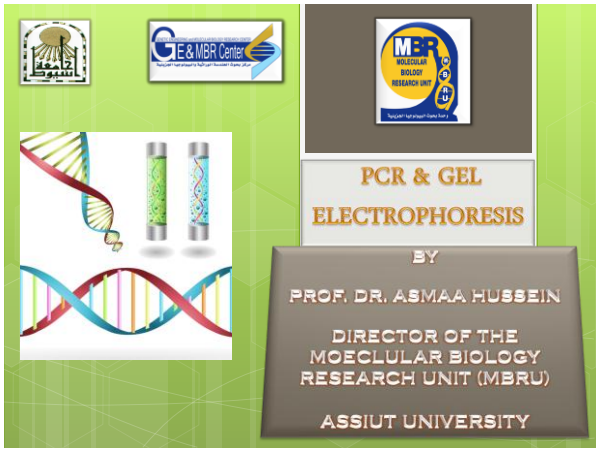
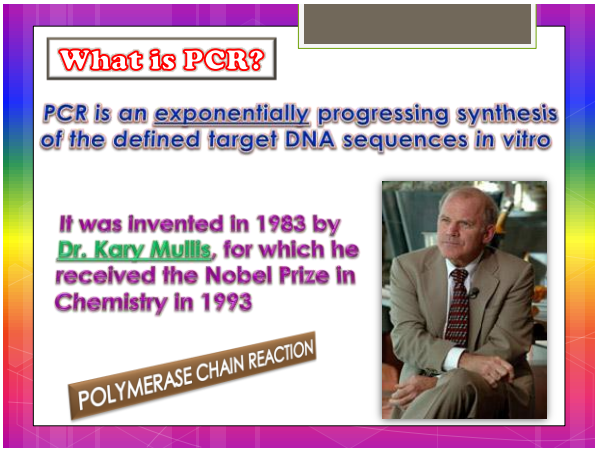
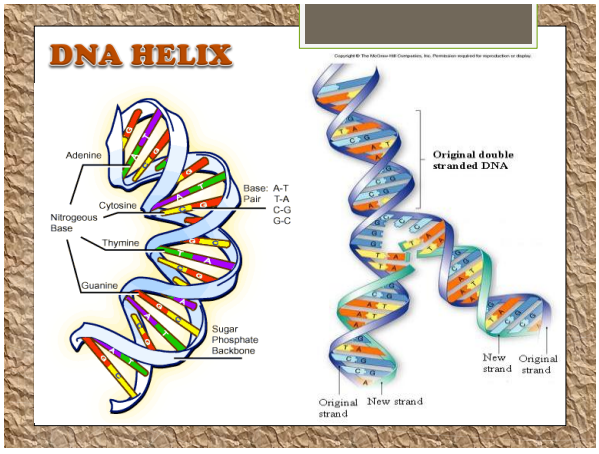
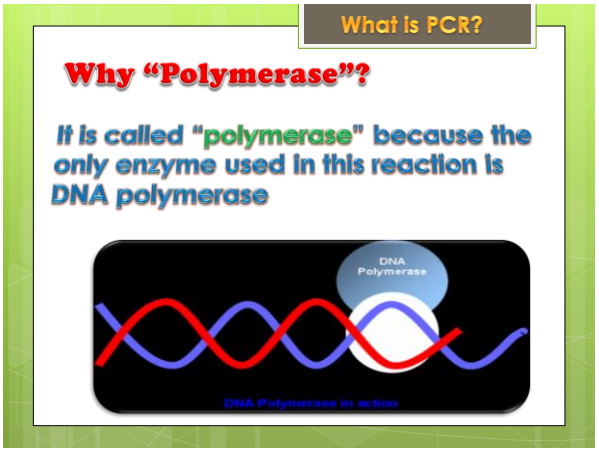

E&MBR Center
MBRU
MOLECULAR BIOLOGY RESEARCH UNIT
PCR & GEL ELECTROPHORESIS
BY
PROF. DR. ASMAA HUSSEIN
DIRECTOR OF THE MOECLULAR BIOLOGY RESEARCH UNIT (MBRU)
ASSIUT UNIVERSITY


