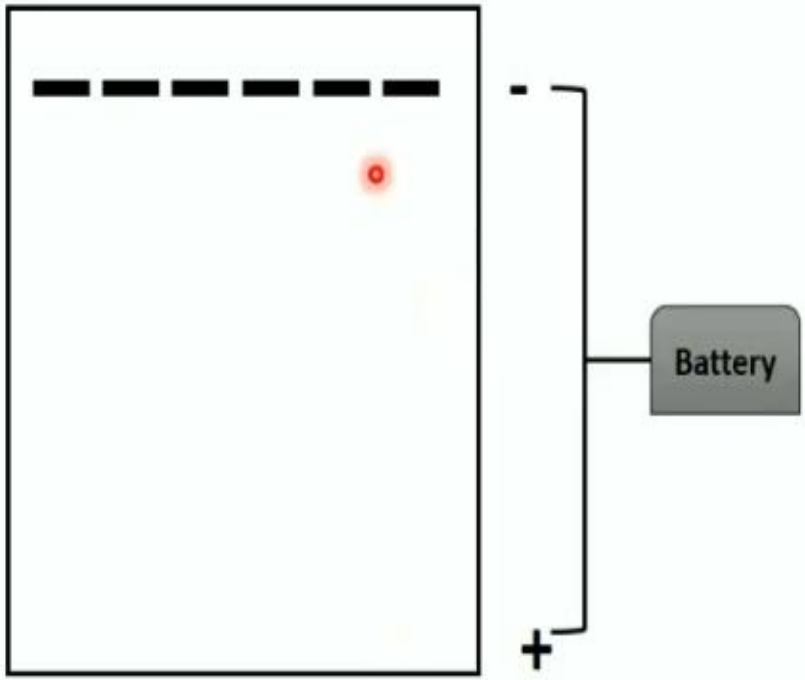
Pulsed Field Gel Electrophoresis(PFGE)

By

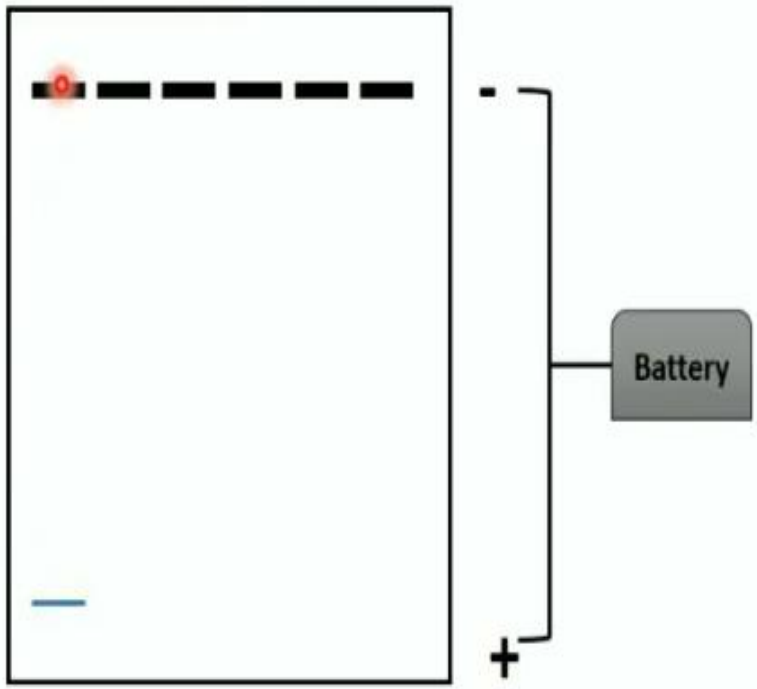
Assist. Prof. Dr. Ali Aldeewan

What is PFGE?



What is PFGE?

- Is a technique developed from agarose gel elechtrophoresis
- Used to separate large fragments of DNA molecules



PFGE(Pulsed Field Gel Electrophorosis)

- Pulsed field gel electrophorosis is a technique used for the separation of large DNA molicules by applying to a gel matrix an electric field that periodically changes direction.
- 1^{ST} developed in 1980 by schwartz and cantor.

- **Principles of PFGE technology**
- It uses specially designed electrophoretic apparatus to separate large DNA fragment ranging from 40 kb to 2000 kb.

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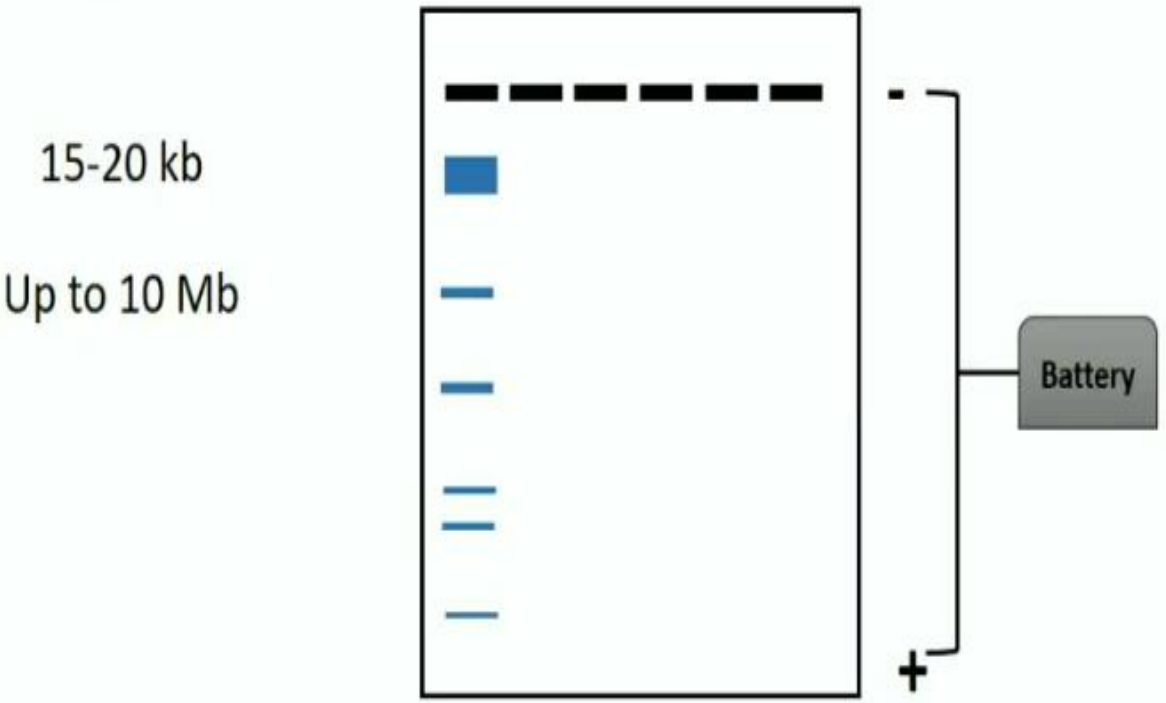
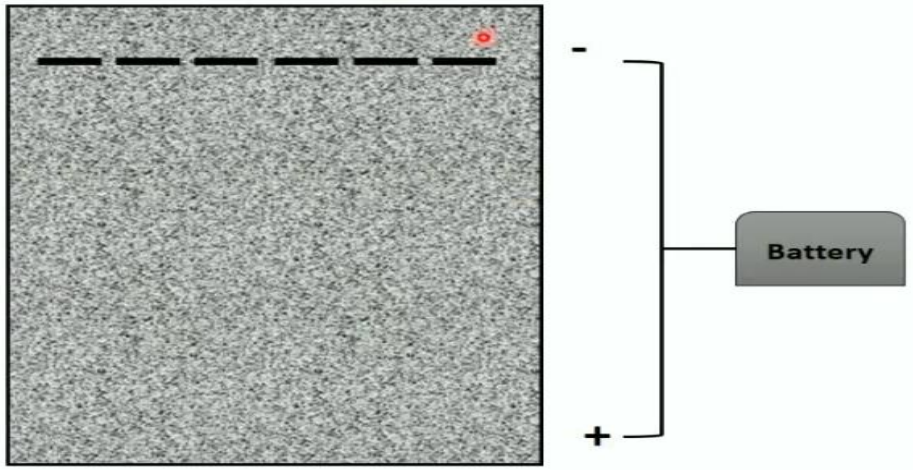


