GEL ELECTROPHORESIS

BY

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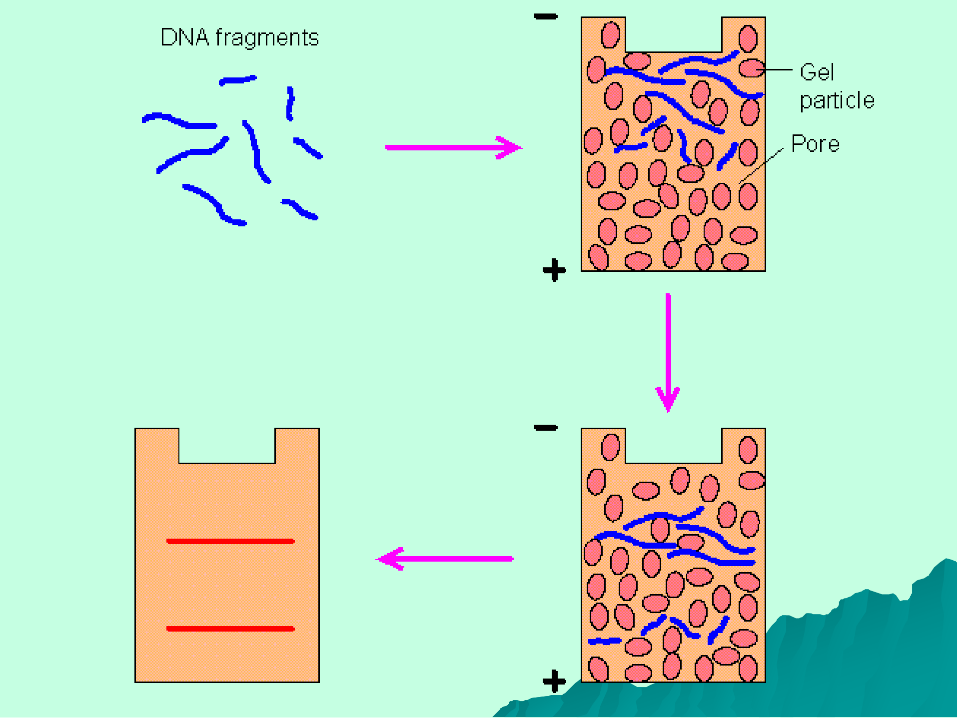
3/19/2013

ELECTROPHORESIS:

Is the migration of charged molecules in solution in response to an electric field

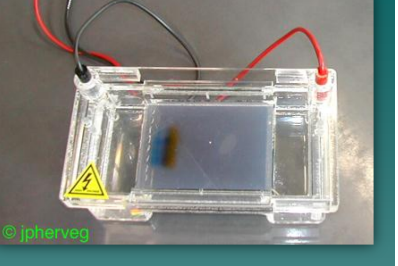
The rate of migration depends on:

- Strength of the field
- Net charge
- Size & shape of the molecules
- Ionic strength, viscosity & temperature of the medium (in which molecules are moving)



AGAROSE

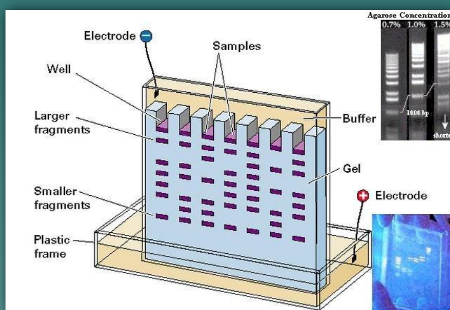
Is used to separate larger molecules such as nucleic acid, large proteins & protein complexes

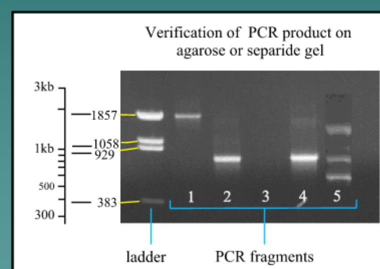


POLYACRYLAMIDE

Is used to separate most proteins & small oligonucleotides that require a small gel pore size for retardation

Dilute agarose gels are generally more rigid & easy to handle than polyacrylamide of the same concentration





There are 2 types of buffer systems in electrophoresis

Continuous System:

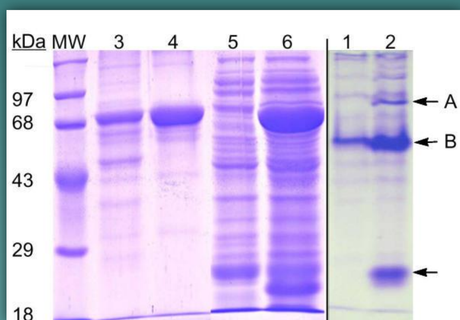
Has only a single separating gel & uses the same buffer in the tank & gel

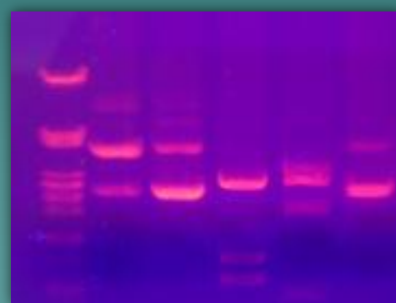
Discontinuous System:

☑A non-restrictive large pore gel (called stacking gel) is layered on top of a separating gel (called a resolving gel)

☑Each gel is made with a different buffer, & the tank buffer are different from the gel buffers

THE RESOLUTION OBTAINED IN A DISCONTINUOUS SYSTEM IS MUCH GREATER THAN THAT OBTAINED WITH A CONTINUOUS SYSTEM

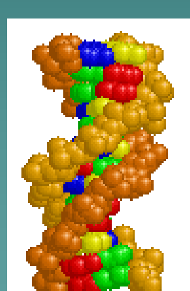




AGAROSE GEL ELECTROPHORESIS

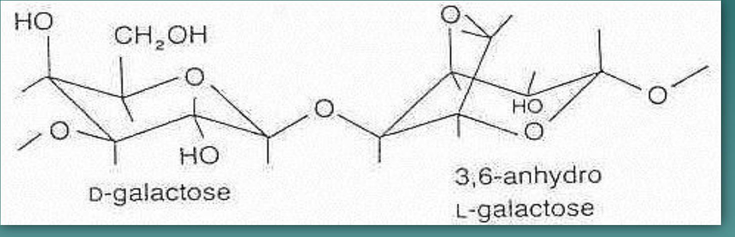
The standard method used to separate, identify & purify DNA fragments is **electrophoresis** through agarose gels

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 & is a linear polymer whose basic structure is

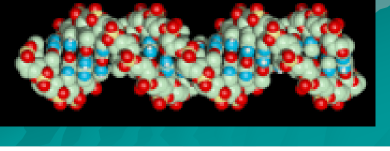


Commercially available agarose is not completely pure; it is contaminated with other polysaccharides, salts, & proteins

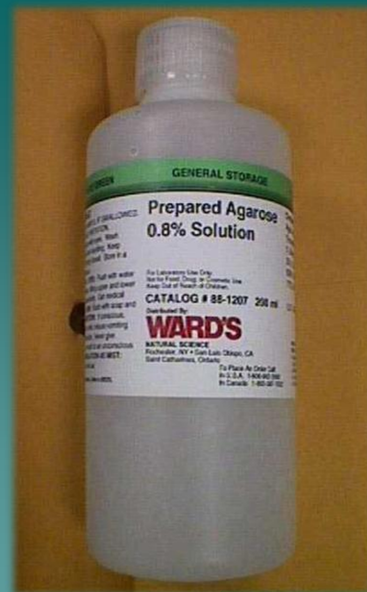
The amount of contamination varies from batch to batch & from manufacture to manufacture

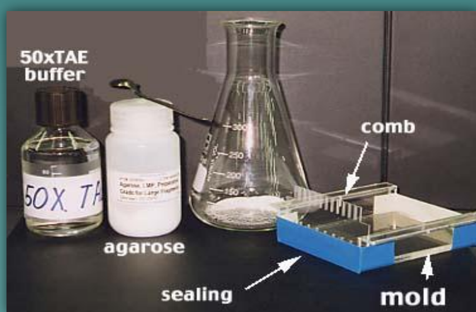
These difference can affect on

- Migration of the DNA
- Ability of the DNA recovered from gel



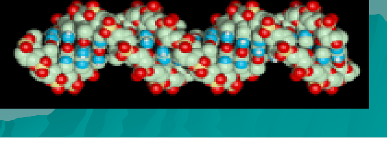
Now, most manufacturers prepare **special grades** of agarose that are screened for the presence of inhibitors & nucleases & for minimal background fluorescence after staining with ethidium bromide