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

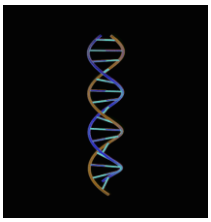

DNA HELIX
Adenine
Cytosine
Nitrogenous Base
Thymine
Guanine
Base Pair
A-T
T-A
C-G
G-C
Sugar Phosphate Backbone
Original double stranded DNA
New strand
Original strand
Original strand
New strand


What is PCR?
Why "Polymerase"?
It is called "polymerase" because the only enzyme used in this reaction is DNA polymerase
DNA Polymerase
DNA Polymerase in action

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

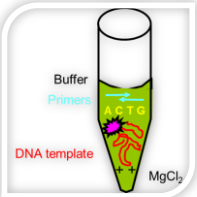
3) dNTPs → Deoxynucleotidetriphosphates: DNA building blocks

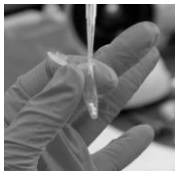
4) Thermostable DNA Polymerase → Enzyme that catalyzes the reaction

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

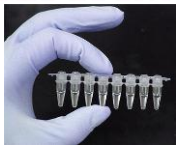
4) Thermostable DNA Polymerase

5) Mg^{++} ions

6) Buffer solution

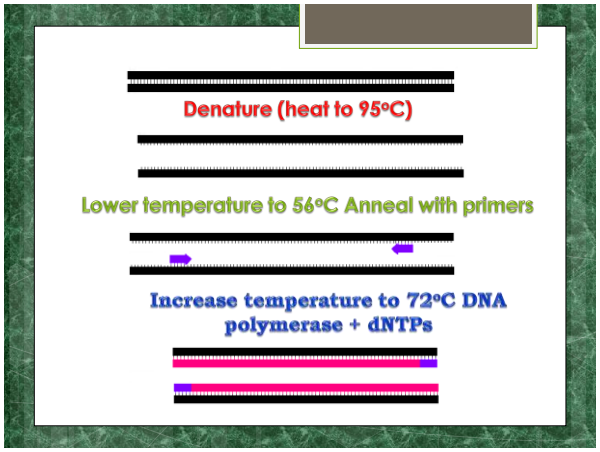
MASTER MIX

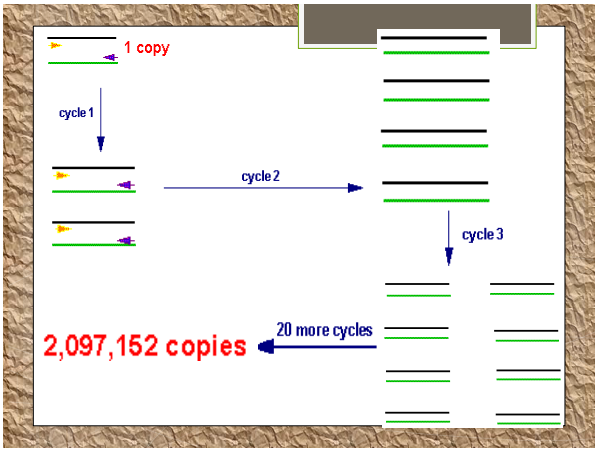
THE REACTION

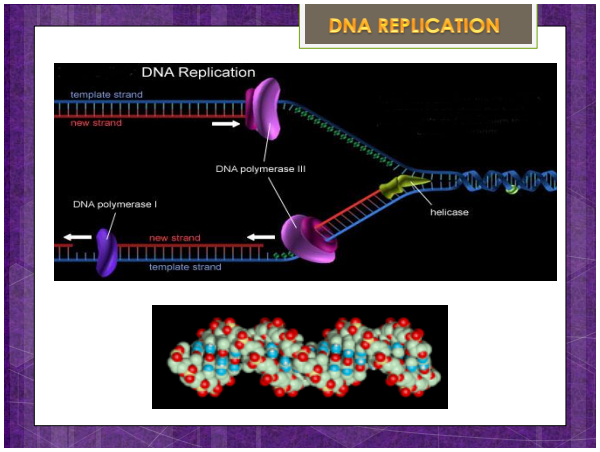


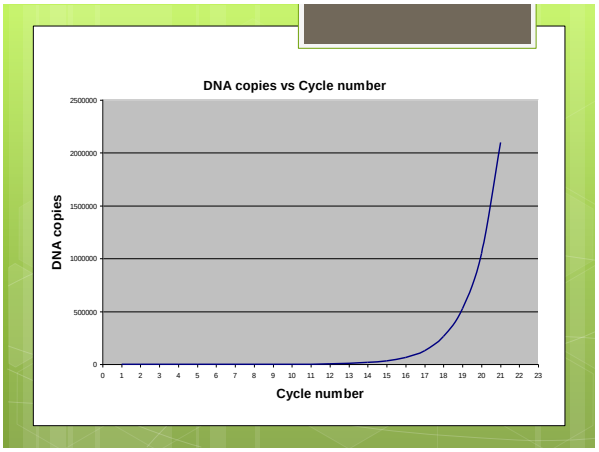
PCR tube

THERMOCYCLER


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


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
2500000
2000000
1500000
1000000
500000
0
0 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23
Cycle number

THERMOCYCLER

PCR - before the thermocycler

95° C
5 min

55° C
3 min

72° C
5 min

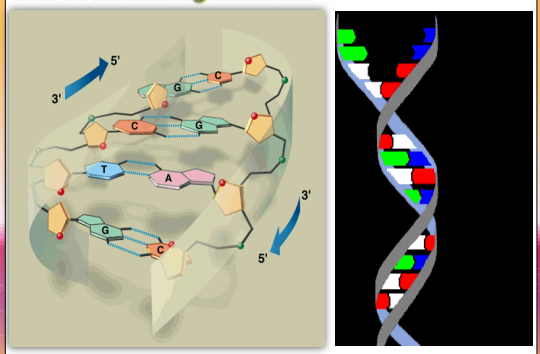
35 times

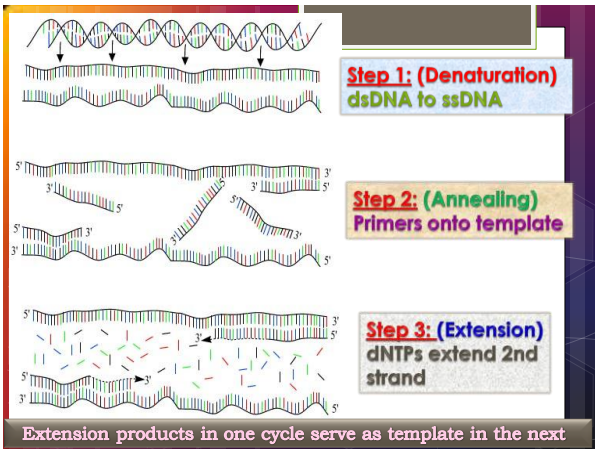
8 BORING hours per PCR!