What Modifications?

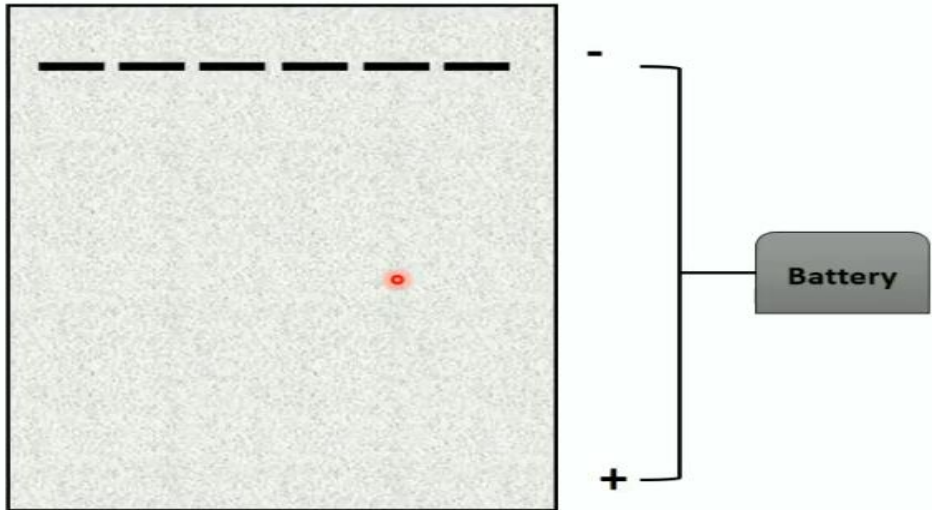
1- the concentration of the agarose solution

(2%)



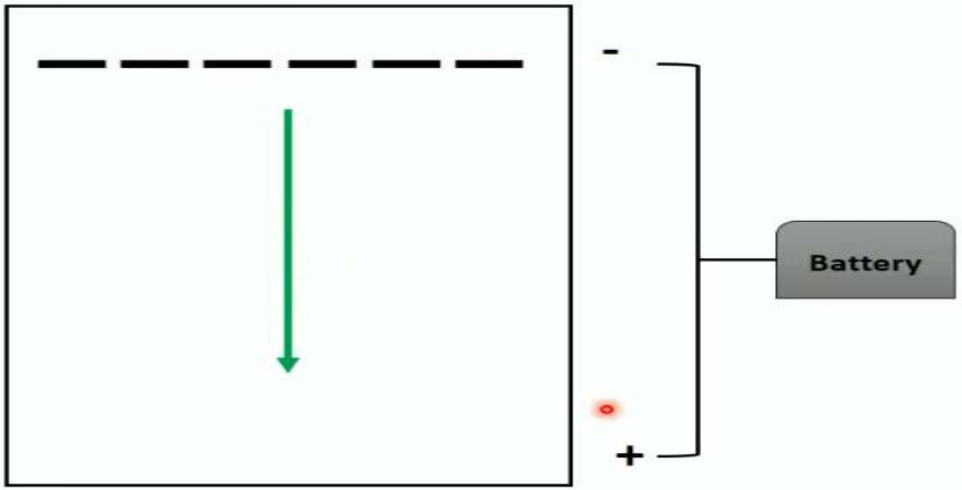
What Modifications?

1- Low concentration of the agarose solution (1%)



What Modifications?

2- Change the **direction** of the electrical field



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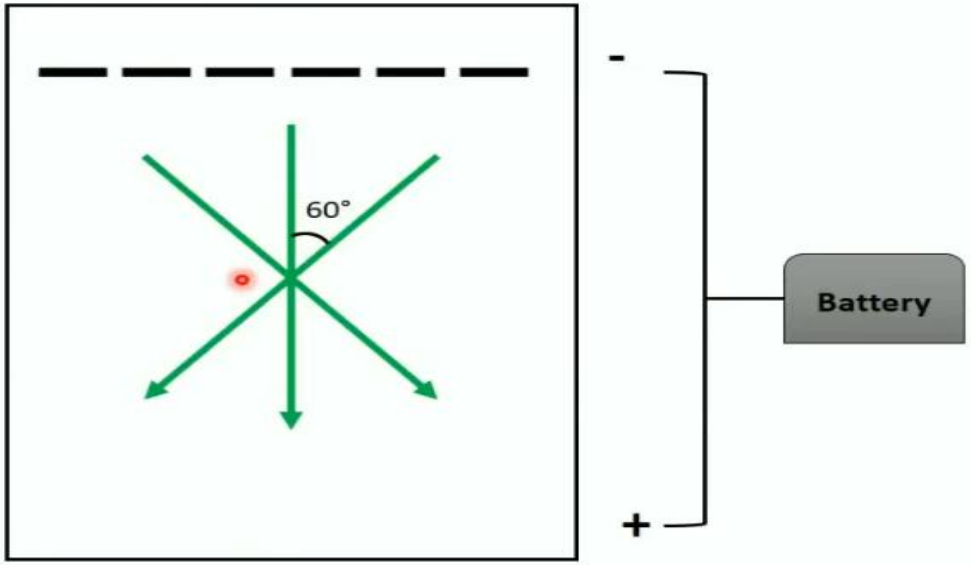


Uses

- strain characterisation
- Gene mapping in microbes and mammalian cells .
- Monitoring and evaluating different microorganisms in clinical samples and in soil and water.
- A reliable and standard method in vaccine preparation
- Epidemological studies
- strain development
- separation of molicules having >50kb molicular weight.
- produce DNA fingerprint for a bacterial isolate.