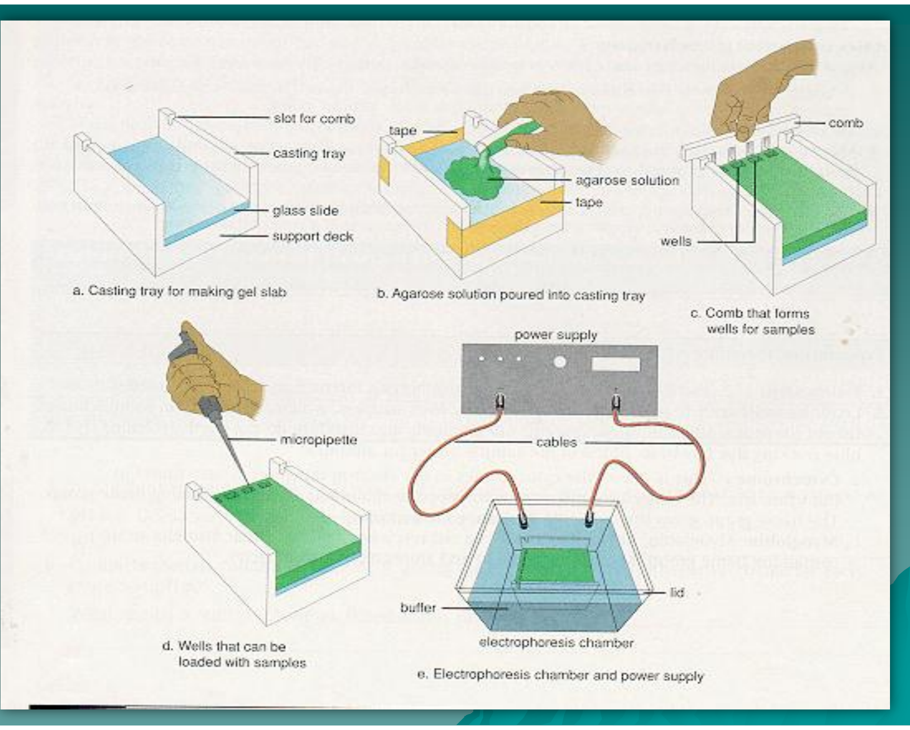




Agarose gels are cast by melting the agarose in the presence of the desired buffer until a clear, transparent solution is achieved

The melted solution is then poured into a mold & allow to harden. The agarose forms matrix & when an electric field is applied across the gel, DNA which is negatively charged at neutral PH, migrates toward the **anode**



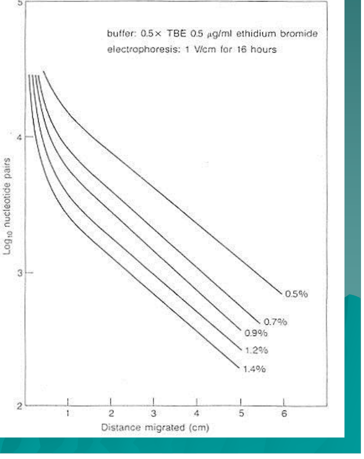


Factors Affecting the Rate of DNA Migration in Agarose Gels

1) Molecular size of the DNA:

Larger molecules migrate more slowly than smaller molecules

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



2) Agarose Concentration:

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:

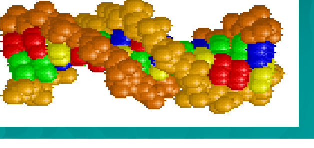
Superhelical circular DNAs

Niked circular DNAs

Linear DNAs

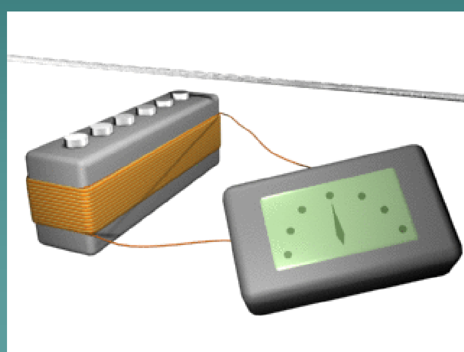
Of the same molecular weight

Migrate through agarose gels at different rates



4) Applied Voltage:

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



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:

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 & exposing it to UV light, distinct bands of DNA become visible

Ethidium Bromide is a carcinogen & 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 & to give cleaner, higher powered staining

