An Introduction to Polymerase Chain Reaction (PCR)

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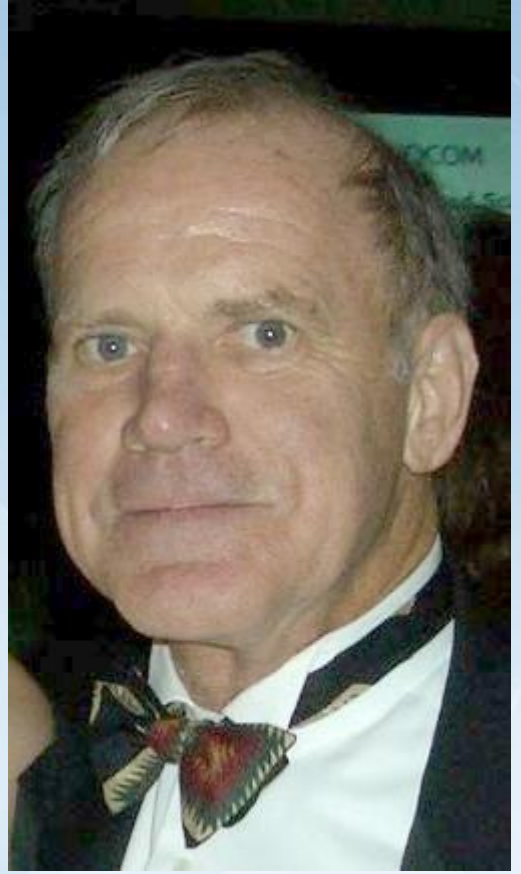
Introduction

- The technique was invented by Dr. Kary Mullis, 1986
- for which he received the Nobel Prize in Chemistry in 1993.



PCR Achieves Fame and Fortune

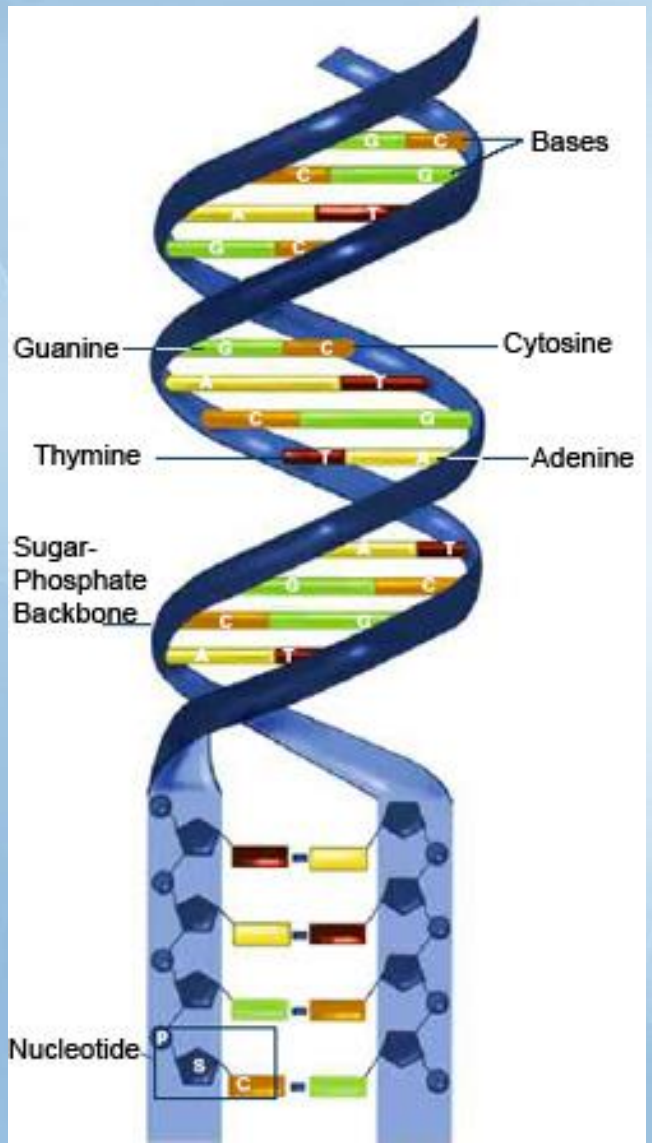
--becomes standard in molecular biology tool box--

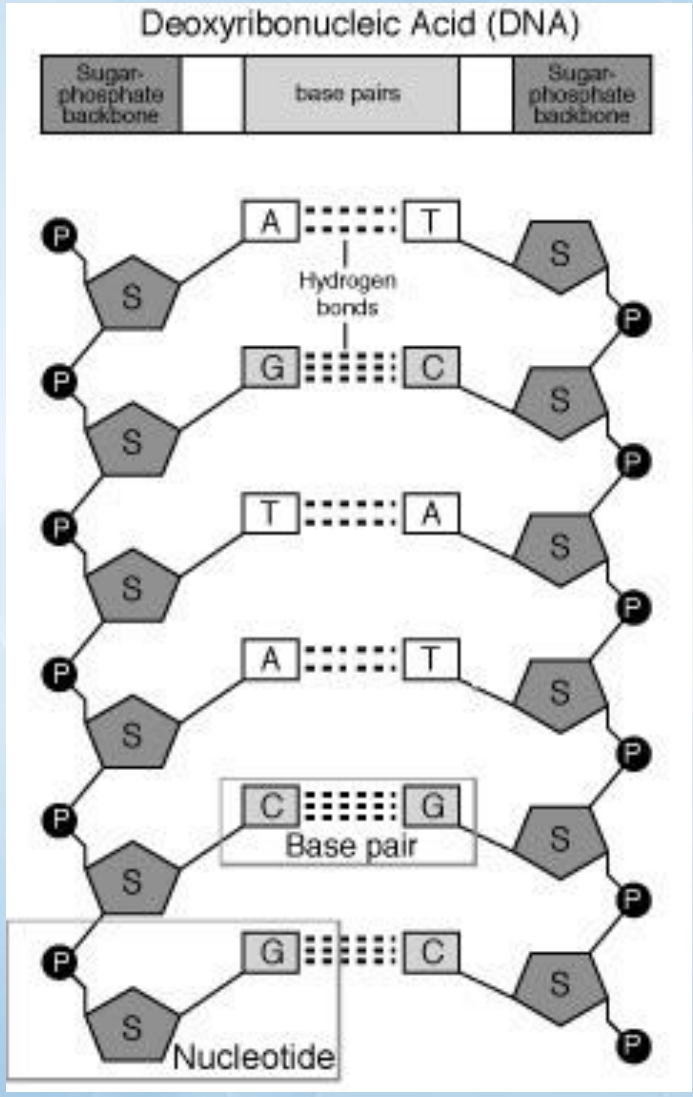
The Molecule of the Year

Ruth Levy Guyer and Daniel E. Koshland, Jr.

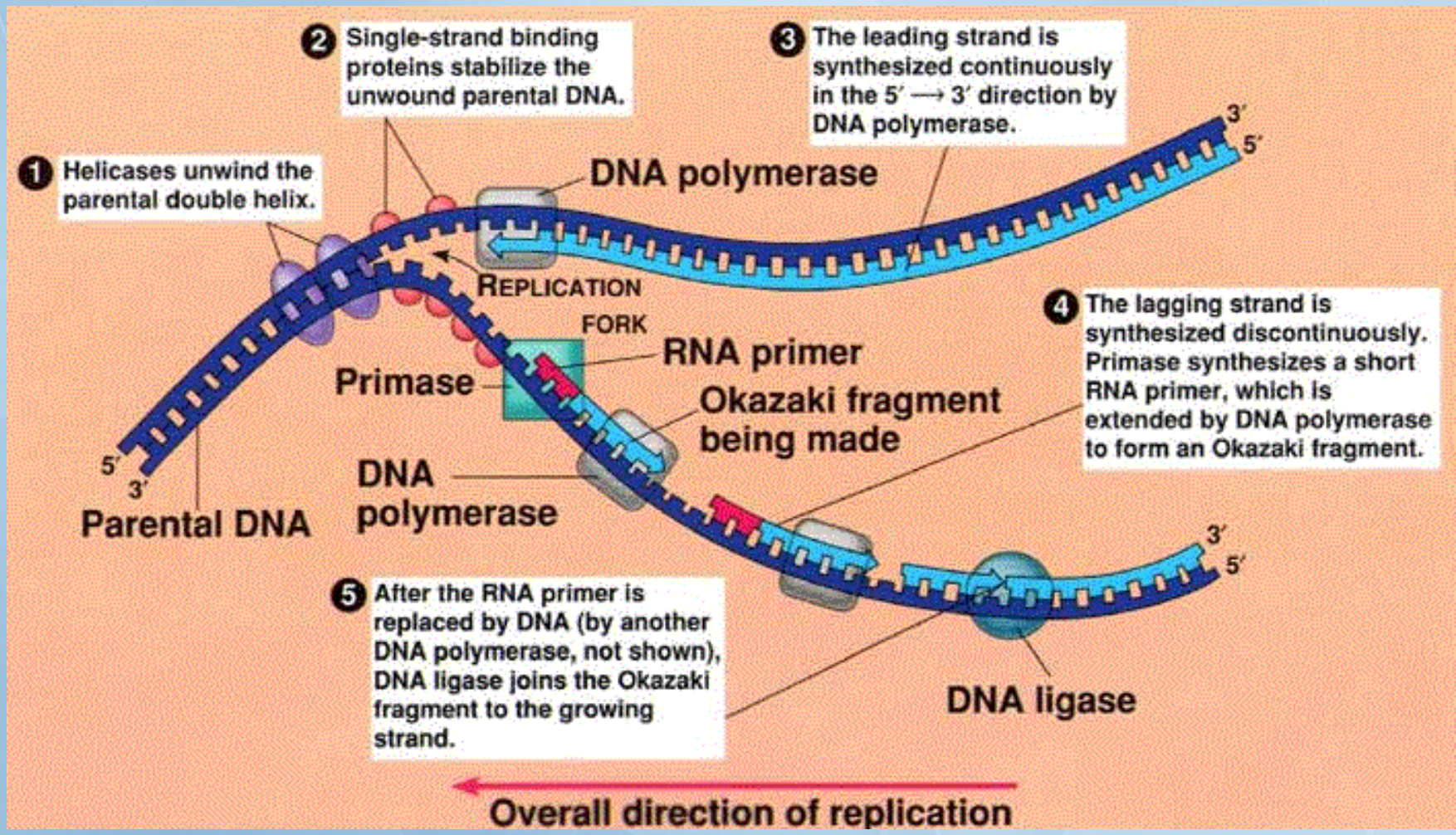
Science has selected the polymerase chain reaction as the major scientific development of 1989 and has chosen for its first "Molecule of the Year" the DNA polymerase molecule that drives the reaction. The list from which the polymerase chain reaction (PCR) was chosen included an impressive array of accomplishments in many areas of science and technology; additional kudos are therefore conferred below to 17 of the other big "stories" that made 1989 an exciting year for scientists and for followers and beneficiaries of science. Although the PCR procedure was introduced several years ago, use of the technique truly burgeoned in 1989; in much the same way, the full potentials of many of the interesting "runner-up" scientific achievements of this year are likely to be realized sometime in the years to come.