DEVELOPMENT OF PFGE

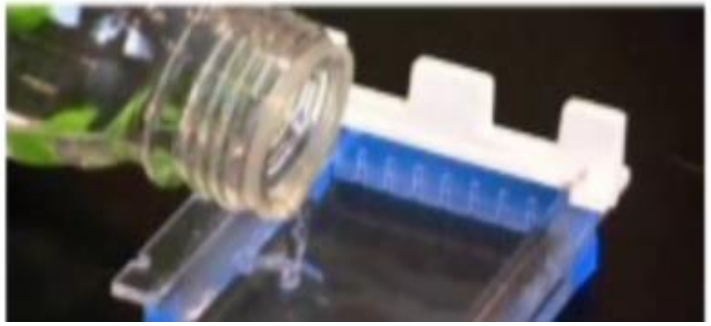
Conventional electrophorosis.....>PFGE

PFGE Procedure

I. Cell lysis and release of intact chromosomal DNA
II. Restriction Endonuclease digestion of chromosomal DNA
III. Separation of large DNA fragments
IV. Analysis of DNA fragment length polymorphism

1.Cell lysis and release of intact chromosomal DNA

- Overnight culture of the bacterial isolate(10^9 cells /ml)
- Add detergents and enzymes to the bacterial suspension
- Mix bacterial cells with warm agarose and pipette into plastic mold to form agarose plugs
- Wash with preheated water and TE buffer
- Agarose gel matrix keep chromosomal DNA and remove the other components.



2.Restriction Endonuclease digestion of chromosomal DNA

- R.E ENZYME(molicular scissors): to cleave ds DNA at restriction sites.
- The choice of RE enzyme depend on bacterial sp.
- Once the recognition site is located , the enzyme catalyses the digestion of DNA at defined position.....>produce restriction fragments
- Restriction Endonuclease Sma 1 recognises CCC/GGG sequence, that cleave DNA of most gram positive bacteria.
- After digestion, plugs are cut into appropriate size ....>loaded onto comb teeth.....>sealed.....>placed in electrophoresis chamber.

3. Separation of large DNA fragments

- **Resolution of DNA depend upon**:

1. Concentration and composition of gel and buffer
2. Temperature
3. Pulsed field condition(electrophoresis duration, electric field strength, pulse angle, and switching time)

- **Migration rate vary in different buffer**
- **Agarose concentration**: 0.8-1%
- **Voltage** 6 V /cm

- **Problems associated with the detection of band:**

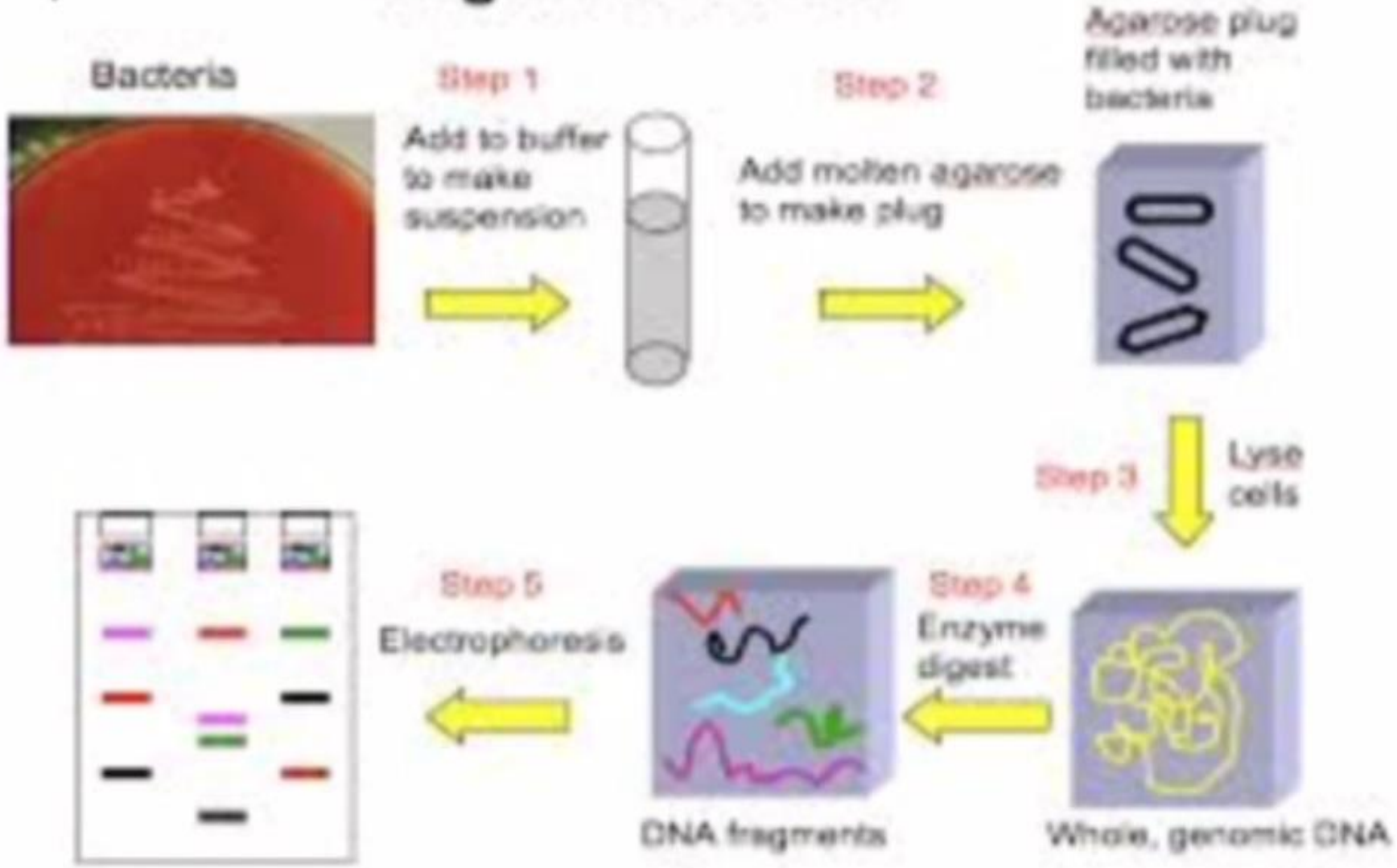
1. DNA digestion in gel
2. Incomplete digestion by Restriction endonuclease
3. Incorrect electrophoresis condition