THERMOCYCLERS

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

Directional Synthesis



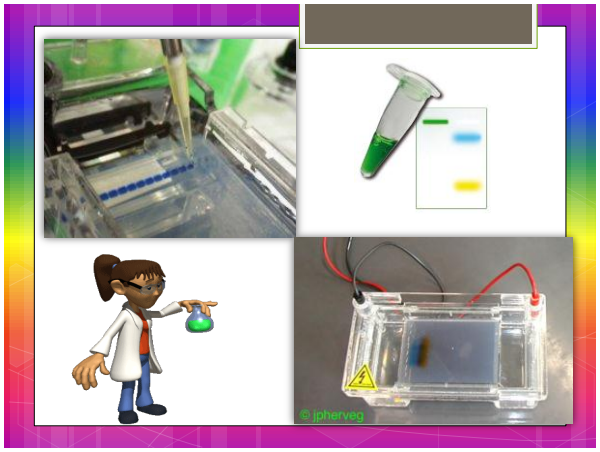


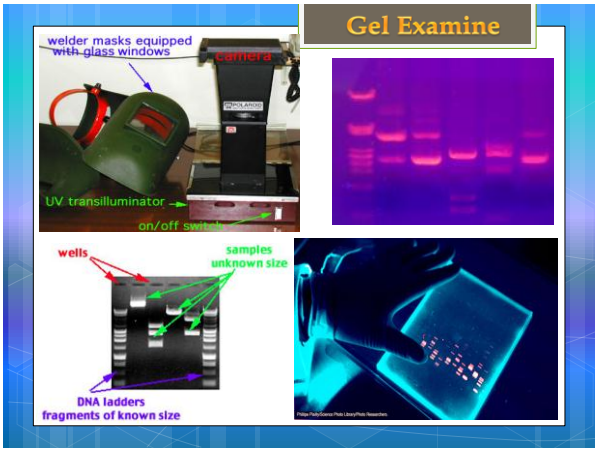
Step 1: (Denaturation)
dsDNA to ssDNA

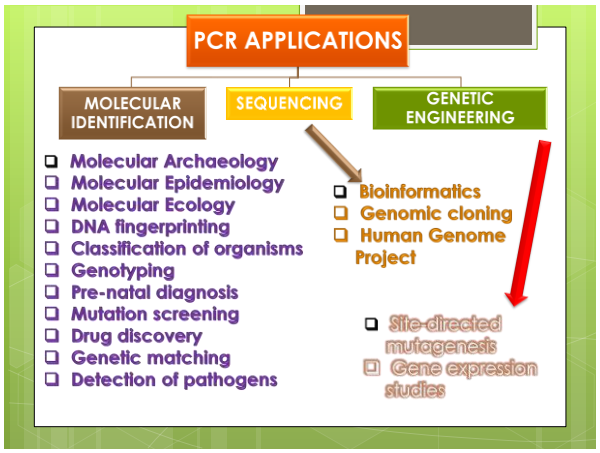
Step 2: (Annealing)
Primers onto template

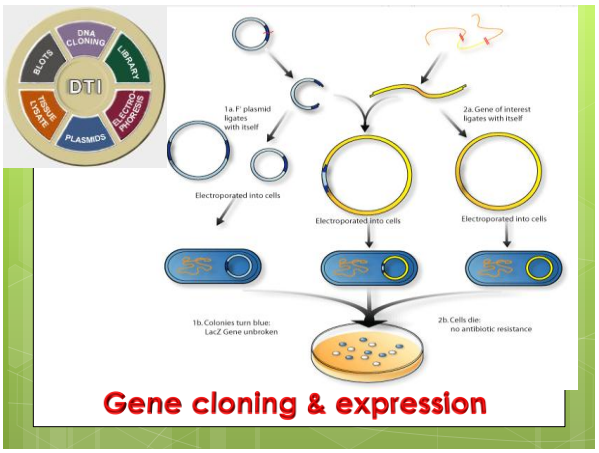
Step 3: (Extension)
dNTPs extend 2nd strand

Extension products in one cycle serve as template in the next


© jpherveg


Gel Examine
welder masks equipped with glass windows
camera
UV transilluminator
on/off switch
wells
samples unknown size
DNA ladders fragments of known size


PCR APPLICATIONS
MOLECULAR IDENTIFICATION
SEQUENCING
GENETIC ENGINEERING
Molecular Archaeology
Molecular Epidemiology
Molecular Ecology
DNA fingerprinting
Classification of organisms
Genotyping
Pre-natal diagnosis
Mutation screening
Drug discovery
Genetic matching
Detection of pathogens
Bioinformatics
Genomic cloning
Human Genome Project
Site-directed mutagenesis
Gene expression studies