DNA Structure





DNA Replication



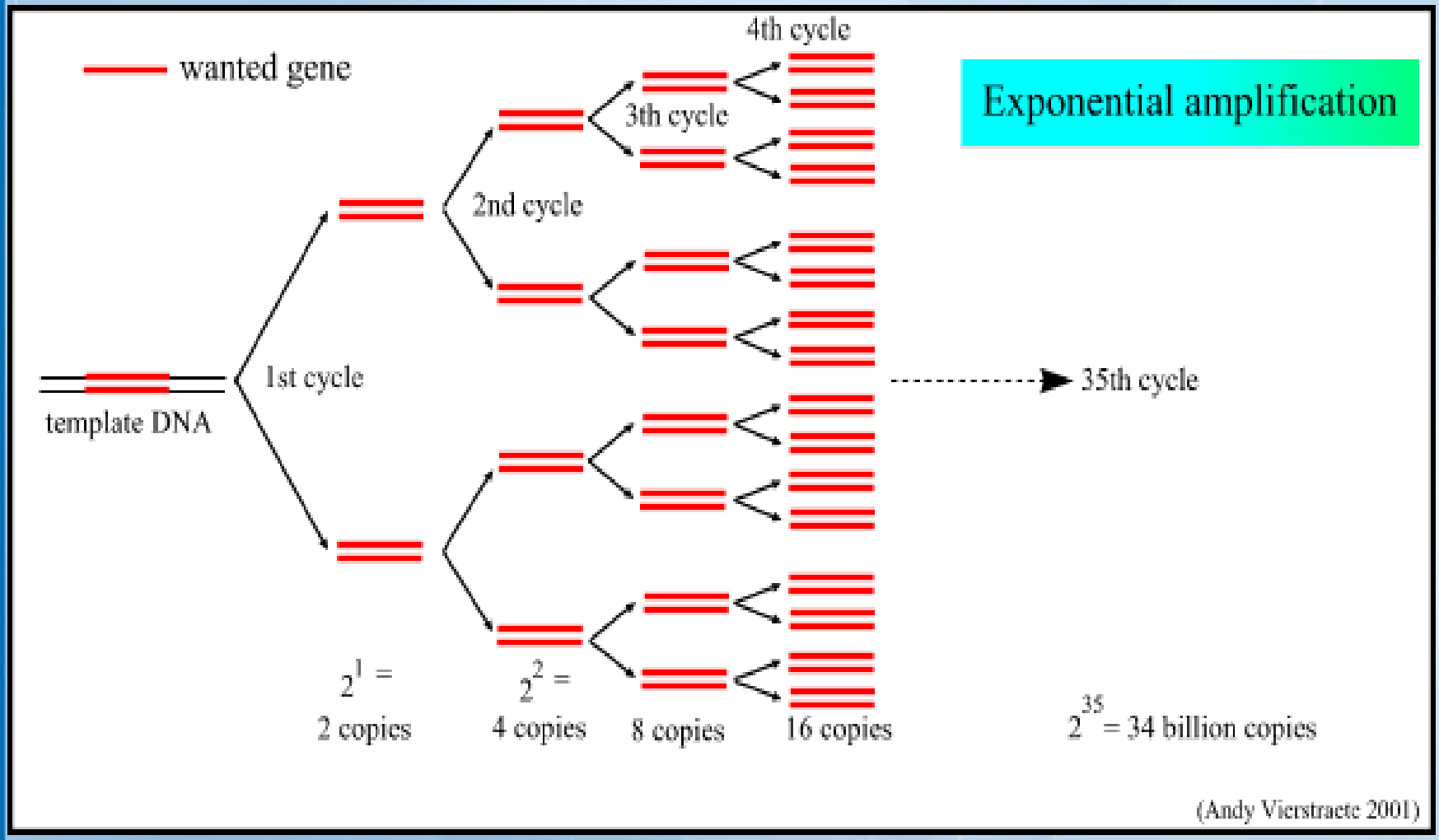
Polymerase Chain Reaction (PCR)

- PCR is a technique which is used to amplify the number of copies of a **specific region of DNA**, (usually fewer than 3000 base pairs) in order to produce enough DNA to be adequately tested.

- **Millions** of copies of a segment of DNA can be made within a few hours

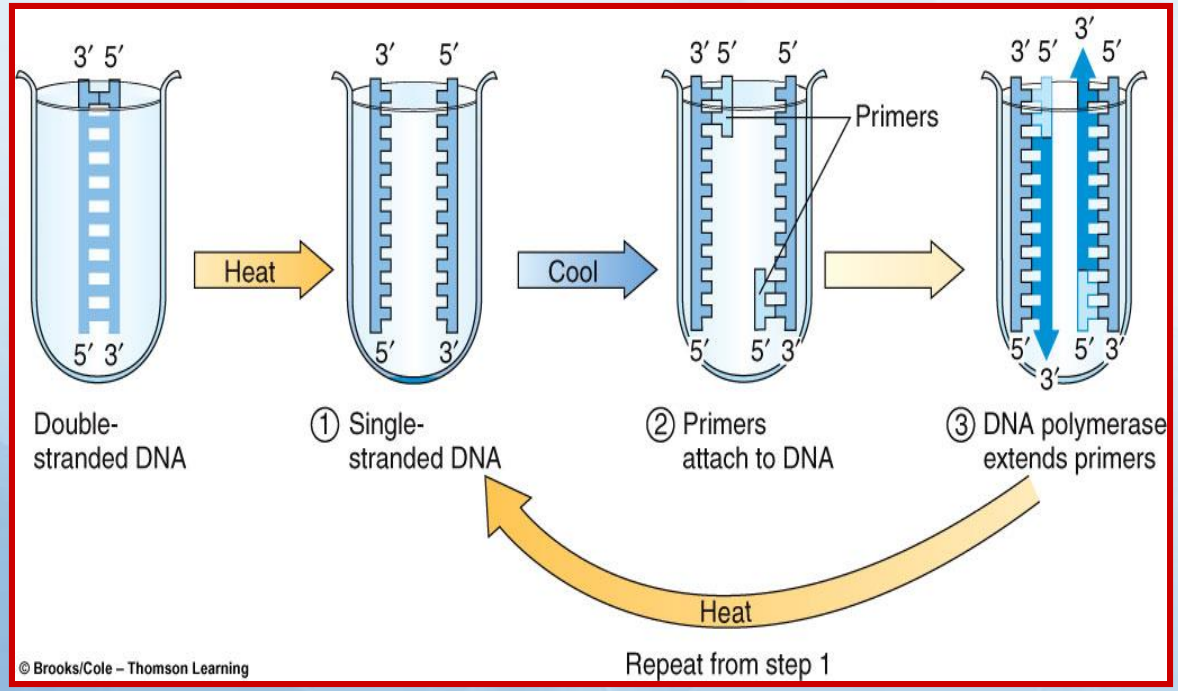
- As a result, it now becomes possible to analyze and characterize the DNA.

