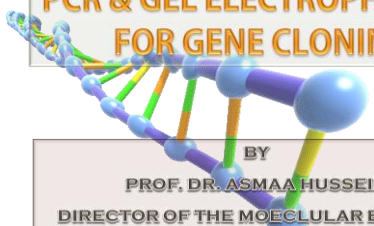
PCR & GEL ELECTROPHORESIS FOR GENE CLONING



BY
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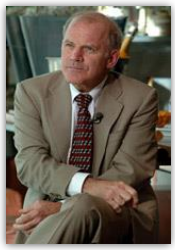
DIRECTOR OF THE MOECLULAR BIOLOGY RESEARCH UNIT (MBRU)
ASSIUT UNIVERSITY

What is PCR?

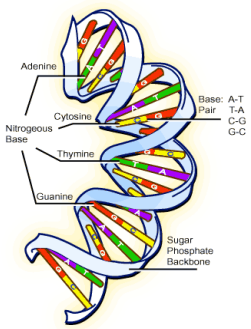
PCR is an exponentially progressing synthesis of the defined target DNA sequences in vitro

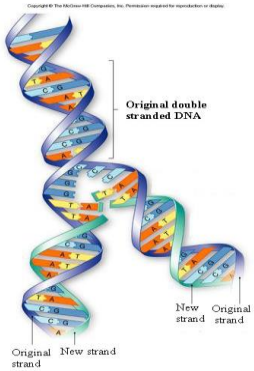
It was invented in 1983 by Dr. Kary Mullis, for which he received the Nobel Prize in Chemistry in 1993



POLYMERASE CHAIN REACTION

DNA HELIX

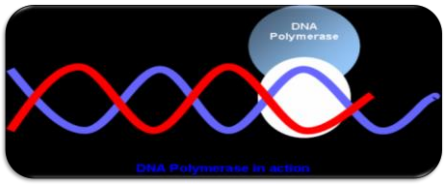




What is PCR?

Why "Polymerase"?

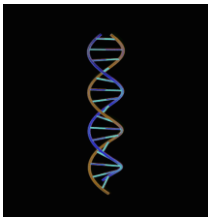
It is called "polymerase" because the only enzyme used in this reaction is DNA polymerase



What is PCR?

Why "Chain"?

IT IS CALLED "CHAIN" BECAUSE THE PRODUCTS OF THE FIRST REACTION BECOME SUBSTRATES OF THE FOLLOWING ONE, AND SO ON



What is PCR?

THE "REACTION" COMPONENTS

1) Target DNA → Contains the sequence to be amplified

2) Pair of Primers → Oligonucleotides that define the sequence to be amplified

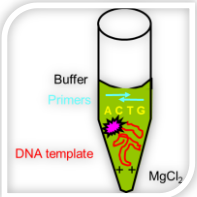
3) dNTPs → Deoxynucleotidetriphosphates: DNA building blocks

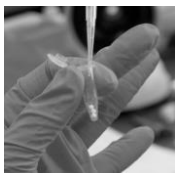
4) Thermostable DNA Polymerase → Enzyme that catalyzes the reaction

5) Mg^{++} ions → Cofactor of the enzyme

6) Buffer solution → Maintains pH & ionic strength of the reaction solution suitable for the activity of the enzyme

What is PCR?