Midori Green DNA Stain

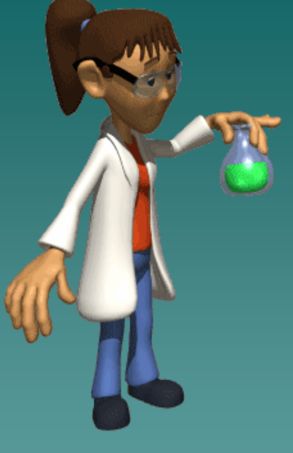
Left side Gel stained with Midori green stain & Right side Gel Stained with Ethidium Bromide

8) Electrophoresis Buffer:

The electrophoretic mobility of DNA is affected by the composition & ionic strength of the electrophoresis buffer

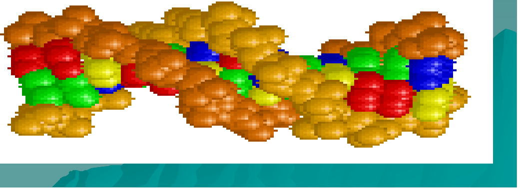
In the absence of ions → electrical conductance is minimal & DNA migrates very slowly

In buffers of high ionic strength → electrical conductance is very efficient & 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 & stored at room temp



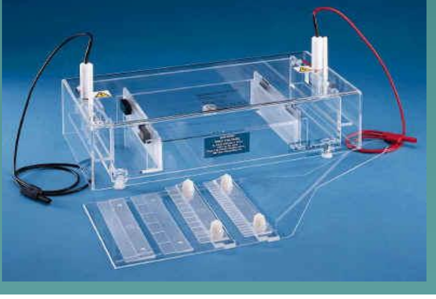
Commonly used Electrophoresis Buffers

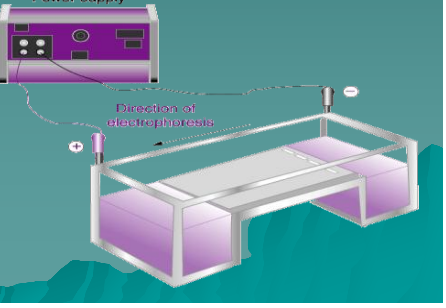
Buffer	Working solution	Conc. Stock solution (Per Liter)
Tris-acetate (TAE)	1X: 0.04 M Tris-acetate 0.001 M EDTA	50X: 243 g Tris base 57.1 ml glacial acetic acid 100 ml 0.5M ETDA (PH 8.0)
Tris-phosphate (TPE)	1X: 0.09 M Tris-phosphate 0.002 M EDTA	10X: 108 g Tris base 15.5 ml 85% phosphoric acid (1.679g/ml) 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 electrophoresis tank**