4. Analysis of DNA fragment length polymorphism

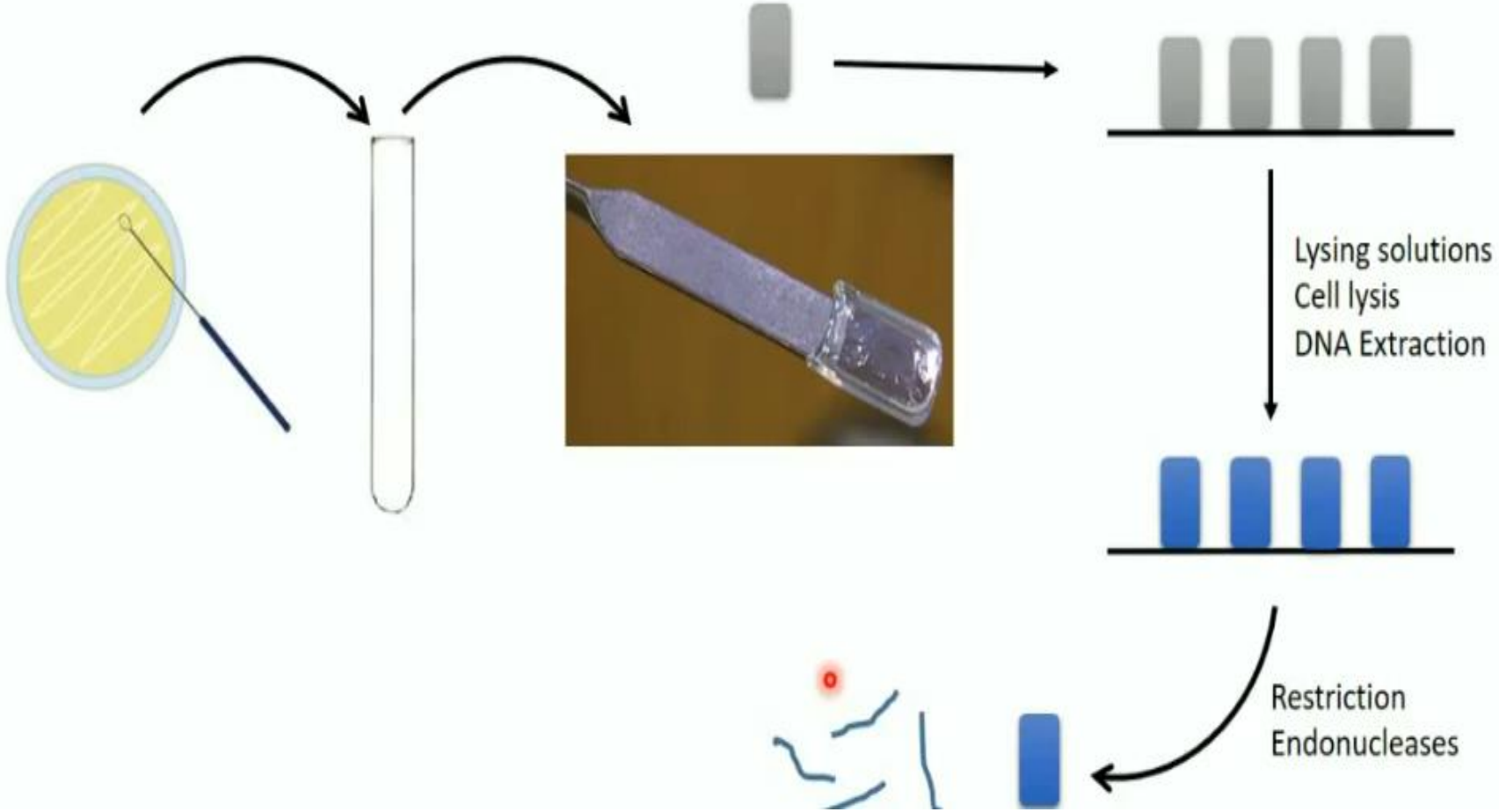
- Visualisation: staining with **ethidium bromide**
- Each lane on gel represents the chromosomal pattern of one bacterial isolate.
- Same band pattern: **indistinguishable**
- 1-3 band difference**: closely related**
- 4-6 band difference: **possibly related**
- 6 or more difference: **unrelated**
- Computerised gel scanning and data anlysis:eg: **Dendrogram.**

- .