DNA amplification by PCR (overview)

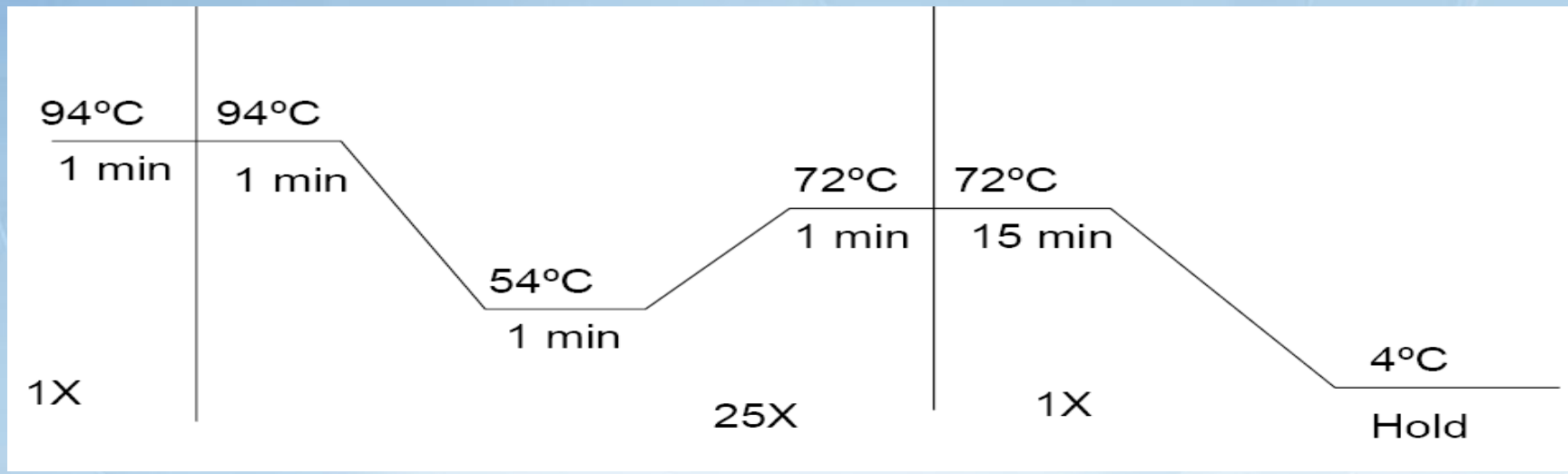


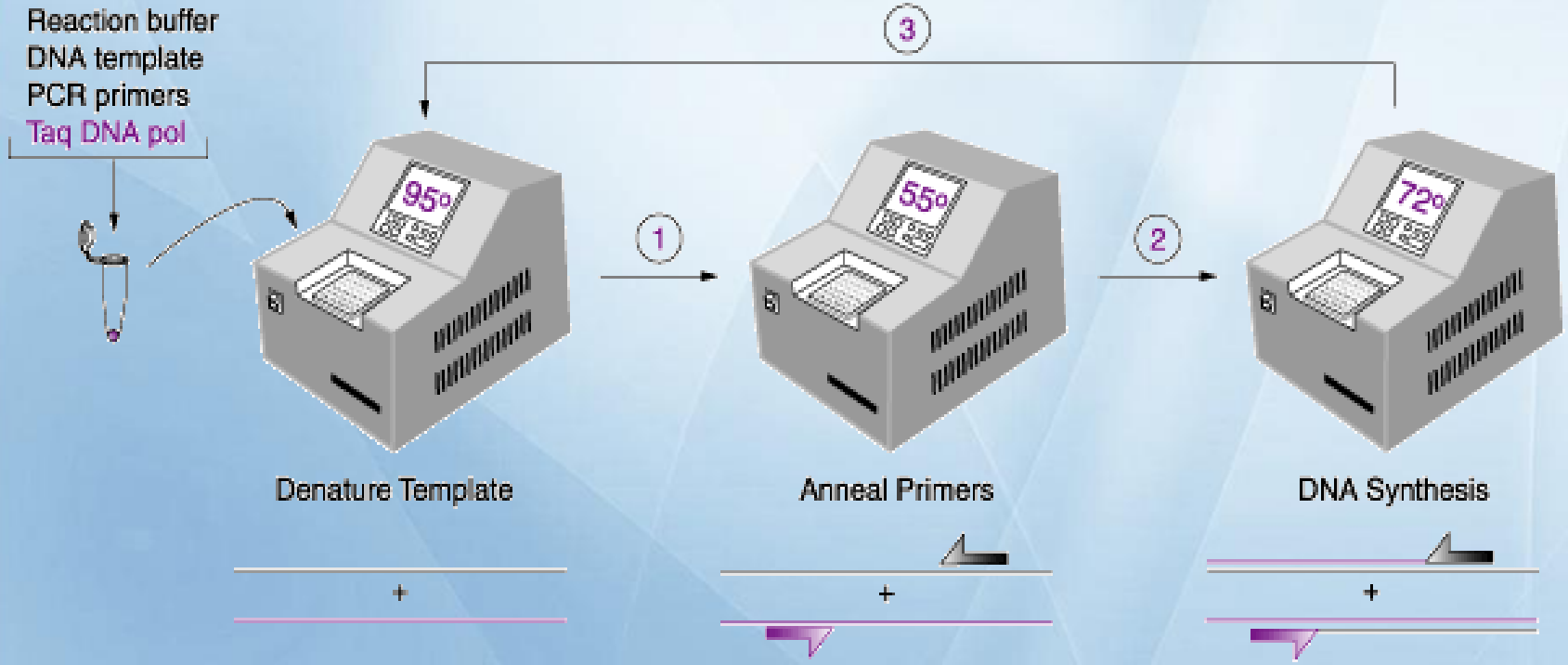
PCR Cycle

- **Each cycle (Round) of PCR contains 3 steps:**
  - **1- Denaturation**
  - **2- Primer annealing**
  - **3- Primer extension**
- **The cycle usually repeated for 25 – 40 times.**



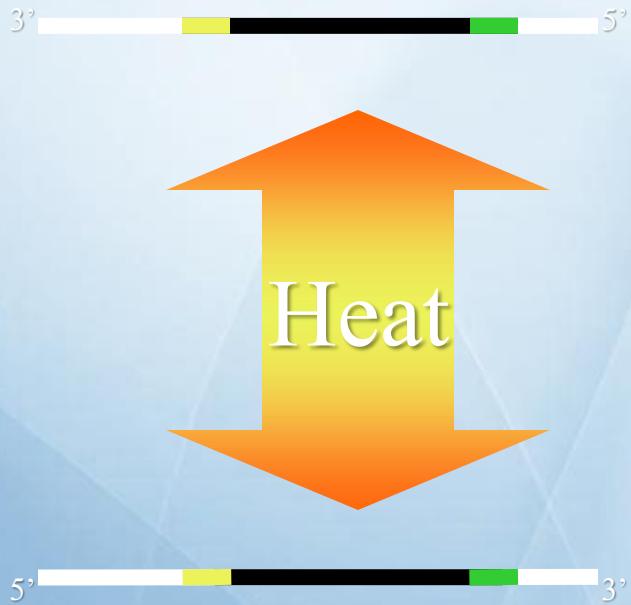
Programming the Thermocycler



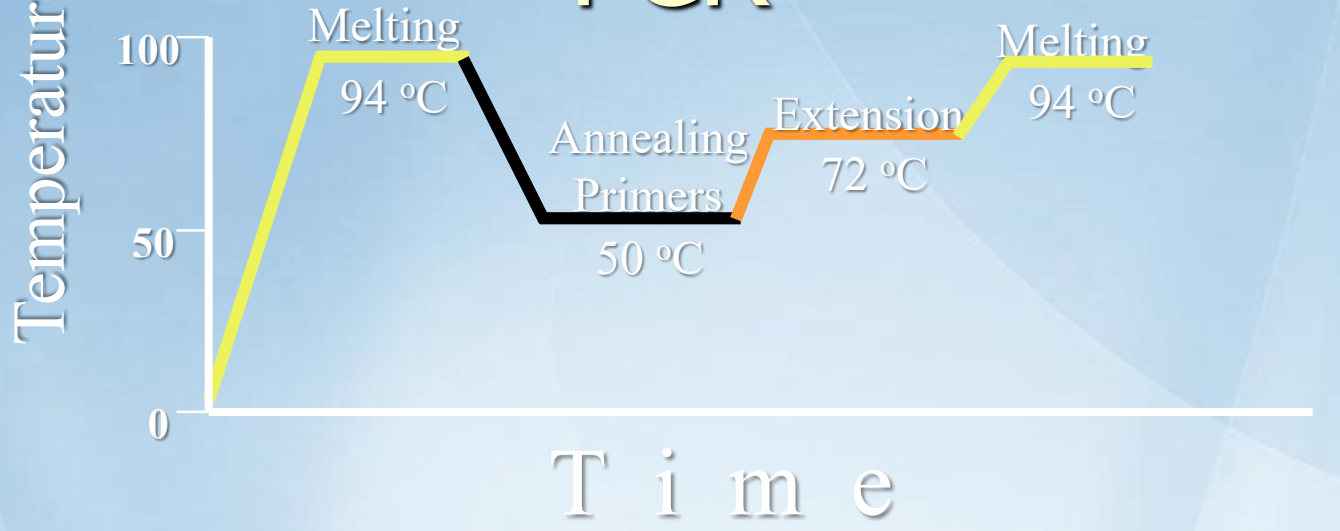


PCR

PCR



PCR


Temperature
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Melting
94 °C
Annealing
Primers
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Extension
72 °C
Melting
94 °C
T i m e