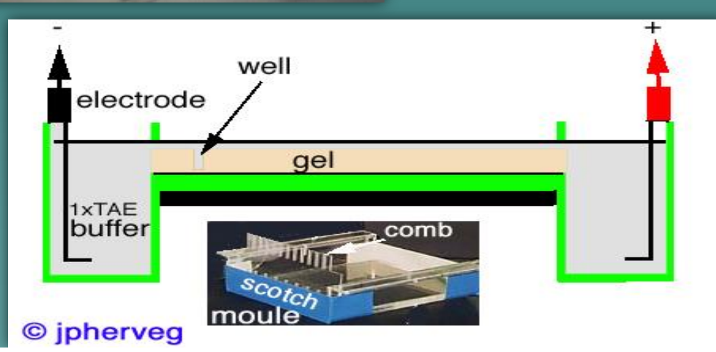




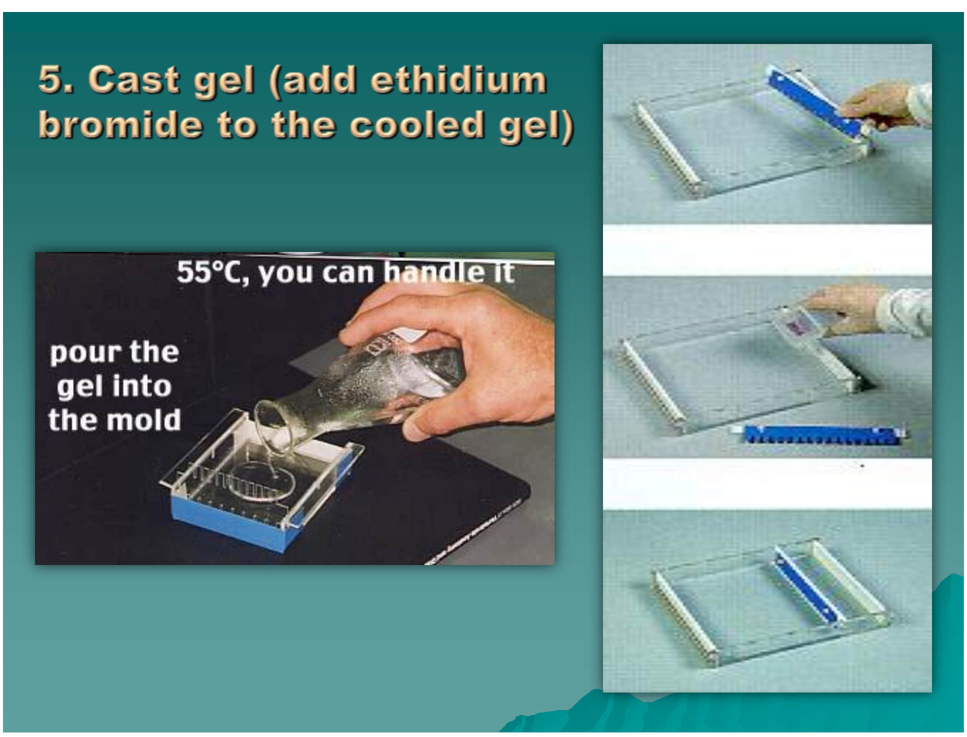
Preparation & Examination of Agarose gels