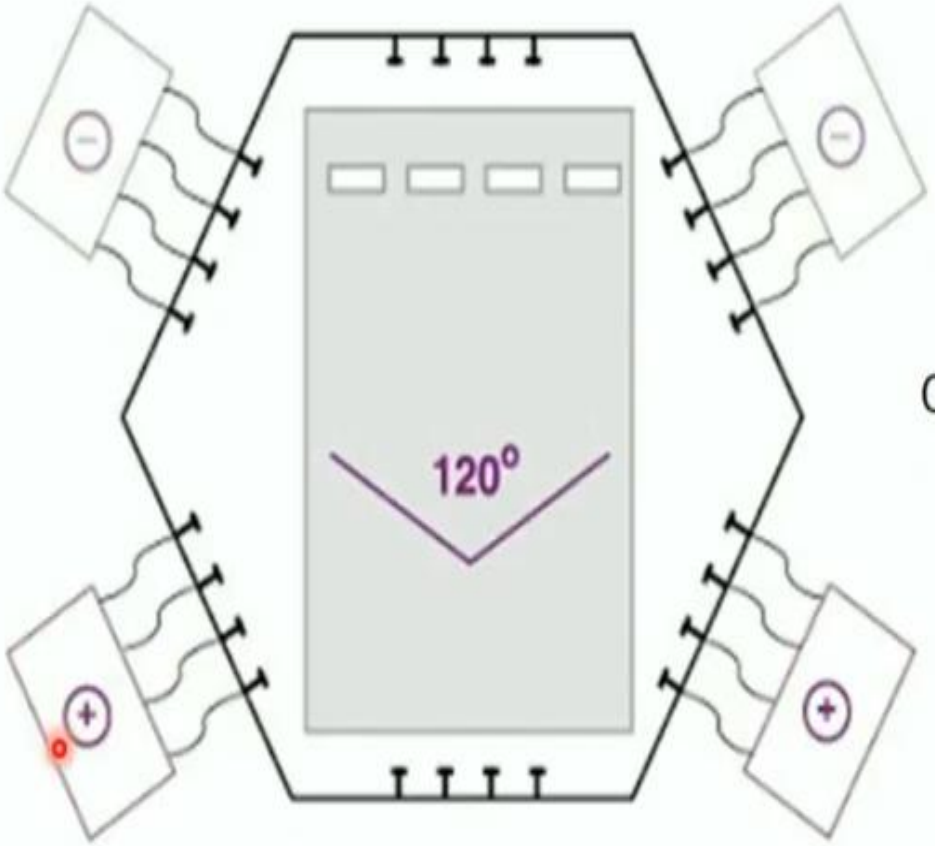
Figure 1 - PFGE



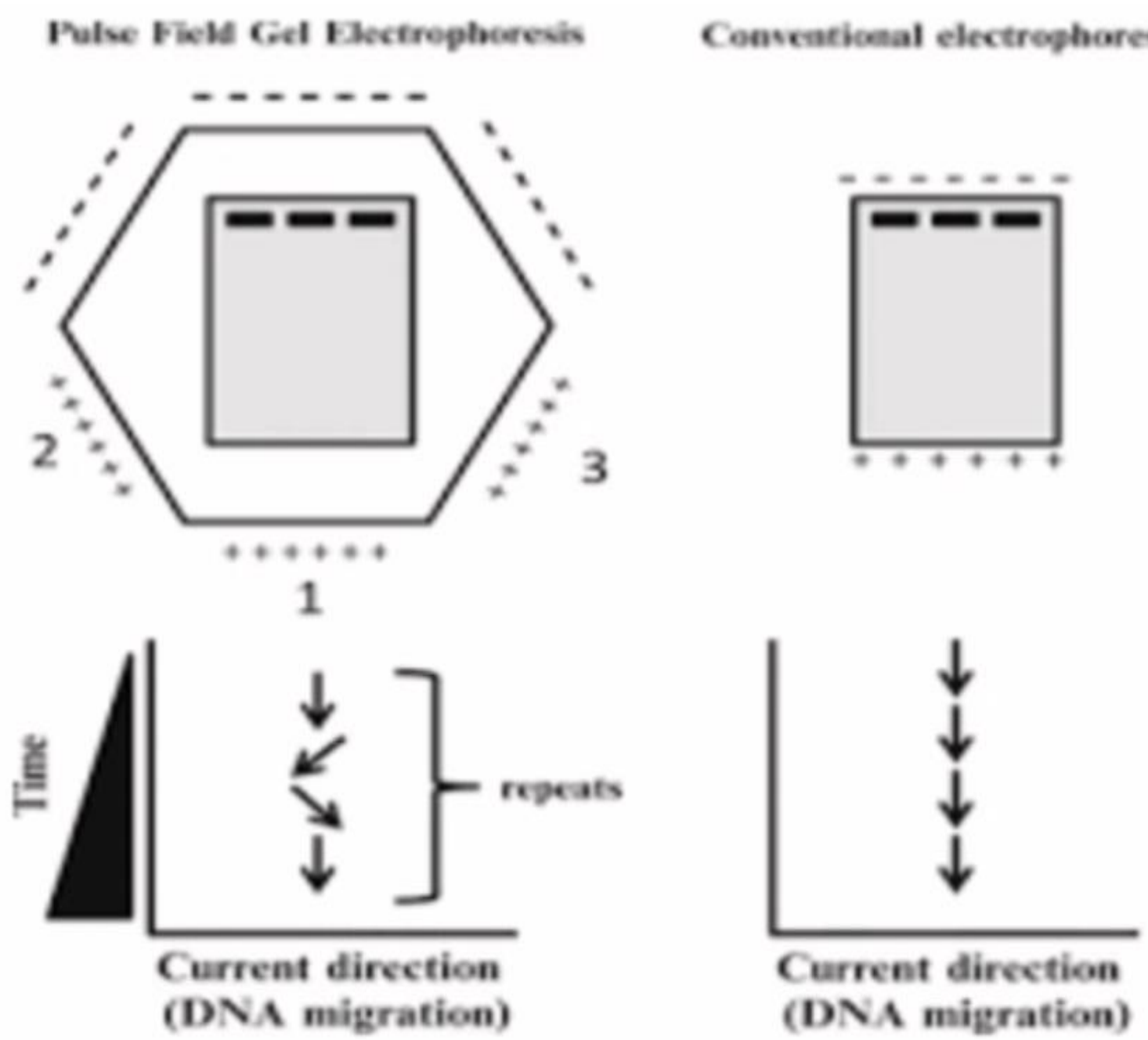
How To Perform It?



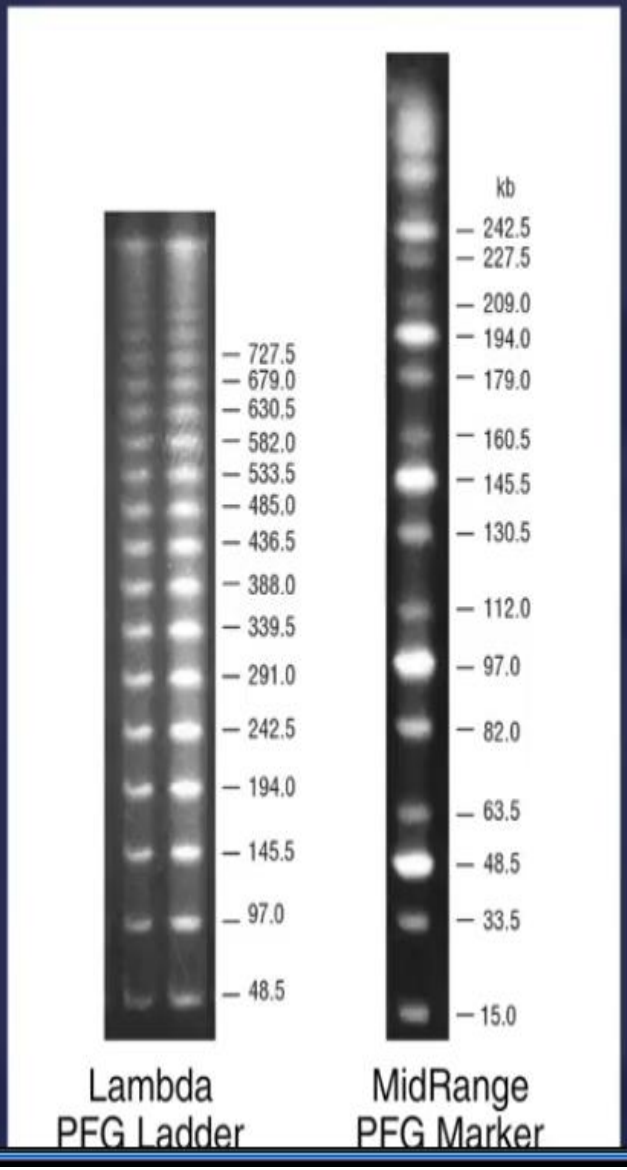
How Does It Work?