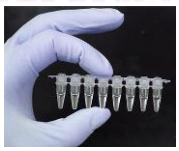
3) dNTPs

4) Thermostable DNA Polymerase

5) Mg^{++} ions

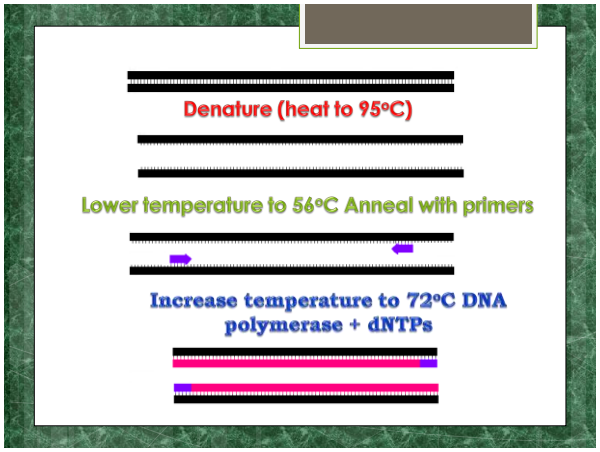
6) Buffer solution

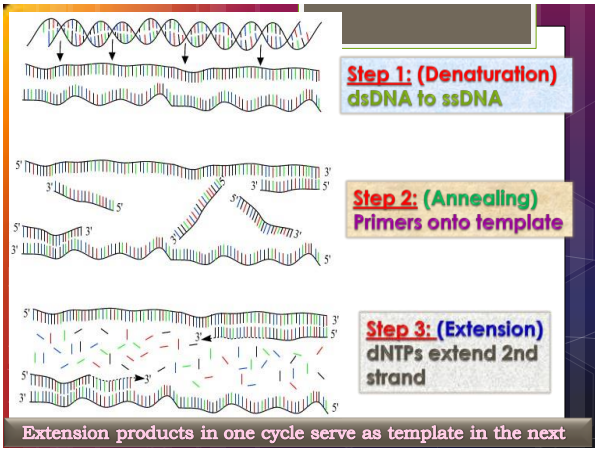
THE REACTION

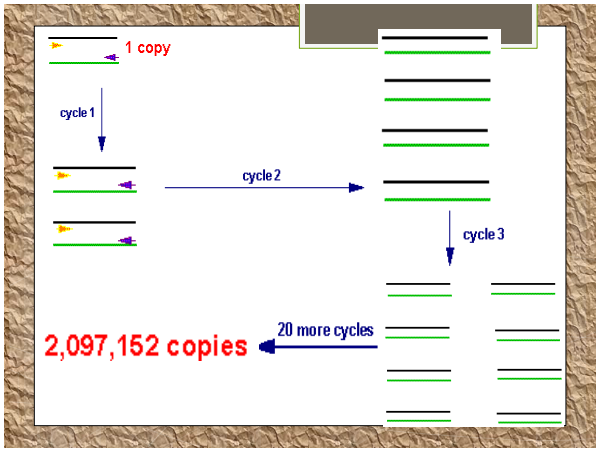


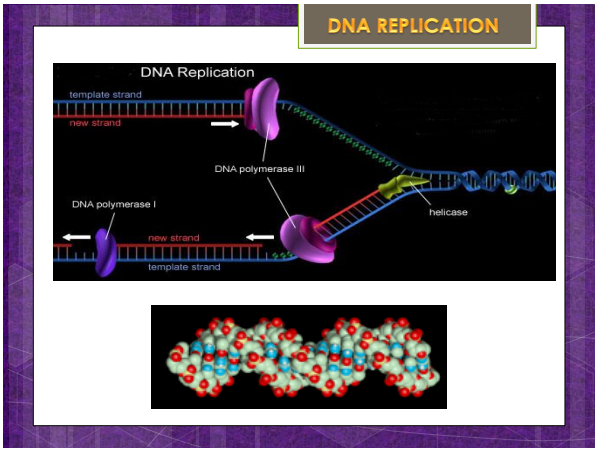
PCR tube

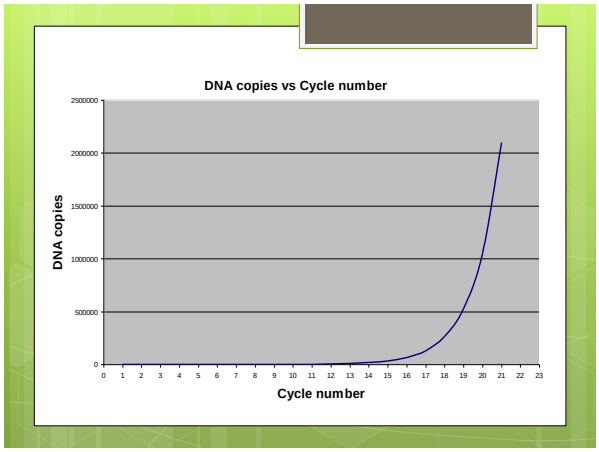
THERMOCYCLER

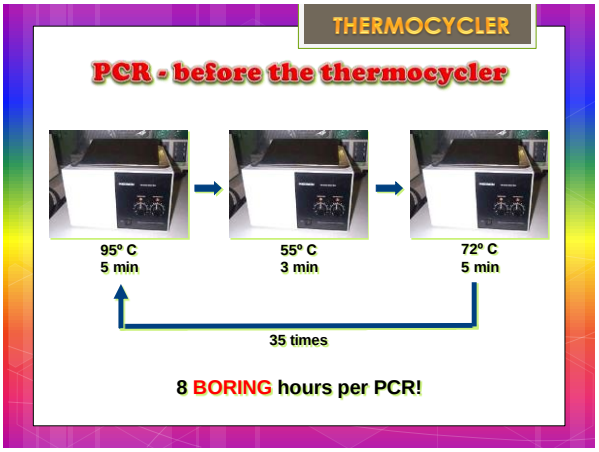

Denature (heat to 95°C)
Lower temperature to 56°C Anneal with primers
Increase temperature to 72°C DNA polymerase + dNTPs


Step 1: (Denaturation) dsDNA to ssDNA
Step 2: (Annealing) Primers onto template
