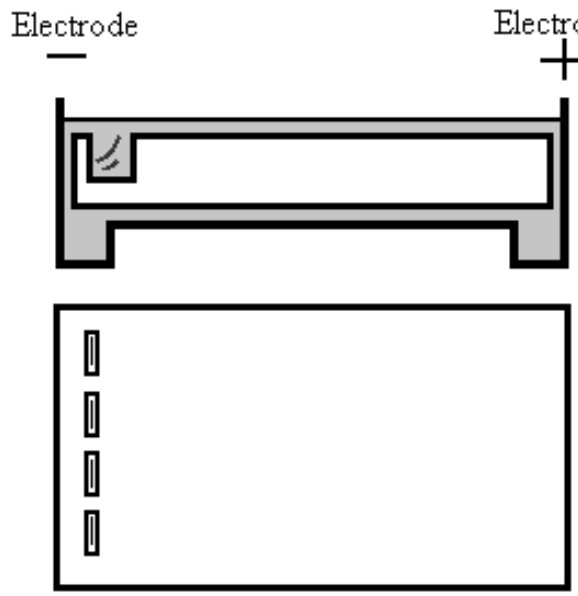
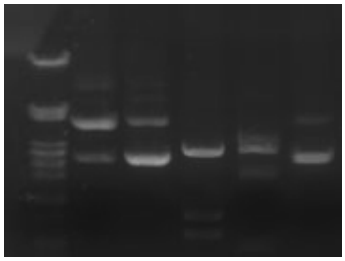
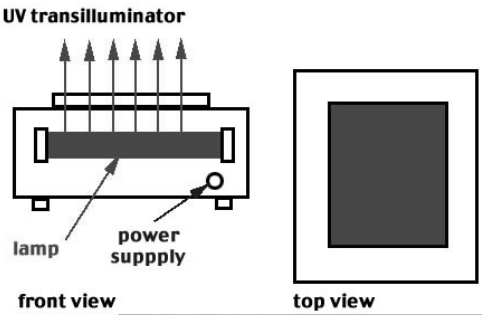
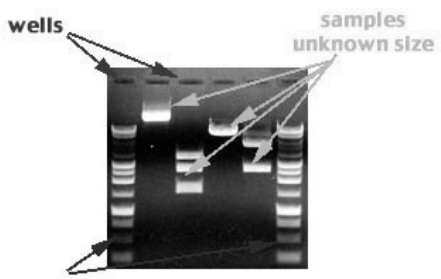

WARD'S
ON
OFF
POWER
OUTPUT
110
75
25


Electrode
Electrode

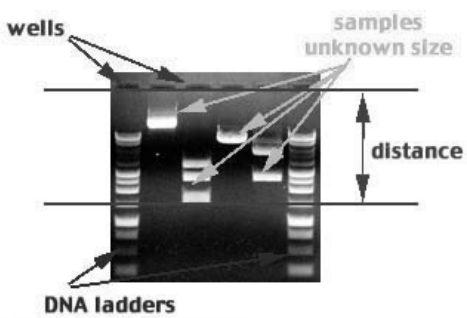
9. Examine the gel





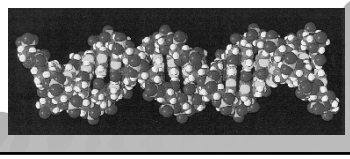


9. Results



Decontamination of Ethidium Bromide Solutions

Ethidium bromide **is a powerful mutagen and is moderately toxic.** ***For this reason,*** **gloves should be worn when working with solutions that contain this dye. Also, after use, this solution should be decontaminated by one of** ***the following methods*****:**



A) Decontamination of concentrated solutions of ethidium bromide:

Method:

a) **Add sufficient water to reduce the concentration of ethidium bromide to < 0.5 mg/ml.**

b) **To the resulting solution, add 0.2 volume of fresh 5% hypophosphorous acid and 0.12 volume of fresh 0.5 M sodium nitrite. Mix carefully (The pH of solution is < 3.0).**

c) **After incubation for 24 hours at room temperature, add a large excess of 1 M sodium bicarbonate. The solution may now be discarded.**

B) Decontamination of dilute solution of ethidium bromide:

Method:

a) **Add 100 mg of powdered activated charcoal for each 100 ml of solution.**

b) **Store the solution for 1 hour at room temperature, shaking it intermittently.**

c) **Filter the solution through a Whatman No.1 filter, and discard the filtrate.**

d) **Seal the filter and activated charcoal in a plastic bag, and dispose of the bag in the hazardous waste.**

Thank you for your attention!