3'
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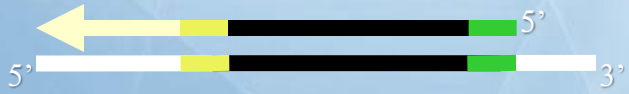
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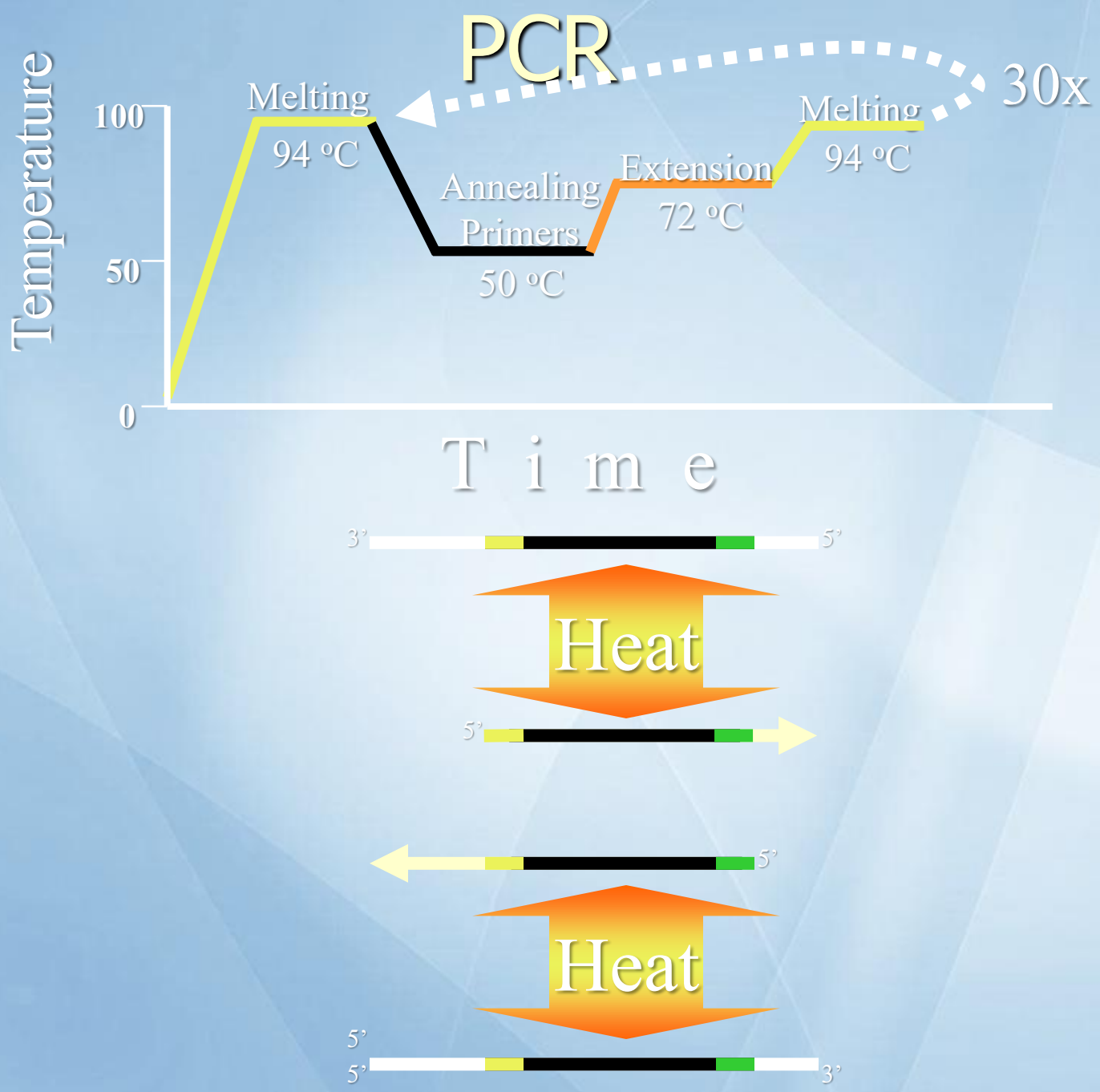
PCR
30x
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Extension
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T i m e
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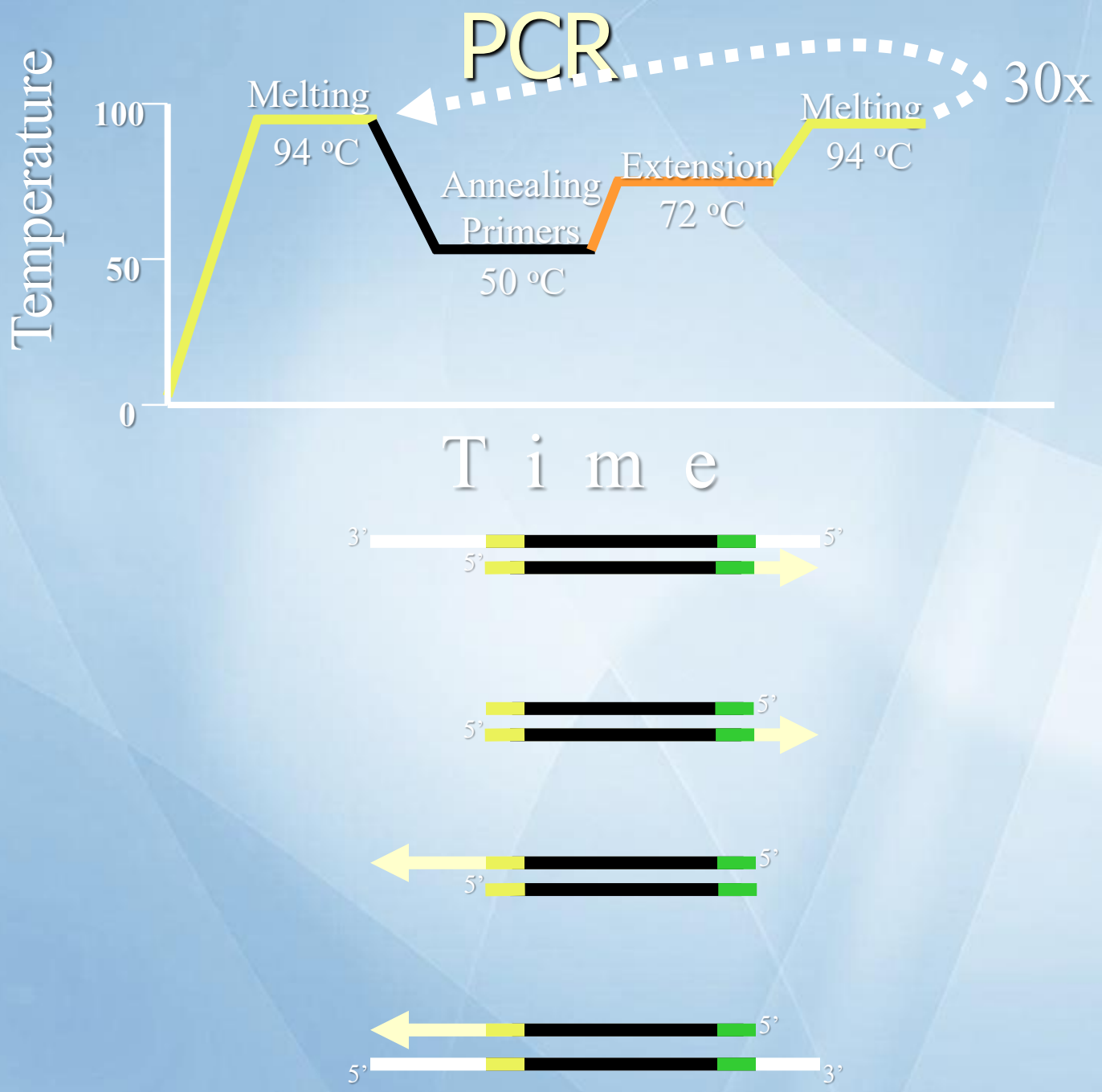