DTI
DNA CLONING
LIBRARY
ELECTRO PHORESIS
PLASMIDS
TISSUE LYSATE
BLOTS
1a. F' plasmid ligates with itself
2a. Gene of interest ligates with itself
Electroporated into cells
Electroporated into cells
Electroporated into cells
1b. Colonies turn blue: LacZ Gene unbroken
2b. Cells die: no antibiotic resistance
Gene cloning & expression

Creating mutation

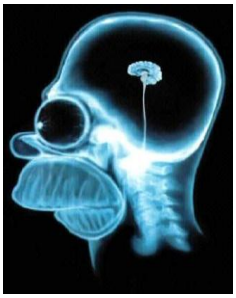
Random or specific

ACCATCGGCCTGCATCA
TGGTAGCCTGACGTAGTCAT
GGTAGCCTGACT----

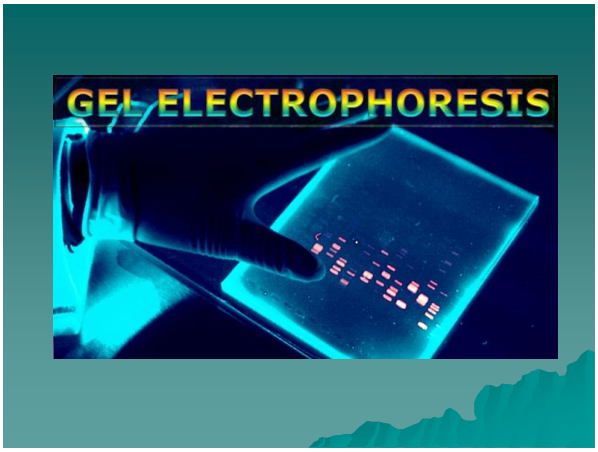
Diagnosis

Samples

PCR is just a tool



How to use it, is up to you

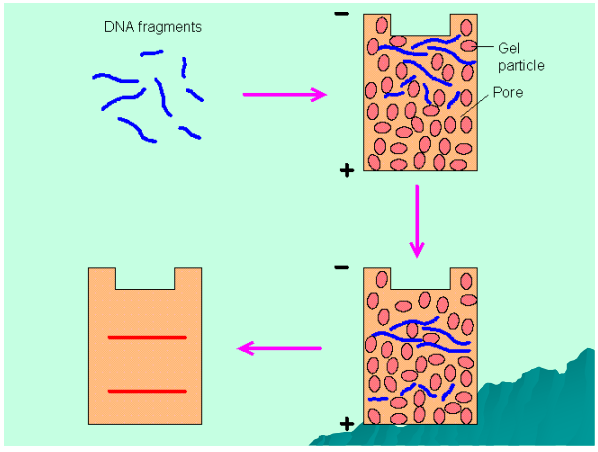


ELECTROPHORESIS:

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

The rate of migration depends on:

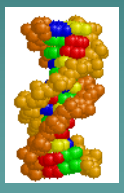
- Strength of the field
- Net charge
- Size & shape of the molecules
- Ionic strength, viscosity & temperature of the medium (in which molecules are moving)



AGAROSE GEL ELECTROPHORESIS

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

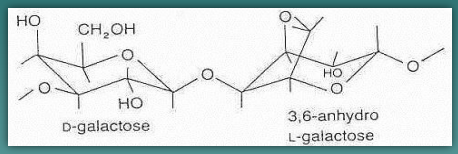
The technique is



- **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 whose basic structure is

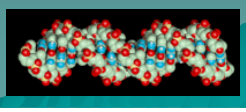


Commercially available agarose is not completely pure; it is contaminated with other polysaccharides, salts, & proteins

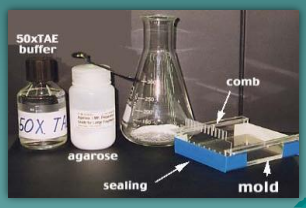
The amount of contamination varies from batch to batch & from manufacture to manufacture