Obtuse reorientation angle

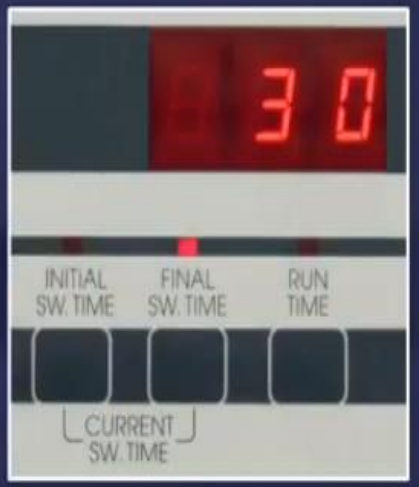

Pulse Field Gel Electrophoresis
Conventional electrophoresis
2
3
1
Time
repeats
Current direction
(DNA migration)
Current direction
(DNA migration)

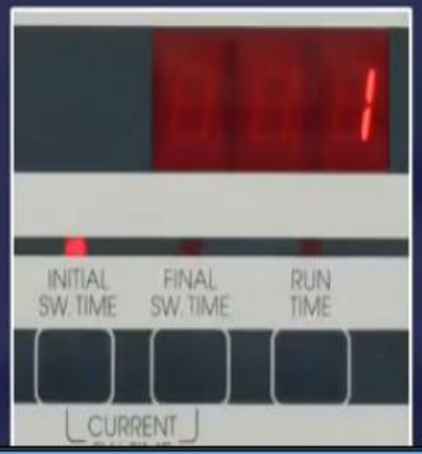
Switch Time Ramp Examples



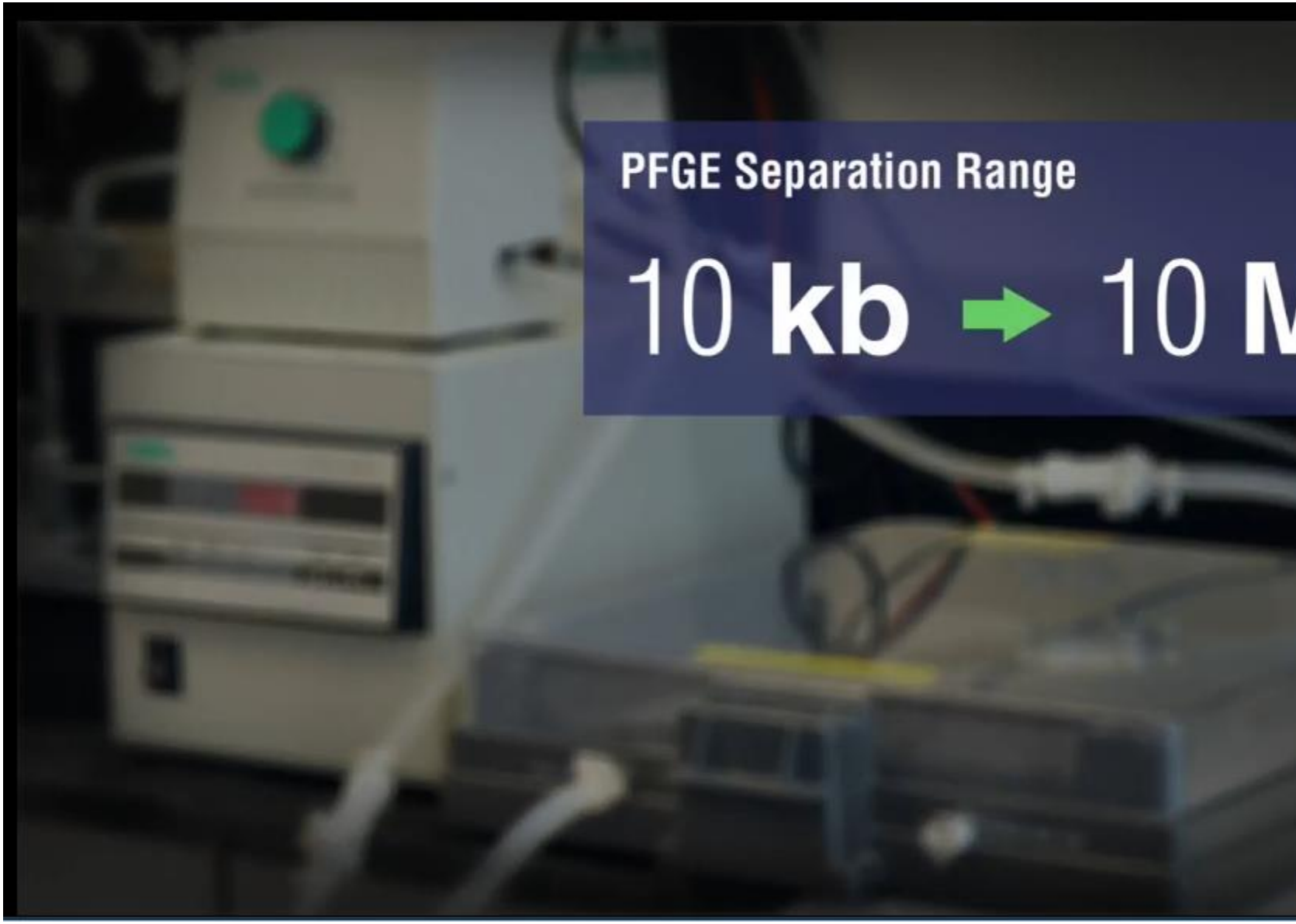
Initial switch time

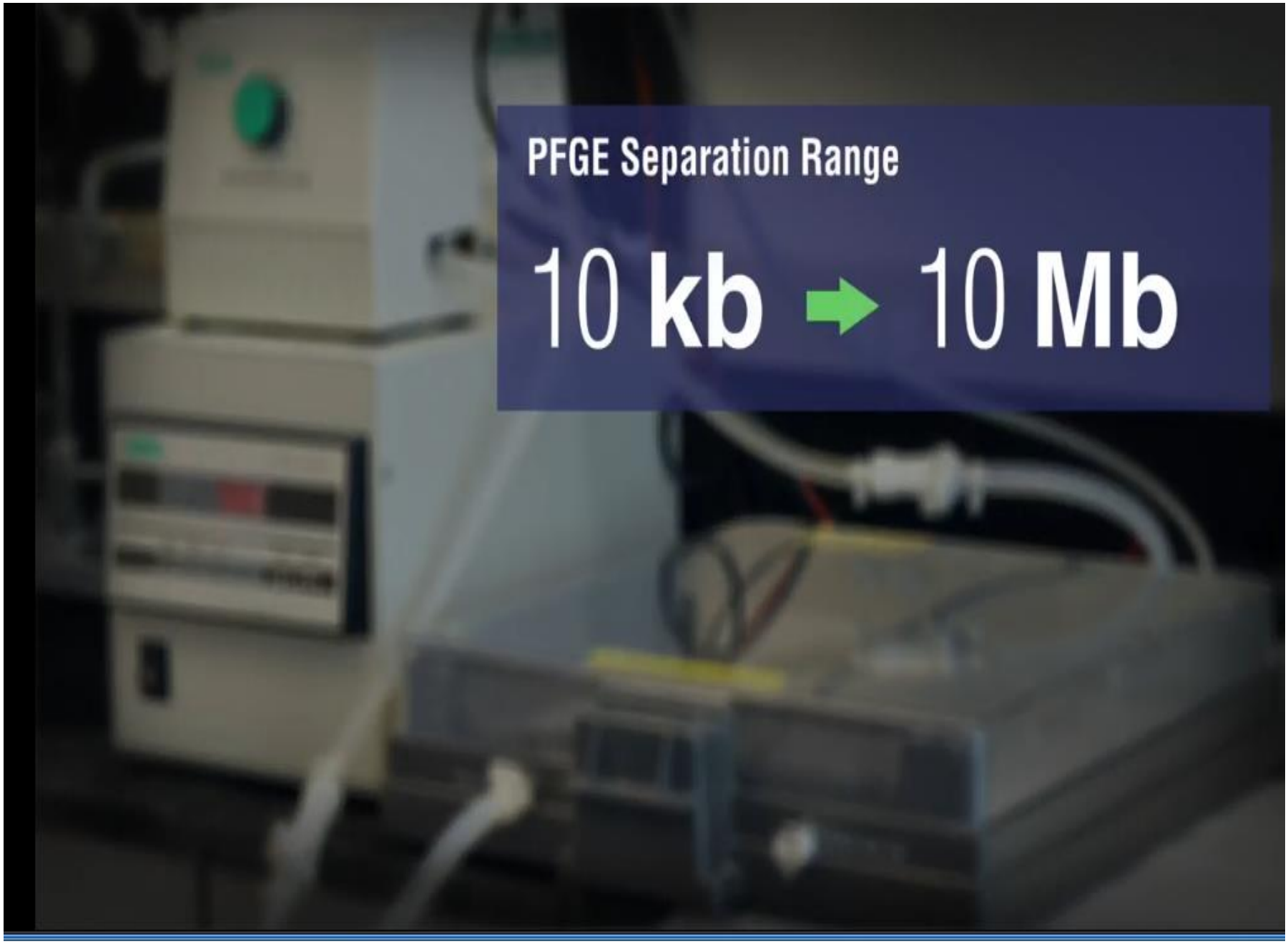
Final switch time






Voltage
A 6.0
VOLTS/CM
INCLUDED ANGLE
ACTUAL CURRENT
PAUSE START RUN
Angle
1 2 0
/CM
INCLUDED ANGLE
ACTUAL CURRENT
PAUSE START RUN


PFGE Separation Range