Step 3: (Extension) dNTPs extend 2nd strand
Extension products in one cycle serve as template in the next

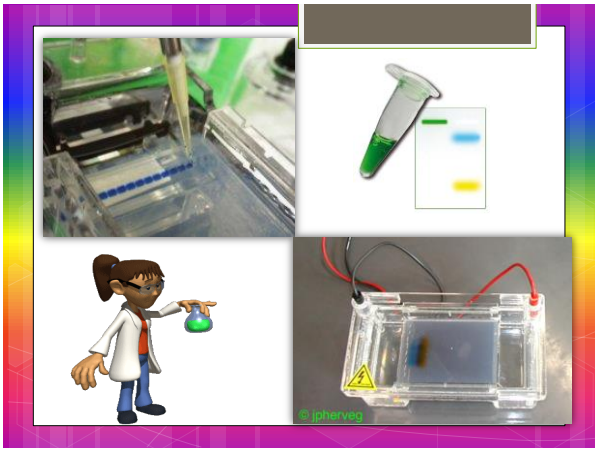

1 copy
cycle 1
cycle 2
cycle 3
2,097,152 copies
20 more cycles


DNA REPLICATION
DNA Replication
template strand
new strand
DNA polymerase III
DNA polymerase I
helicase
new strand
template strand

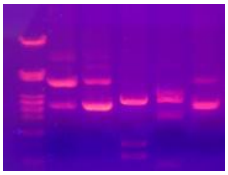

DNA copies vs Cycle number
DNA copies
Cycle number

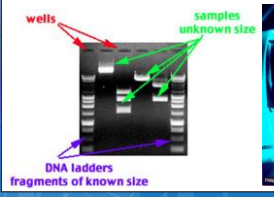

THERMOCYCLER
PCR - before the thermocycler
95º C
5 min
55º C
3 min
72º C
5 min
35 times
8 BORING hours per PCR!