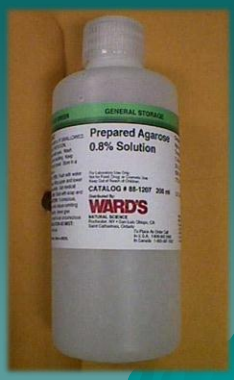
These difference can affect on

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



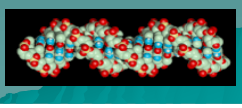
Now, most manufacturers prepare **special grades** of agarose that are screened for the presence of inhibitors & nucleases & for minimal background fluorescence after staining with ethidium bromide

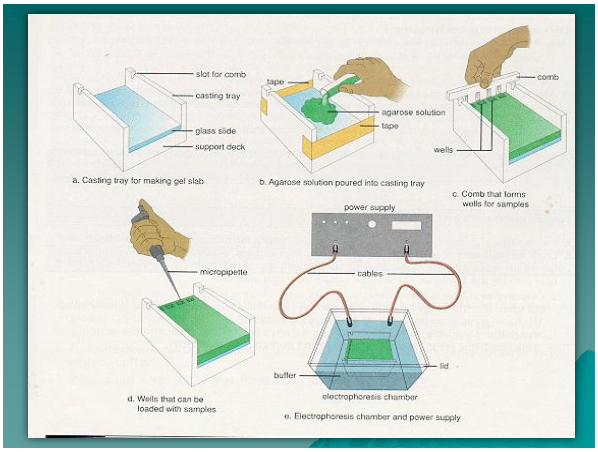




Agarose gels are cast by melting the agarose in the presence of the desired buffer until a clear, transparent solution is achieved

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**





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

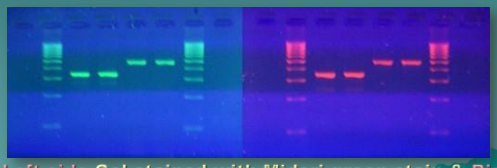
Presence of Intercalating Dyes:

- 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

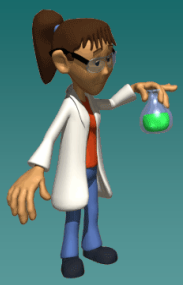
Other dyes are sometimes used including SYBER green or SYBER safe. SYBER dyes are thought to be less carcinogenic than EtBr & to give cleaner, higher powered staining

Midori Green DNA Stain



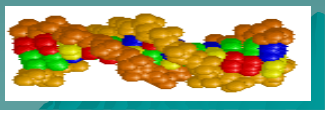
Left side Gel stained with Midori green stain & Right side Gel Stained with Ethidium Bromide

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



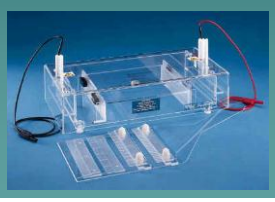
Commonly used Electrophoresis Buffers

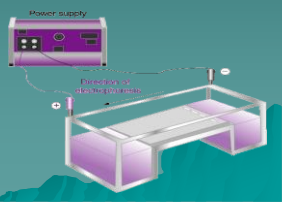
Buffer	Working solution	Conc. Stock solution (Per Liter)
Tris-acetate (TAE)	1X: 0.04 M Tris-acetate 0.001 M EDTA	50X: 243 g Tris base 57.1 ml glacial acetic acid 100 ml 0.5M ETDA (PH 8.0)
Tris-phosphate (TPE)	1X: 0.09 M Tris-phosphate 0.002 M EDTA	10X: 108 g Tris base 15.5 ml 85% phosphoric acid (1.679g/ml)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