10 kb
10 M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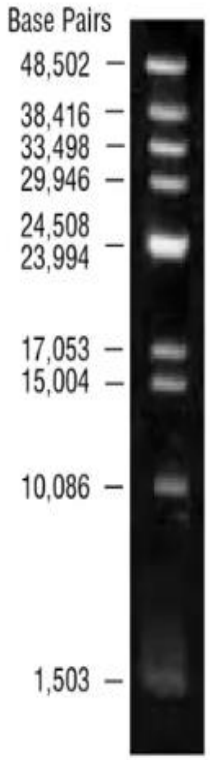
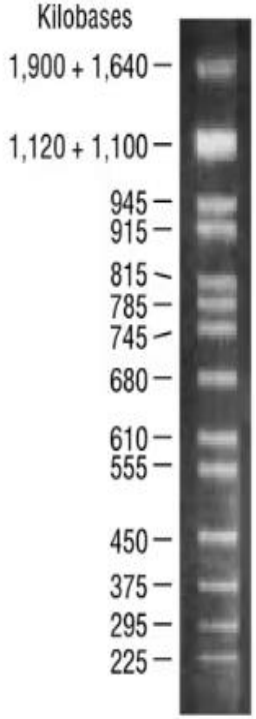
PFGE Separation Examples



λ DNA-Mono Cut Mix
1.5 to 48.5 kb



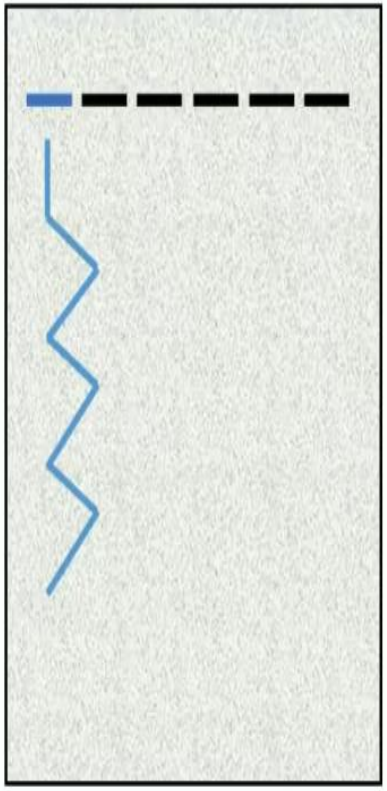
Yeast Chromosomes
225 to 1,900 kb

Principle of PFGE

DNA segments elongate in the presence of an electrical field

The relaxation rate depends on the size

Principle of PFGE



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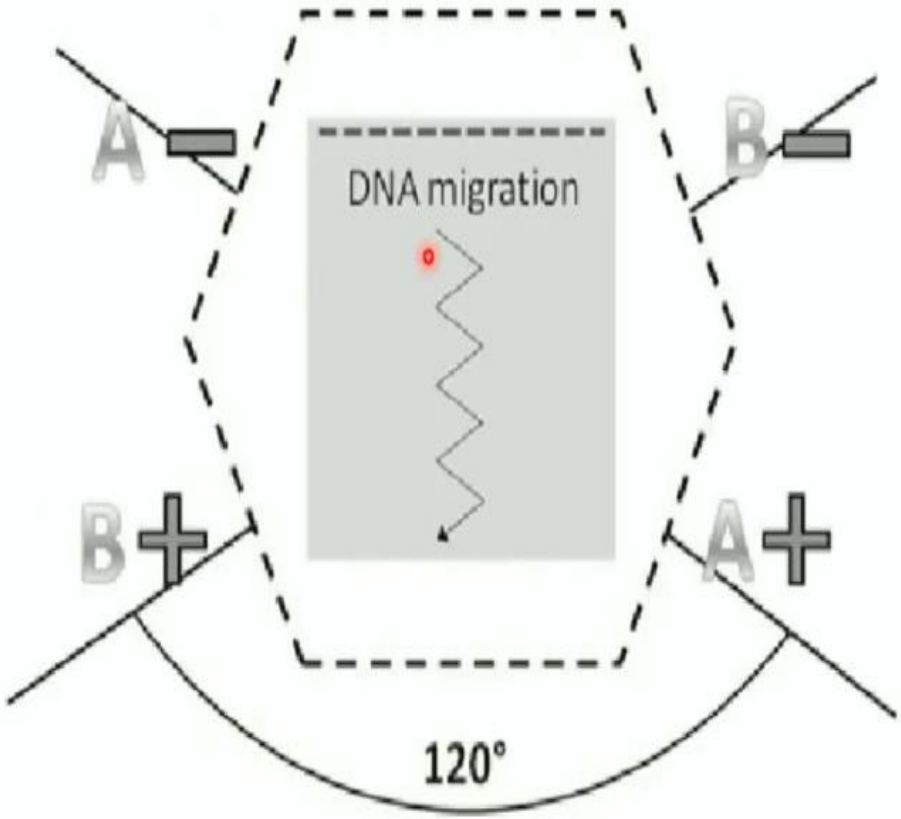
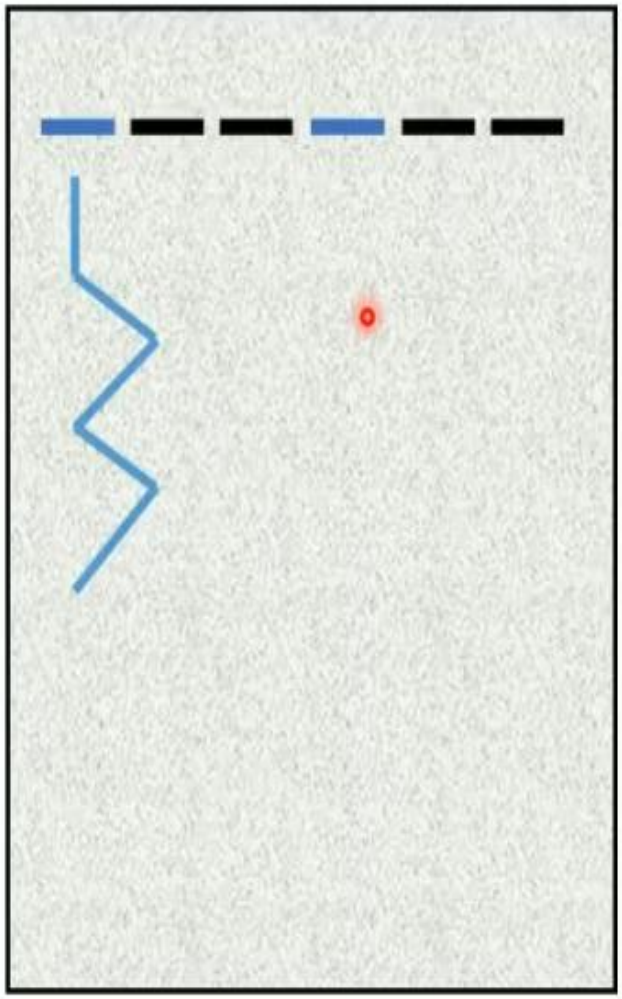