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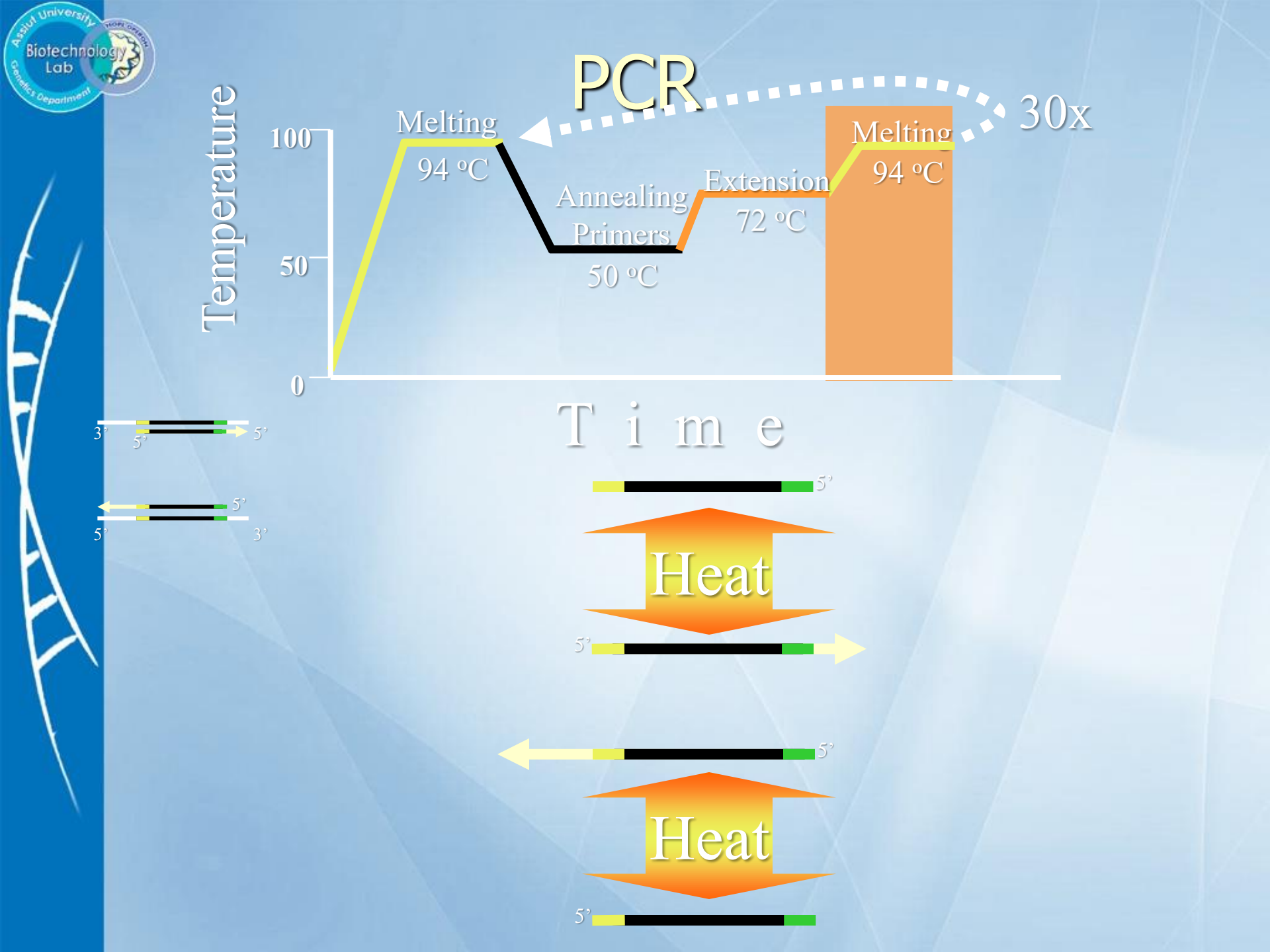
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Heat
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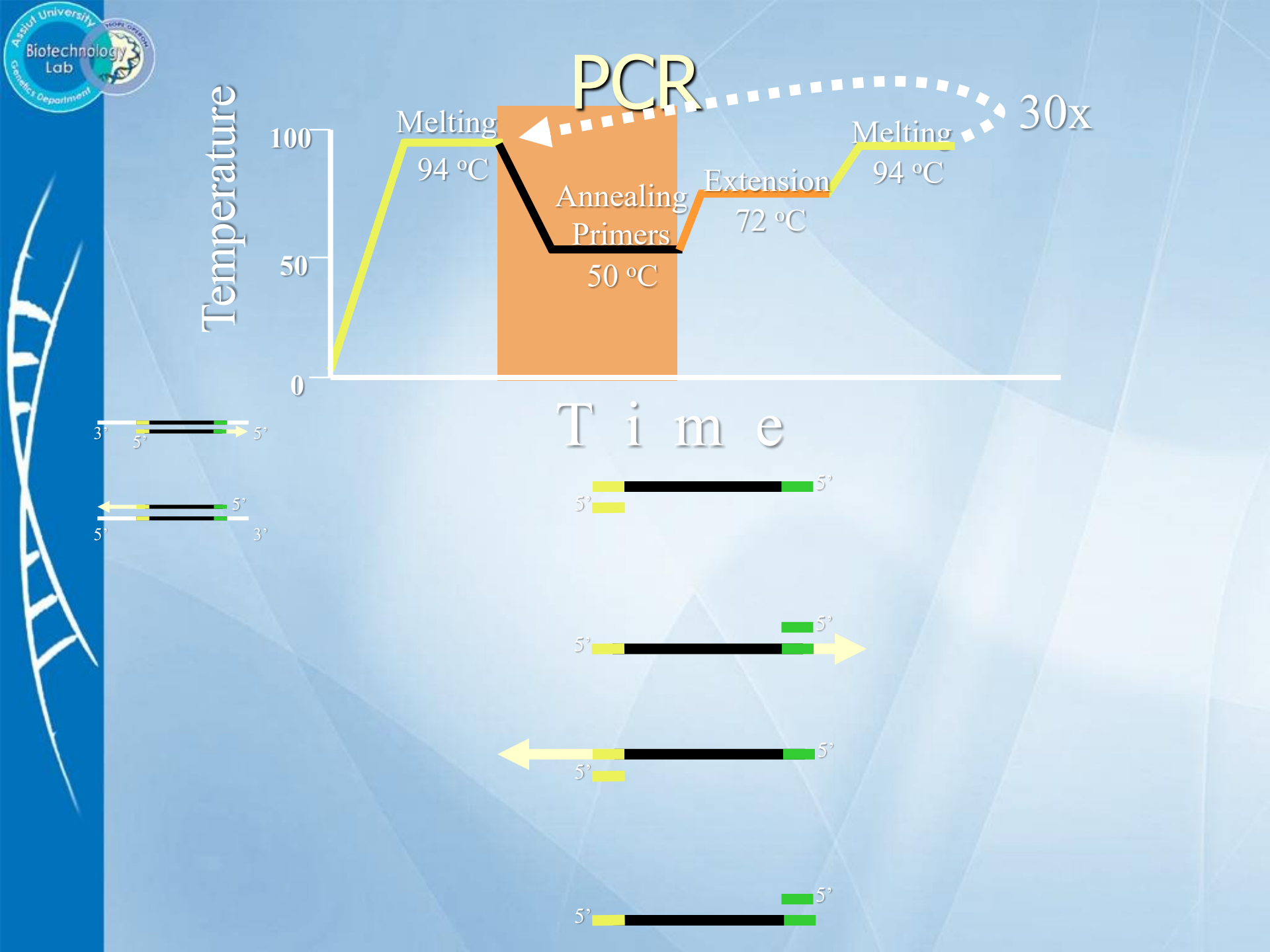
PCR
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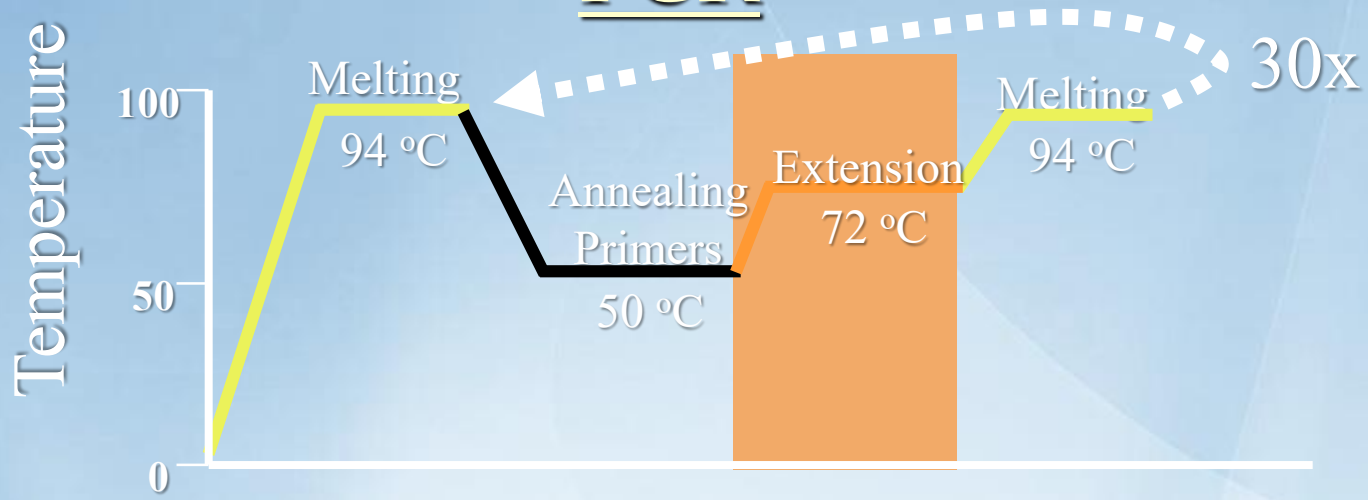
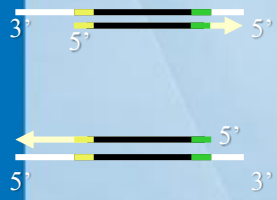
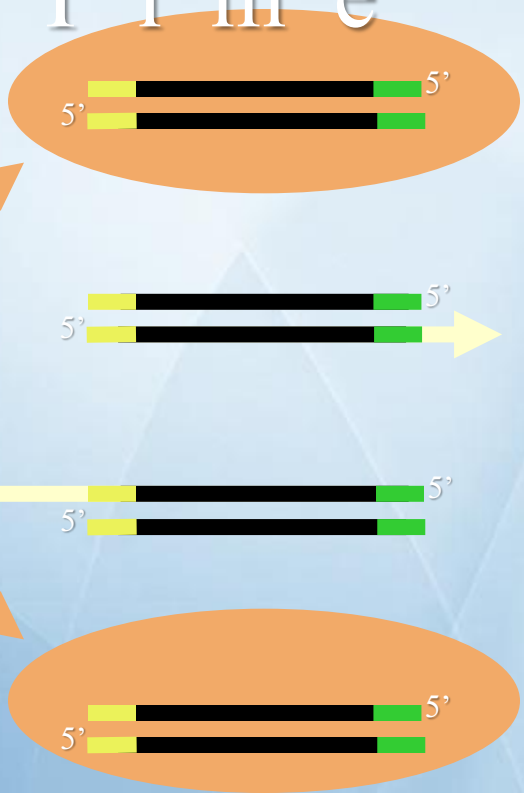
PCR