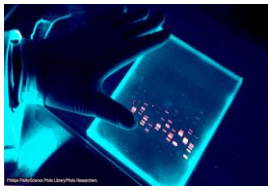

THERMOCYCLERS
Heated lids
Adjustable ramping times
Single/multiple blocks
Gradient thermocycler blocks


© jpherveg

Gel Examine







PCR APPLICATIONS

MOLECULAR IDENTIFICATION

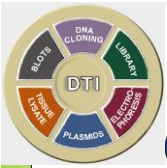
- Molecular Archaeology
- Molecular Epidemiology
- Molecular Ecology
- DNA fingerprinting
- Classification of organisms
- Genotyping
- Pre-natal diagnosis
- Mutation screening
- Drug discovery
- Genetic matching
- Detection of pathogens

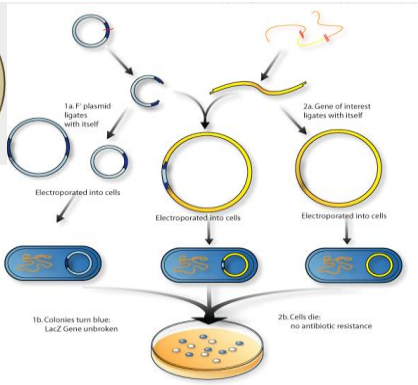
SEQUENCING

- Bioinformatics
- Genomic cloning
- Human Genome Project

GENETIC ENGINEERING

- Site-directed mutagenesis
- Gene expression studies



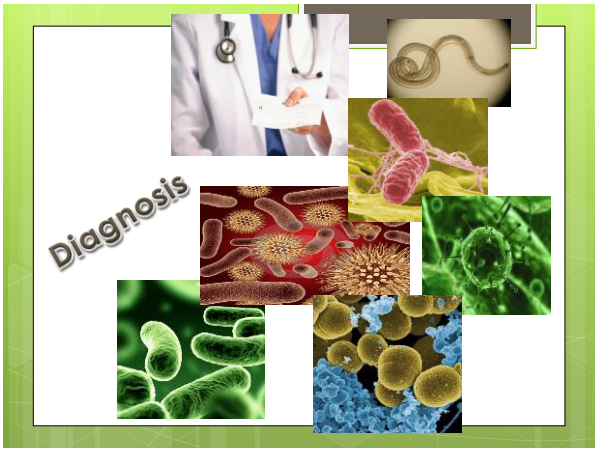


Gene cloning & expression

Creating mutation

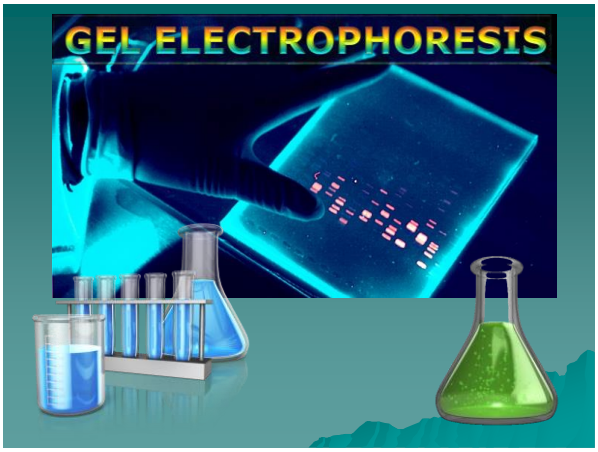
Random or specific

ACCATCGGCCTGCATCA
TGGTAGCCTGACGTAGTCAT
GGTAGCCTGACT----


Diagnosis


Samples


PCR is just a tool
How to use it,
is up to you

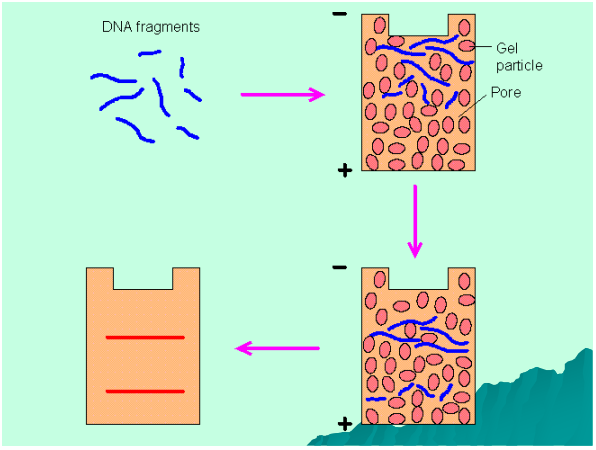

GEL ELECTROPHORESIS

ELECTROPHORESIS:

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

The rate of migration depends on:

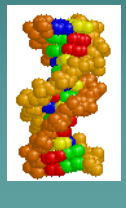
- Strength of the field
- Net charge
- Size & shape of the molecules
- Ionic strength, viscosity & temperature of the medium (in which molecules are moving)



AGAROSE 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)

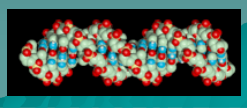
Is extracted from seaweed & is a linear polymer

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



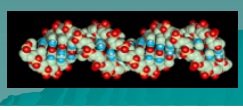
Now, most manufacturers prepare **special grades** of agarose that are screened for the presence of inhibitors & nucleases & for minimal background fluorescence after staining with ethidium bromide

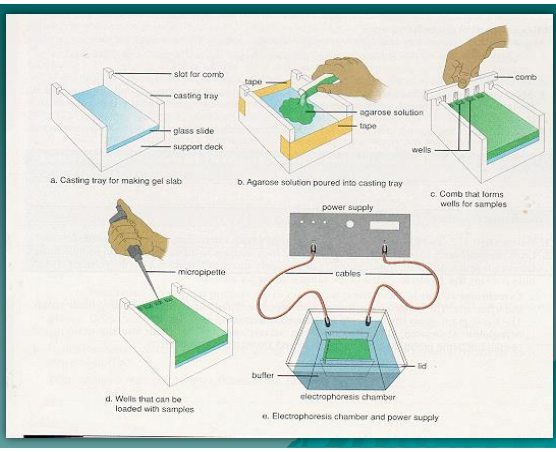
Agarose gels are cast by melting the agarose in the presence of the desired buffer until a clear, transparent solution is achieved

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**





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

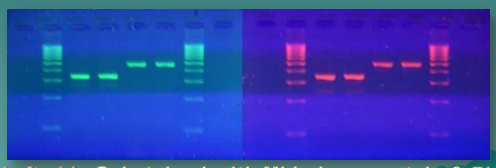
Presence of Intercalating Dyes:

- The central dye in agarose gel electrophoresis is *ethidium bromide*
- It has 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

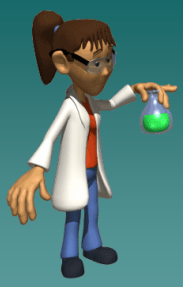
Other dyes are sometimes used including SYBER green or SYBER safe. SYBER dyes are thought to be less carcinogenic than EtBr & to give cleaner, higher powered staining

Midori Green DNA Stain



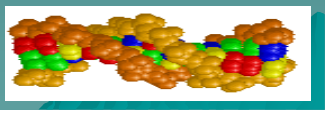
Left side Gel stained with Midori green stain & Right side Gel Stained with Ethidium Bromide

Electrophoresis Buffer:



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

Electrophoresis buffers are usually made up as concentrated solutions & stored at room temp



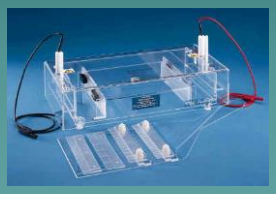
Commonly used Electrophoresis Buffers

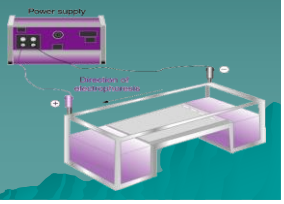
Buffer	Working solution	Conc. Stock solution (Per Liter)
Tris-acetate (TAE)	1X: 0.04 M Tris-acetate 0.001 M EDTA	50X: 243 g Tris base 57.1 ml glacial acetic acid 100 ml 0.5M ETDA (PH 8.0)
Tris-borate (TBE)	0.5X: 0.045 M Tris-borate 0.001 M EDTA	5X: 54 g Tris base 27.5 g boric acid 20 ml 0.5M ETDA (PH 8.0)