What Important Parameters

1- Voltage (V/cm)

2- The angle:
Smaller angle (106°) gives better resolution with larger segments.



3- The switching time:
The longer the switching time is, the further the large segments will migrate throw the gel.

4- Temperature:
DNA stays in the gel overnight.
Pumping the running buffer through a chiller during the run
Switch the buffer.

PFGE performance characteristics

- Performance is measured by

1. Discriminatory power
2. Reproducibility
3. Stability
4. Typeability

➢ **Application to gram positive bacteria**

For molecular typing of nosocomial pathogens

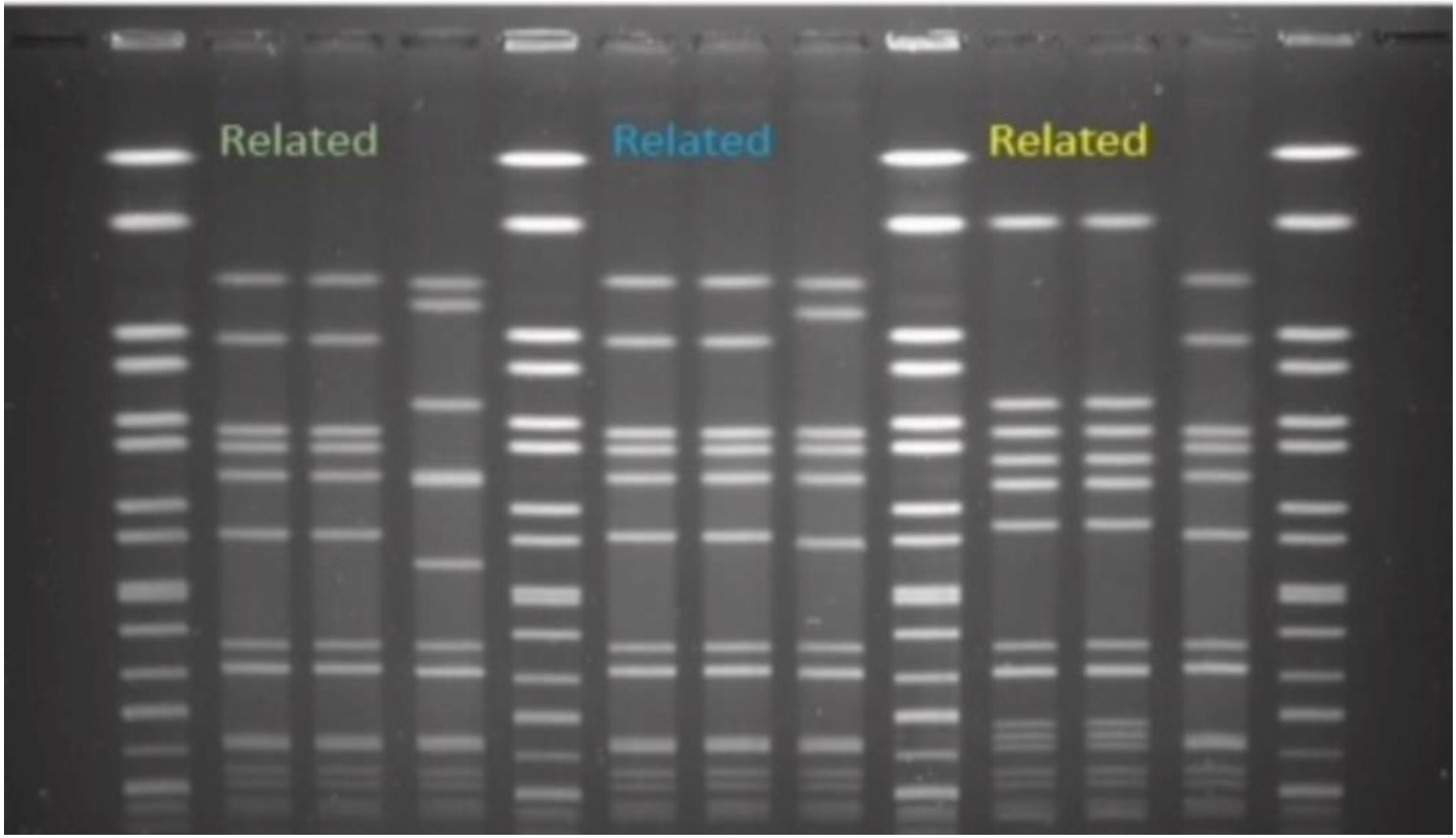
To examine genetic identity of MRSA

➢ **Application to gram negative bacteria**

To investigate epidemiological relatedness of strains of gram negative bacteria.

Epidemiological investigations

Thank you all .....



How to Prepare and Load a Pulse Field Gel Marker.mp4