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

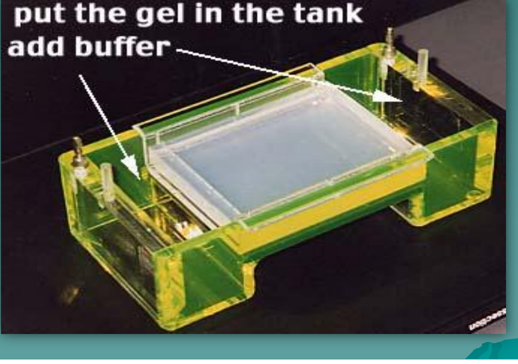
2. Prepare the materials you will need

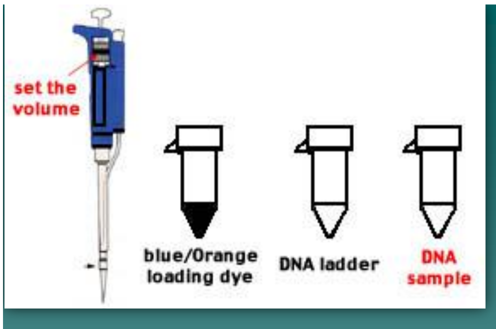



3. Prepare mixture
50xTAE buffer
comb
50X TA
agarose
sealing
mold
swirl gently
4. Gel the mixture

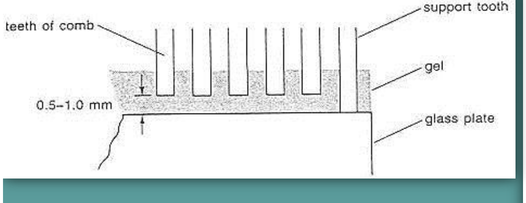

5. Cast gel (add ethidium bromide to the cooled gel)
55°C, you can handle it
pour the gel into the mold

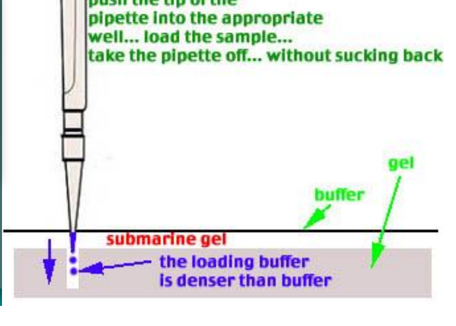