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**



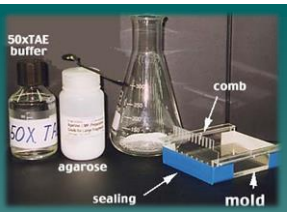


Preparation & Examination of Agarose gels

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

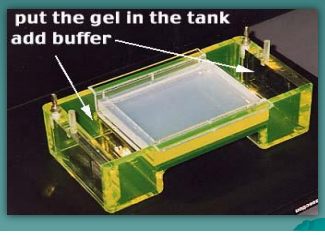
3. Prepare mixture



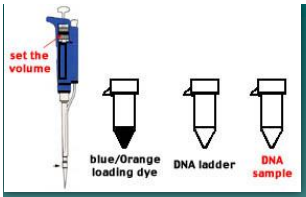
4. Gel the mixture

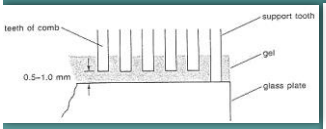
5. Cast gel (add ethidium bromide to the cooled gel)

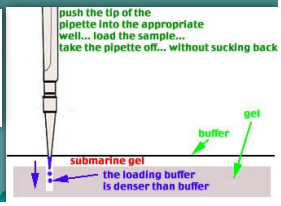
6. Place mold in the tank

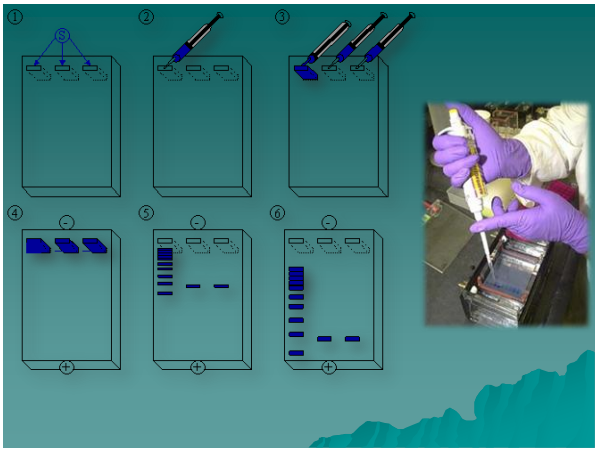


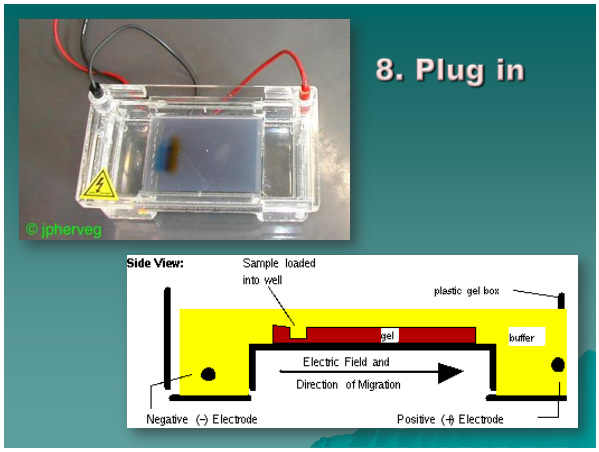
7. Charge the wells

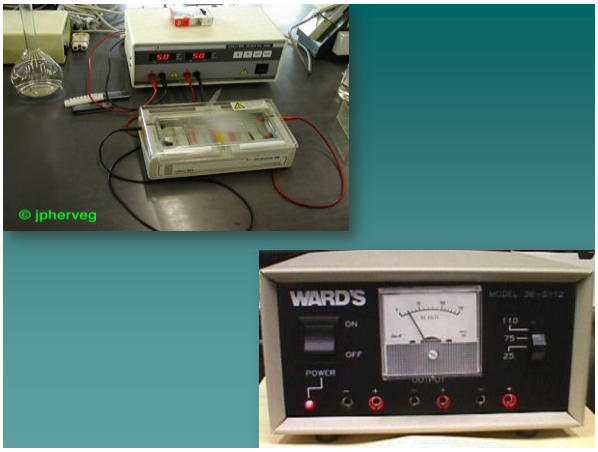