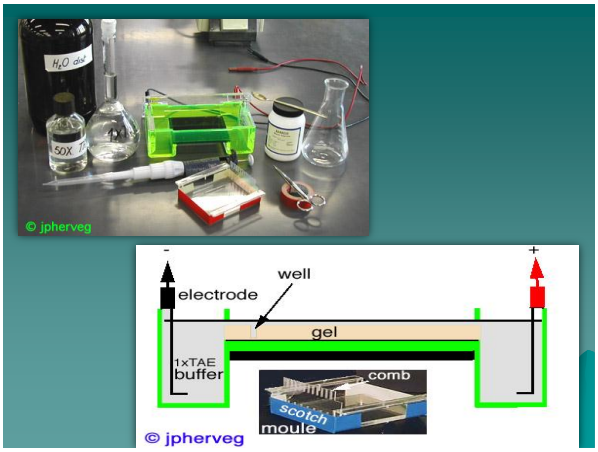




Preparation & Examination of Agarose gels

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

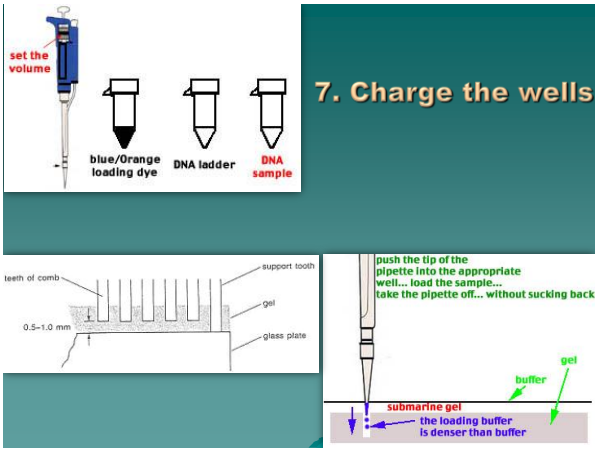
2. Prepare the materials you will need

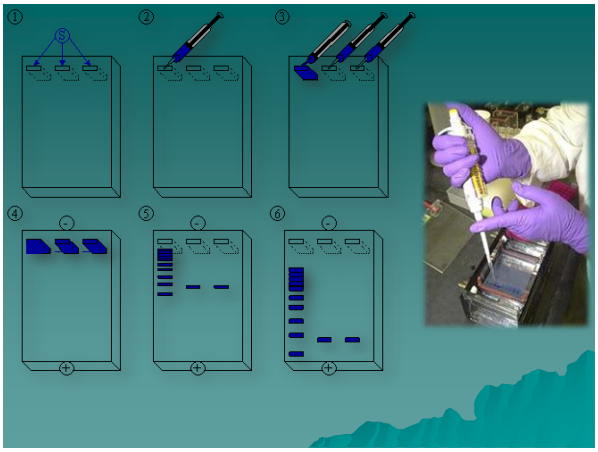

© jpherveg
-
+
well
electrode
gel
1xTAE
buffer
comb
scotch
moule
© jpherveg

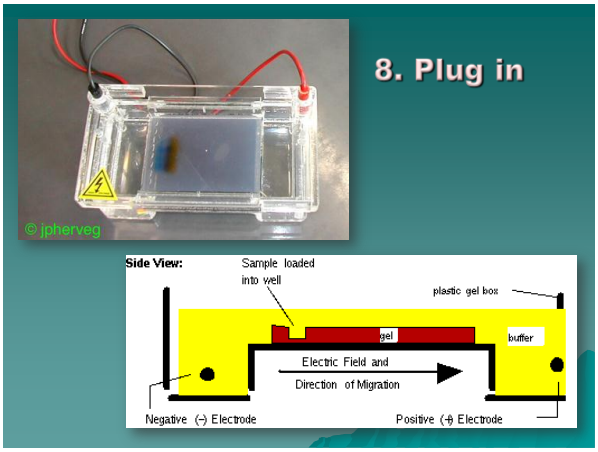

3. Prepare mixture
50xTAE
buffer
comb
agarose
sealing
mold
swirl
gently
4. Gel the mixture

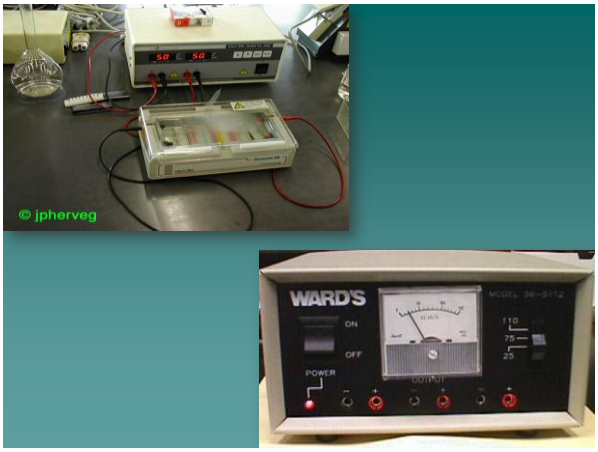

5. Cast gel (add ethidium
bromide to the cooled gel)
55°C, you can handle it
pour the
gel into
the mold

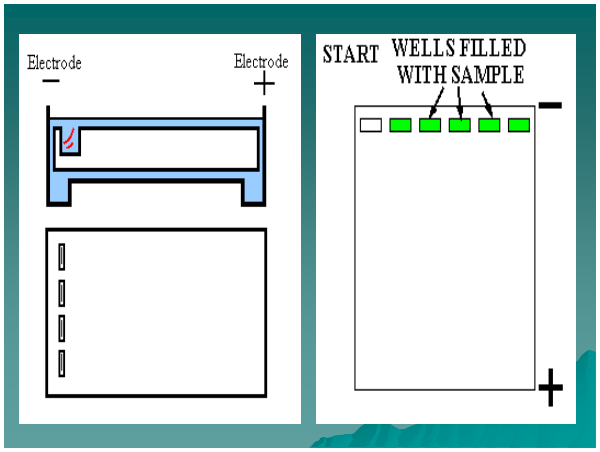

6. Place mold in