Fragments of defined length



Movie

What do we need for PCR?

PCR tube

THERMOCYCLER

متطلبات تقنيه تفاعل البلمره المتسلسل (PCR)

DNA Sample

Primers

نوعان :

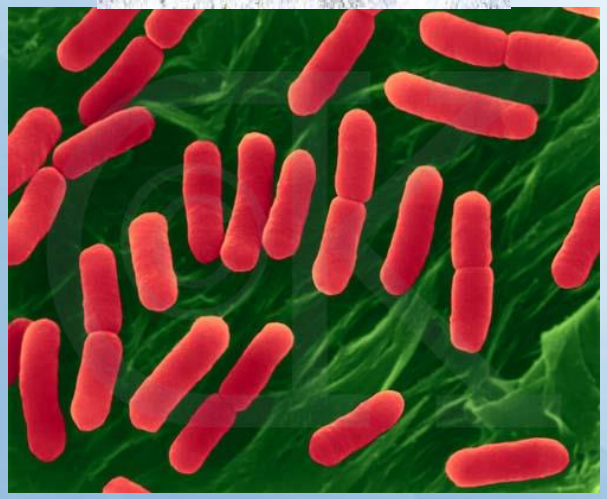
- أمامي (Forward).
- خلفي (Reverse).

وهي تتابع من القواعد النيتروجينيه في شريط واحد قصير(20-25 b) مكمل لبداية الجزء المراد تضخيمه في الـ DNA.

CCGAATGGGATGC

GGCTTACCCTACG

Taq polymerase



- مستخرج من سلالة بكتيريه تسمى *Thermus aquaticus* التي تعيش في المياه الحارة.
- لا يتأثر بدرجات الحرارة المرتفعه.
- درجه الحراة المثلى له ٧٢ ºم.

First reports using DNA polymerase from *Thermus aquaticus* was at (1988)

dNTPs

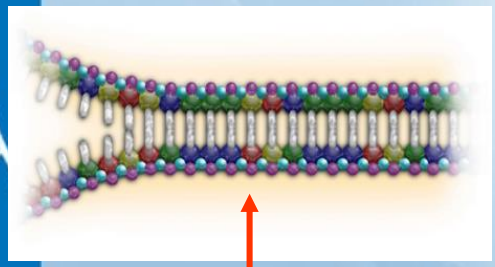
- **Adenine** أدنين
- **Thymine** ثايمين
- **Guanine** جوانين
- **Cytosine** سايتوسين

PCR Buffer 10x

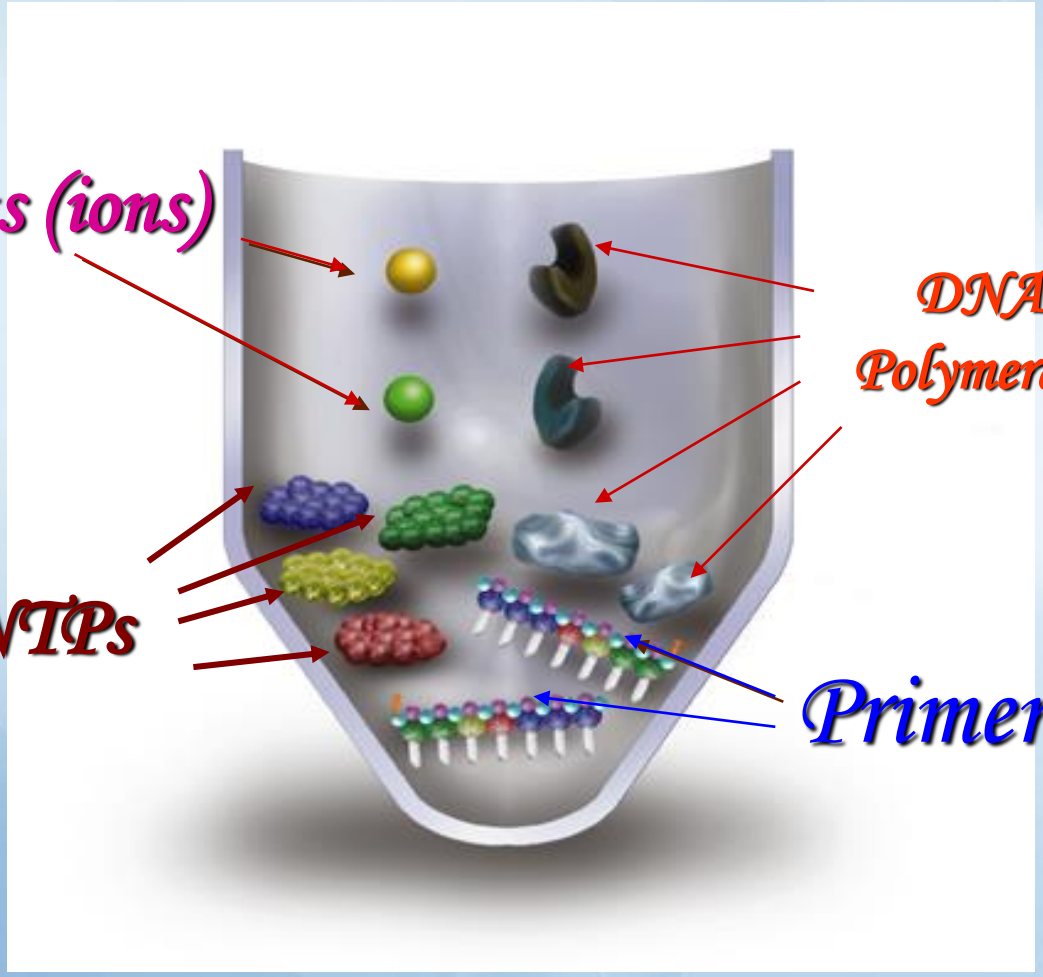
Distilled Water

PCR Procedure

All the required components are inserted into an Eppendorf tube

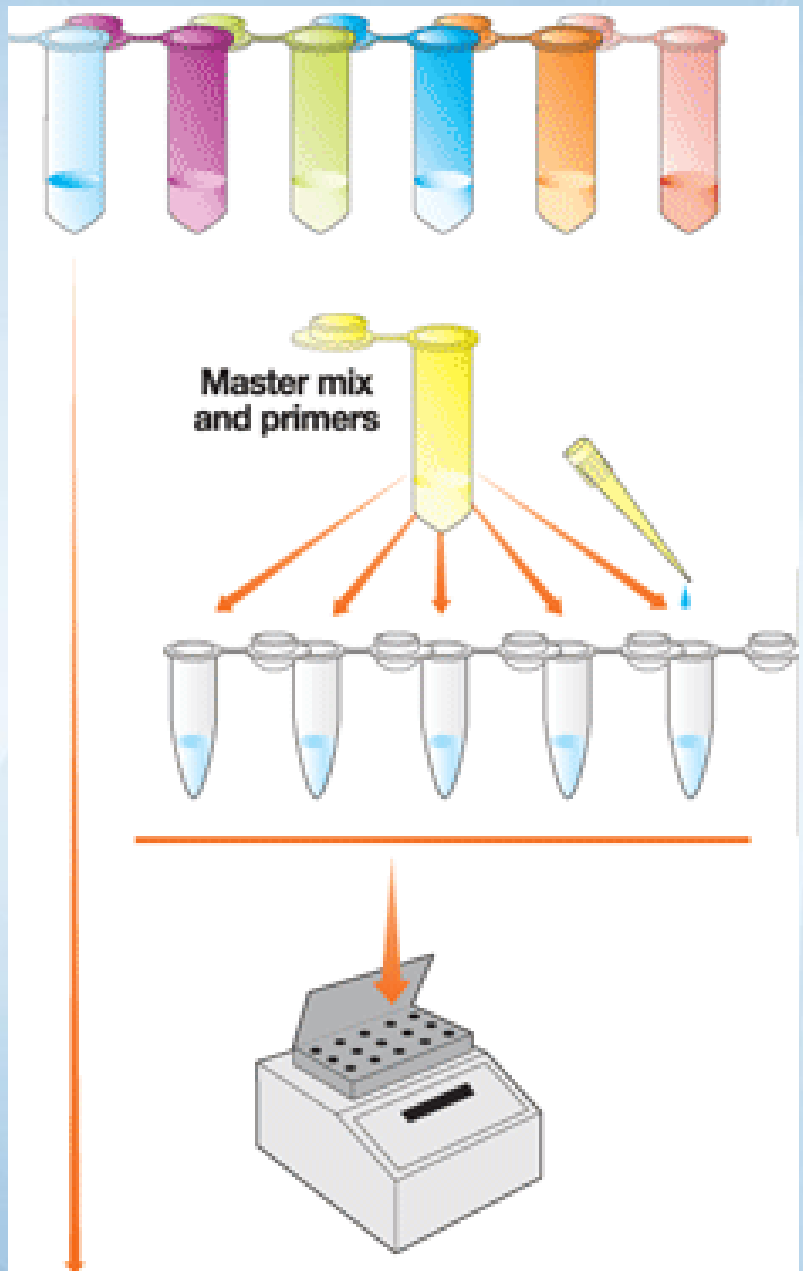


Template DNA



PROCEDURE .....