6. Place mold in the tank

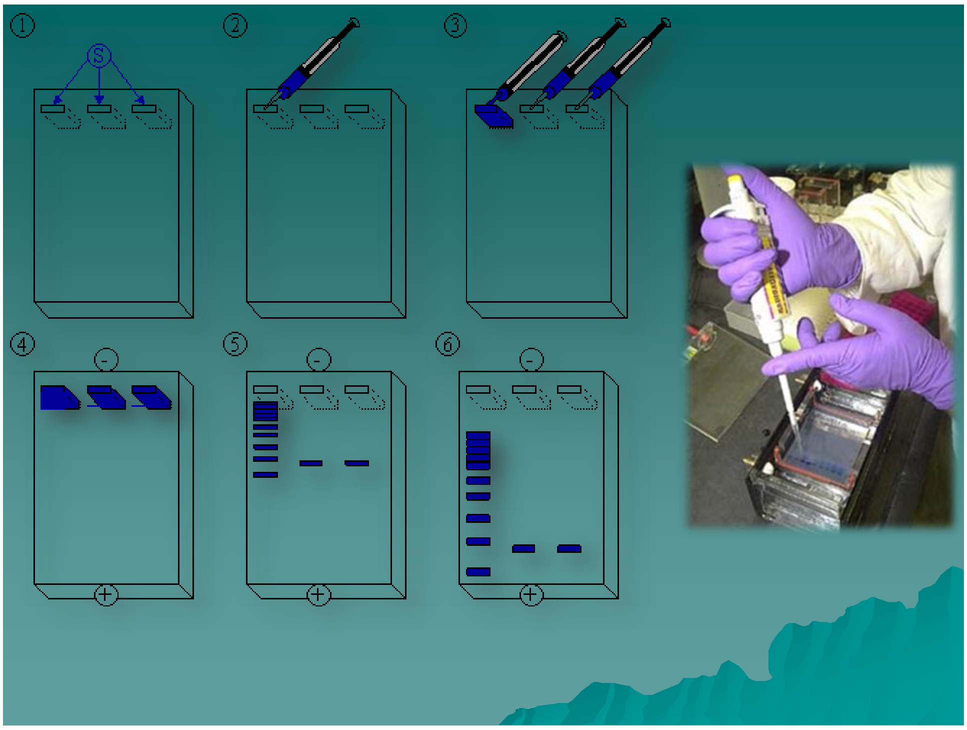




7. Charge the wells






1
2
3
S
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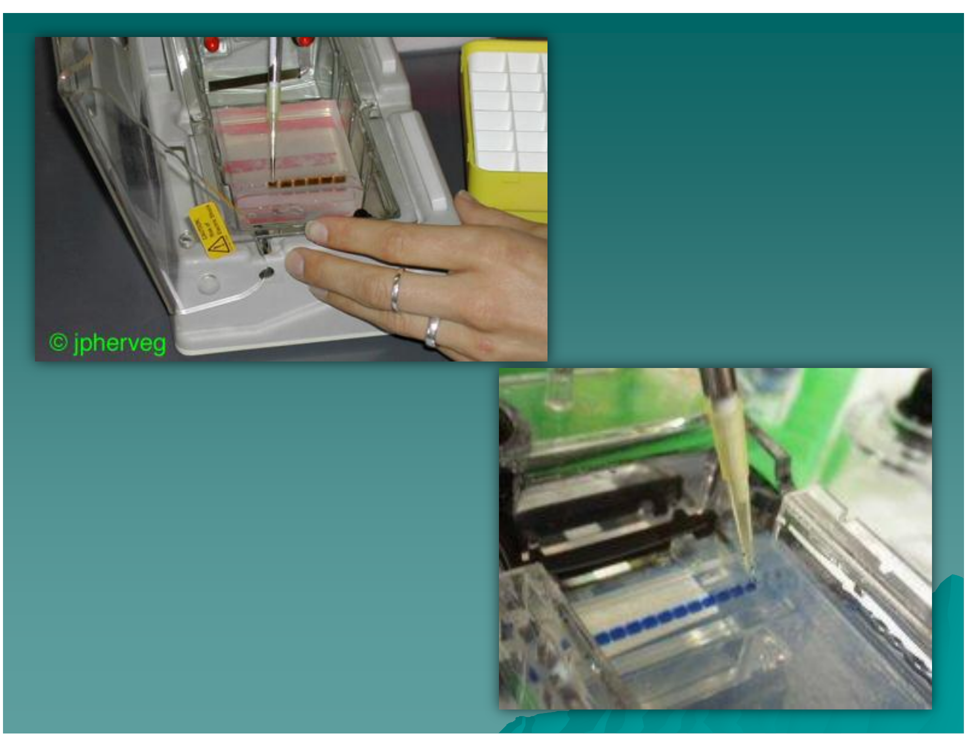
6
-
+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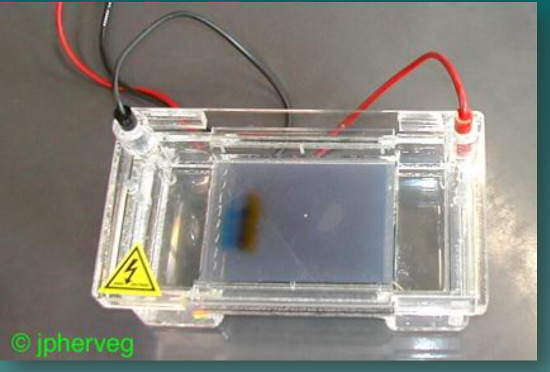
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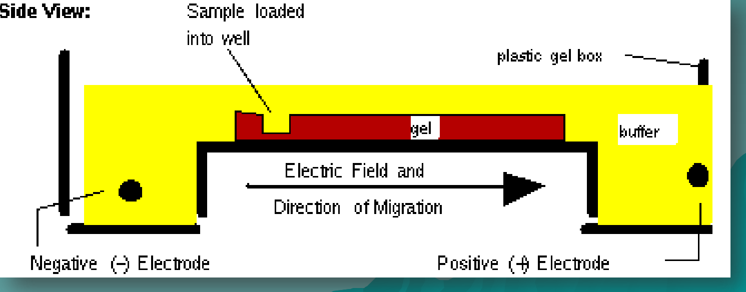
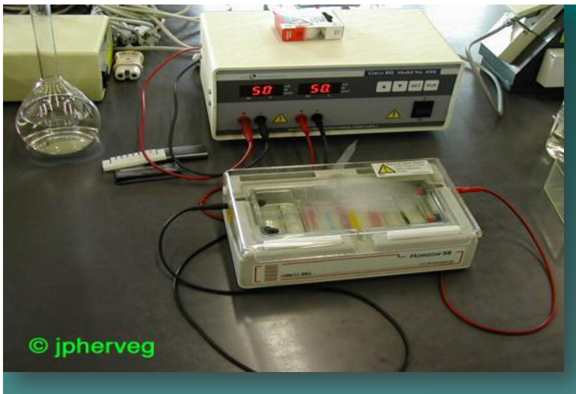
8. Plug in