the tank
10X TBE
buffer
put the gel in the tank
add buffer

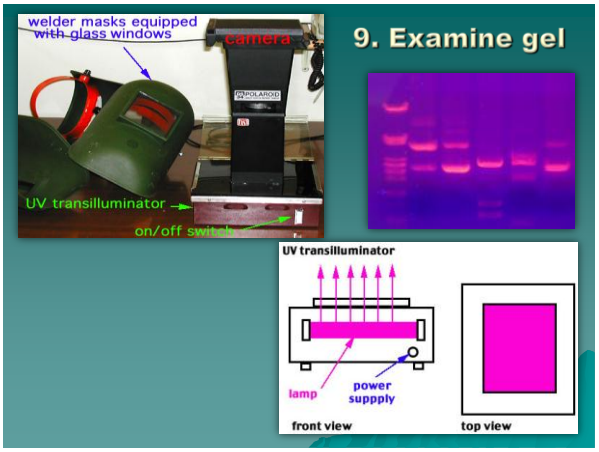

set the volume
blue/Orange loading dye
DNA ladder
DNA sample
7. Charge the wells
teeth of comb
support tooth
gel
0.5–1.0 mm
glass plate
push the tip of the pipette into the appropriate well... load the sample... take the pipette off... without sucking back
buffer
gel
submarine gel
the loading buffer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

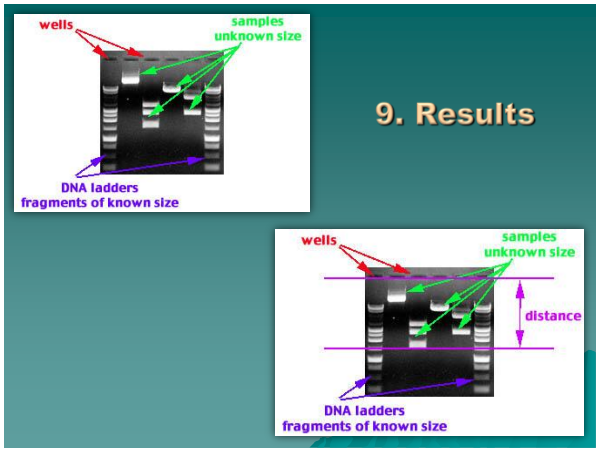

1
2
3
4
5
6
S


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
9. Examine gel
UV transilluminator
on/off switch
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