- DNA (Template).
- Forward primer
- Reverse primer
- dNTP's
- *Taq* DNA Polymerase
- Buffer
- H_2O

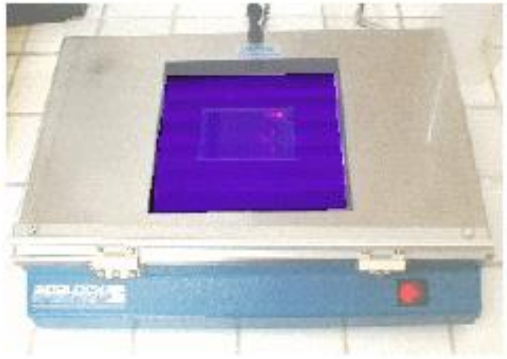


PROCEDURE .....

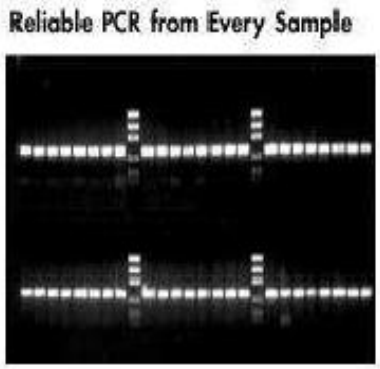
PCR

Agarose gel electrophoresis

3-4 hours

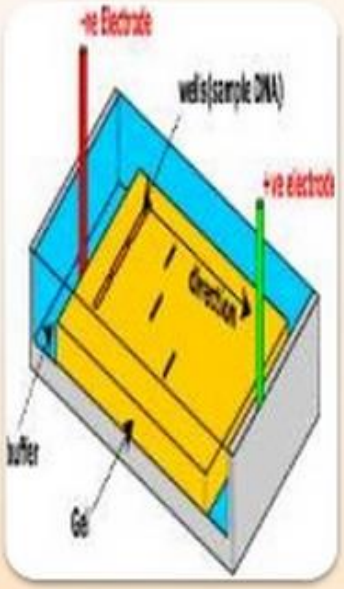


UV visualisation



The final product

Meaning of electrophoresis

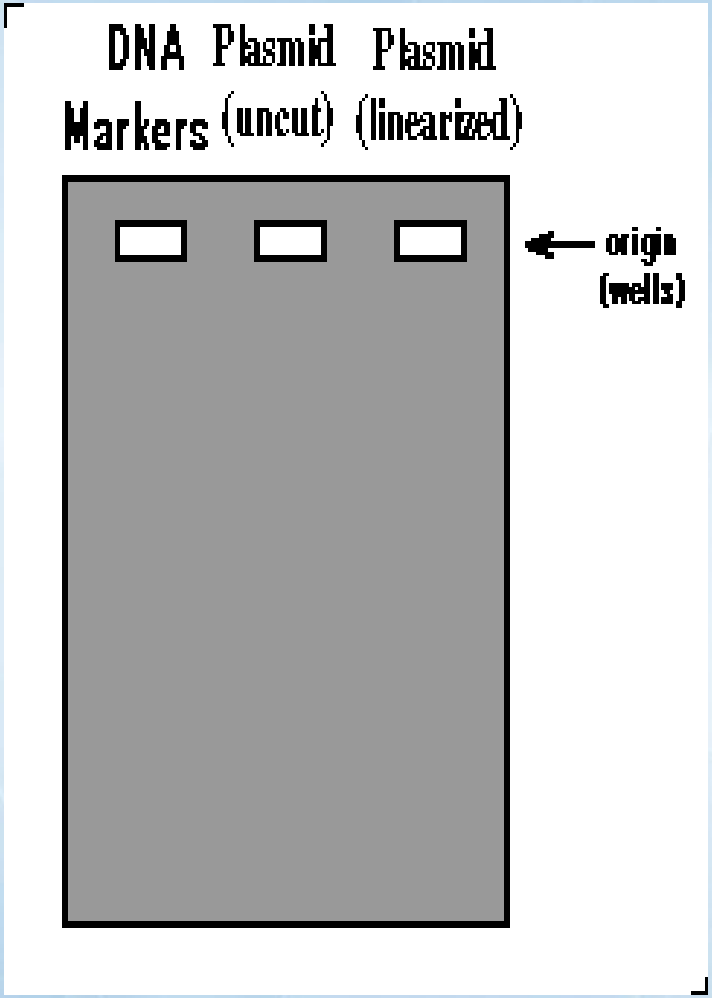


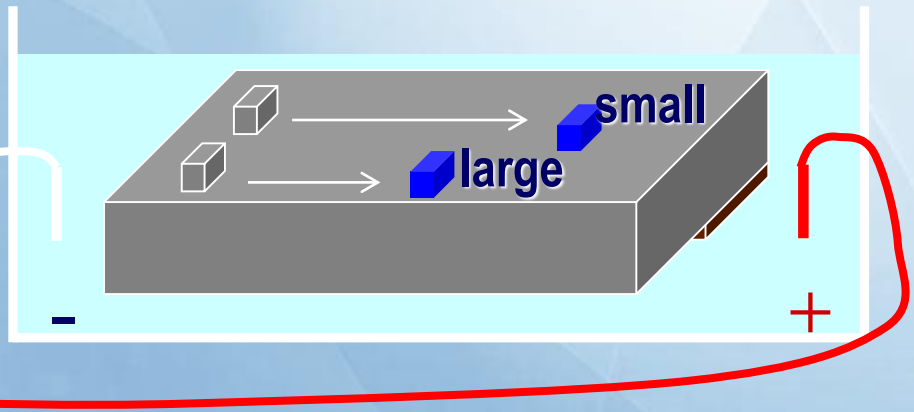
electrophoresis

- The term 'electrophoresis' was coined from the Greek word *'phoresis'*, which means 'being carried'.
- Electrophorosis literally means 'to carry with electricity'.

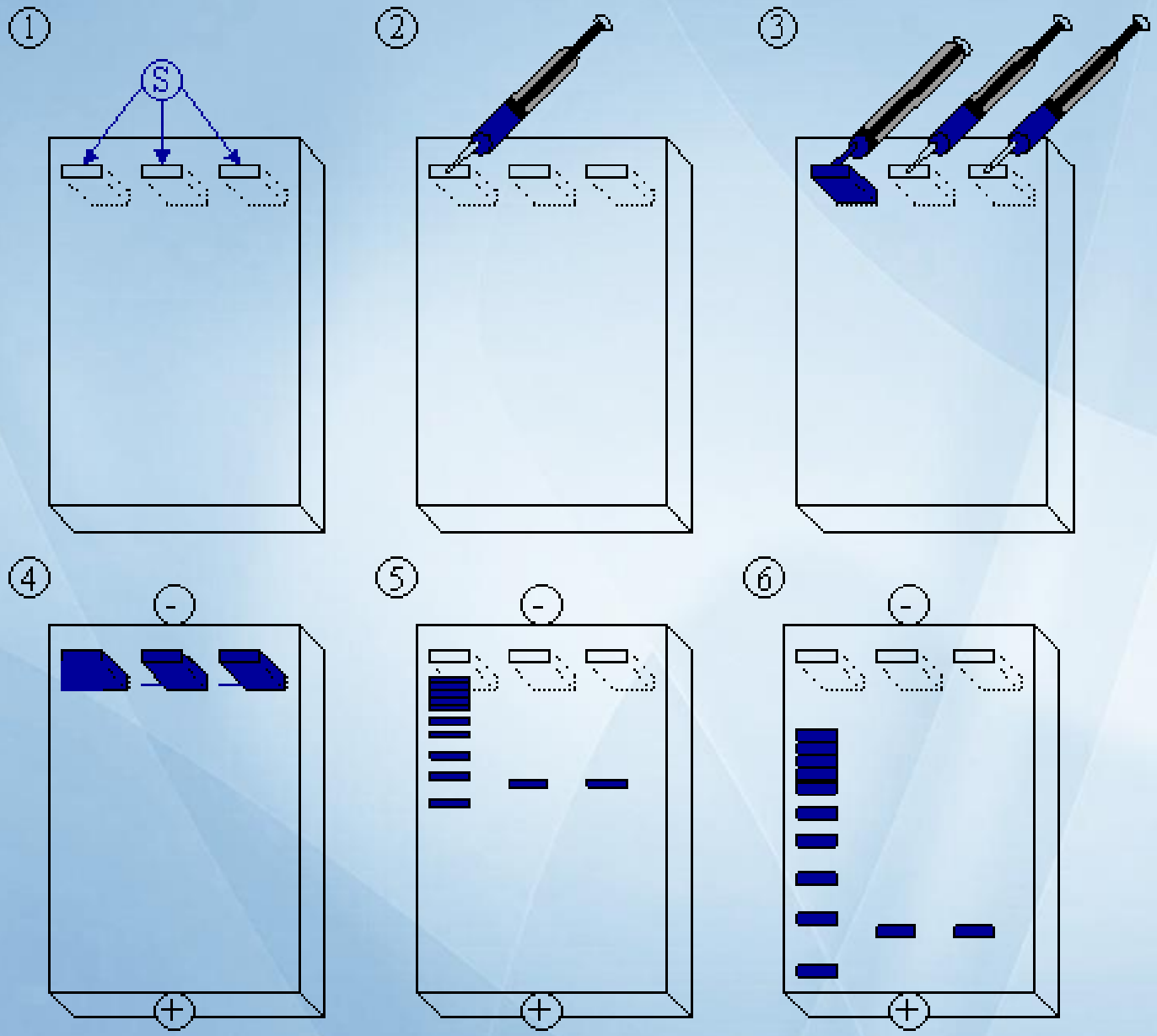
Agarose Gel Electrophoresis

- Agarose electrophoresis is used to Separate DNA by Molecular size (bp.)
- Smaller molecules move faster than large molecules