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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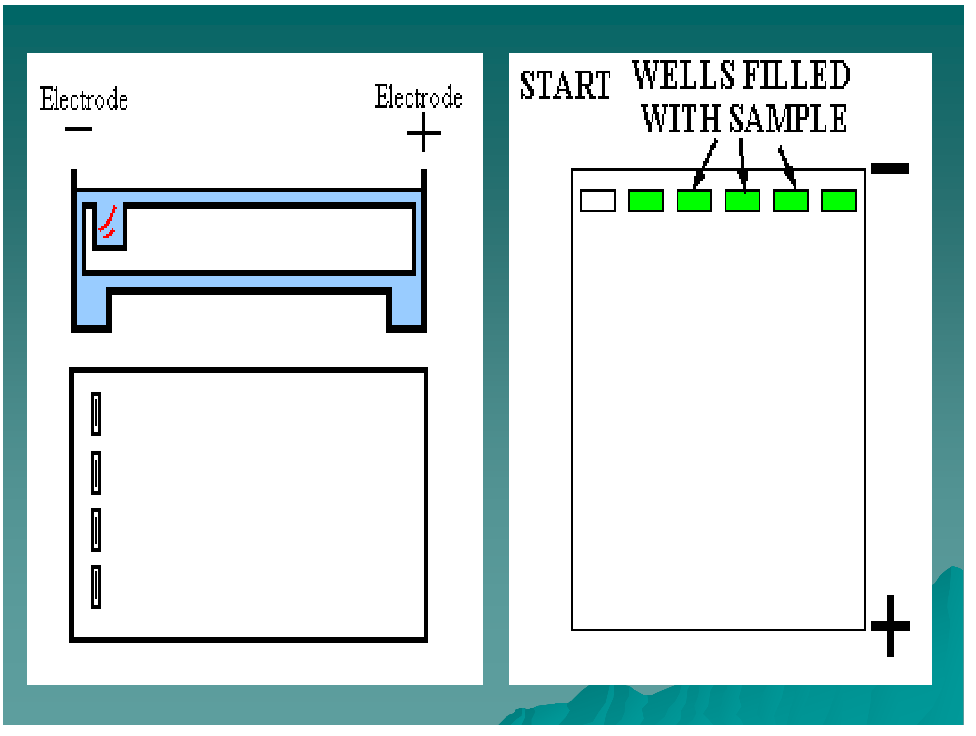
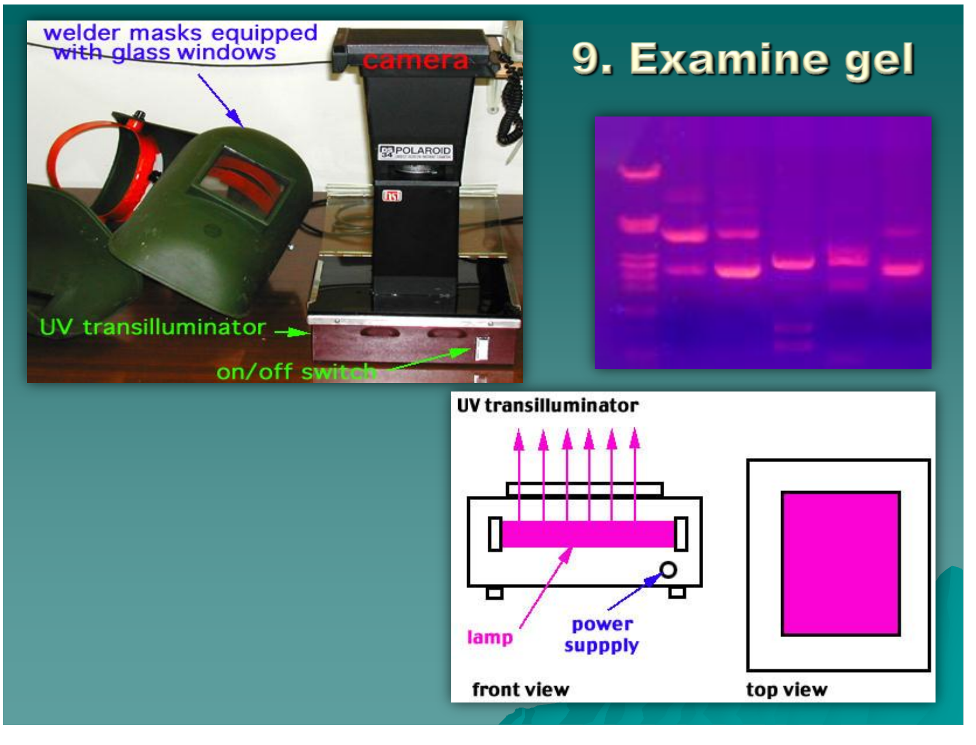
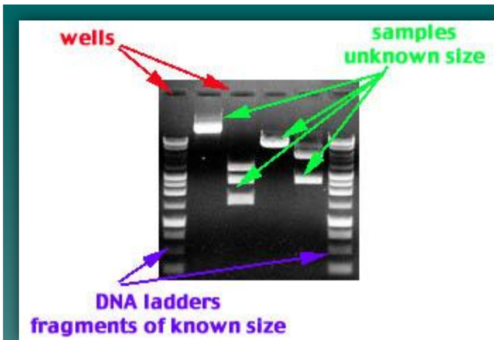
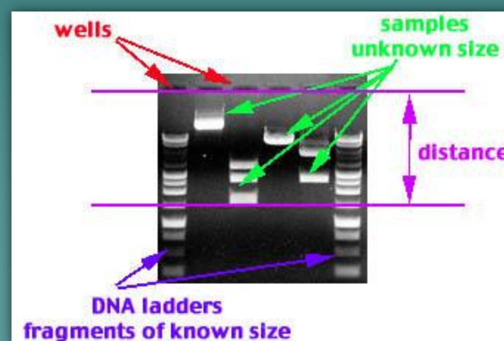
WARD'S
ON
OFF
POWER
OUTPUT
110
75
25


Electrode
Electrode
START
WELLS FILLED WITH SAMPLE


9. Examine gel
welder masks equipped with glass windows
camera
POLAROID
UV transilluminator
on/off switch
UV transilluminator
lamp
power suppply
front view
top view

9. Results



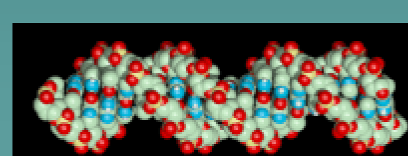


Decontamination of Ethidium Bromide Solutions

Ethidium bromide **is a powerful mutagen & is moderately toxic**

For this reason**, gloves should be worn when working with solutions contain this dye**

Also, after use, this solution should be decontaminated



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) Then add 0.2 volume of fresh 5% hypophosphorous acid & 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, & discard the filtrate

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


Thank you for your attention!