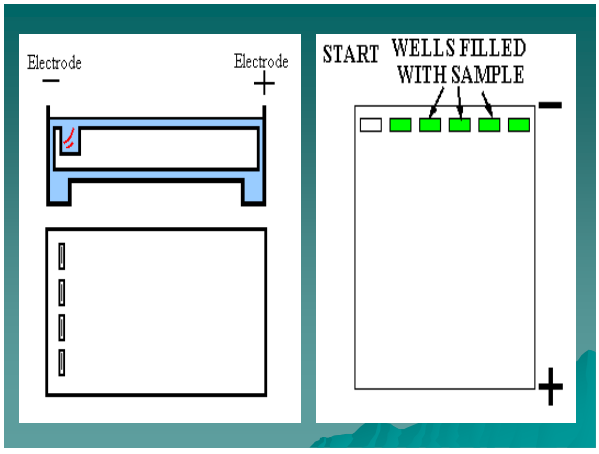


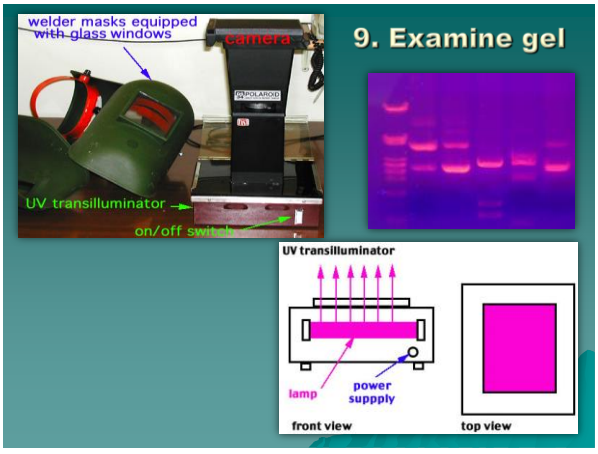


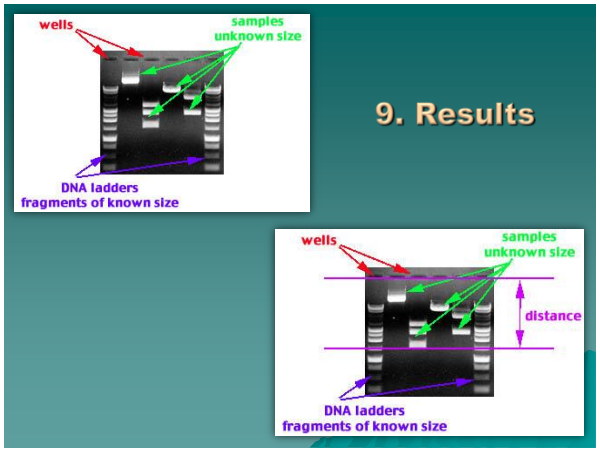



8. Plug in
© jpherveg
Side View:
Sample loaded into well
plastic gel box
gel
buffer
Electric Field and Direction of Migration
Negative (–) Electrode
Positive (+) Electrode


© jpherveg
WARD'S
ON
OFF
POWER


Electrode
–
Electrode
+
START
WELLS FILLED WITH SAMPLE
–
+


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


wells
samples
unknown size
DNA ladders
fragments of known size
9. Results
wells
samples
unknown size
distance
DNA ladders
fragments of known size


Thanks
THANK YOU
GE&MBR Center
ANY QUESTION