Power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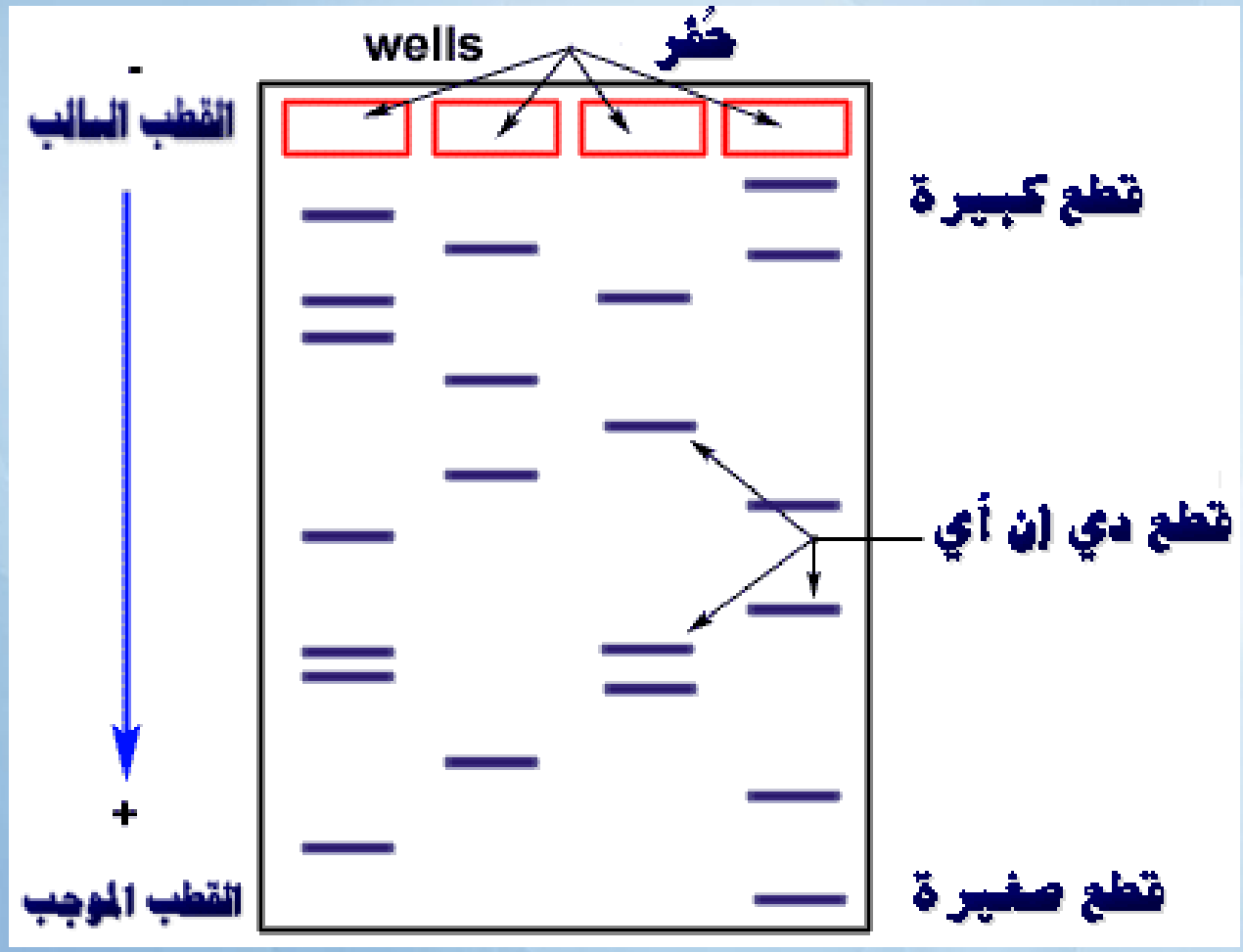
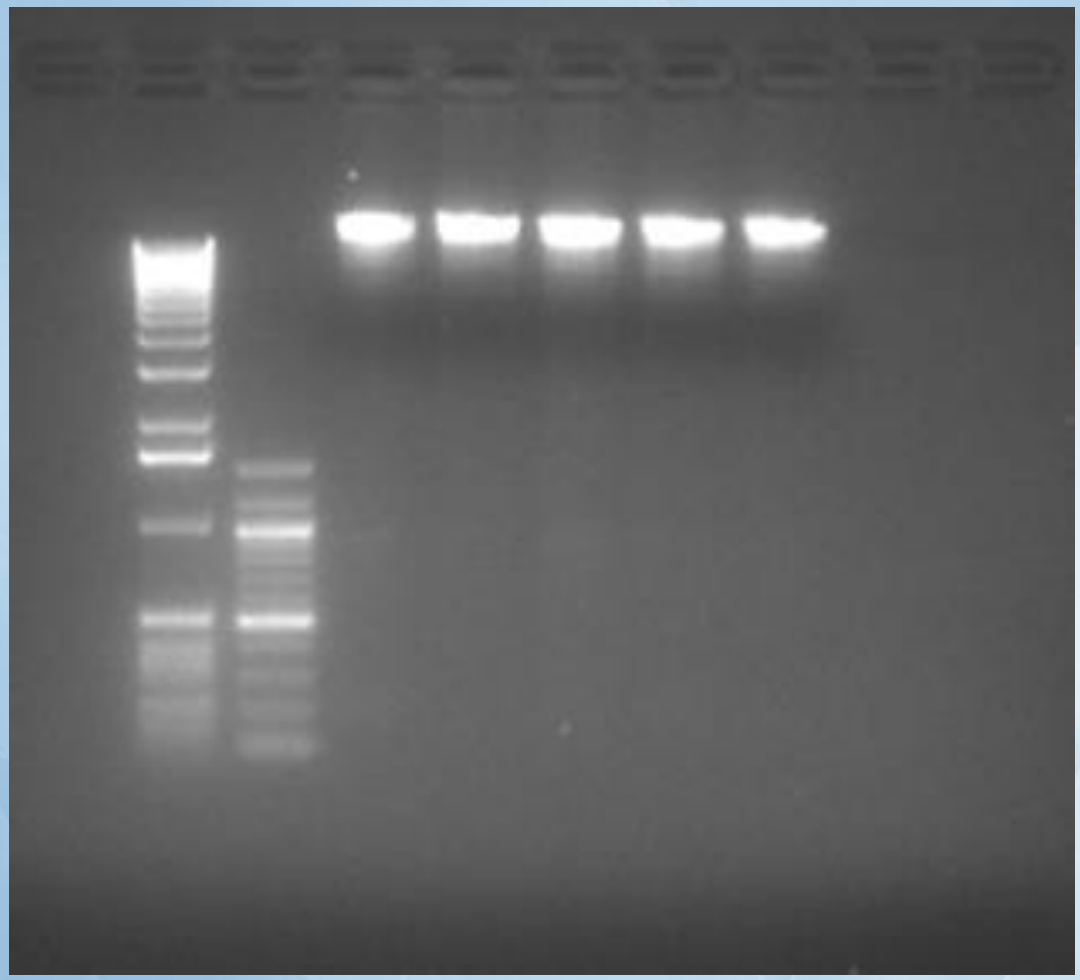
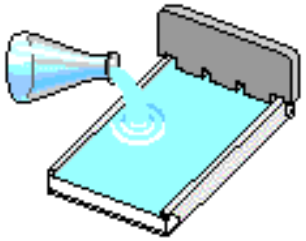
2- تقنية التفريد الكهربي في الجيل
Gel electrophoresis

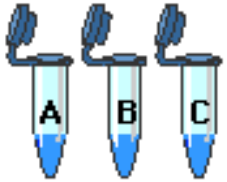




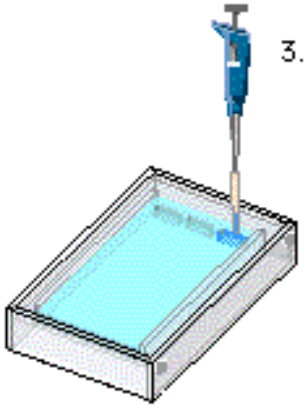
1. Make gel.



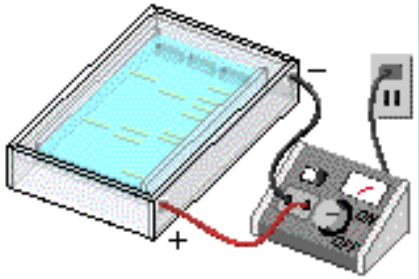
2. Obtain prepared DNA samples.



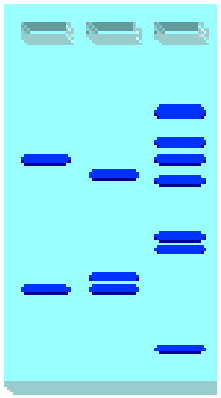
3. Load samples into gel.



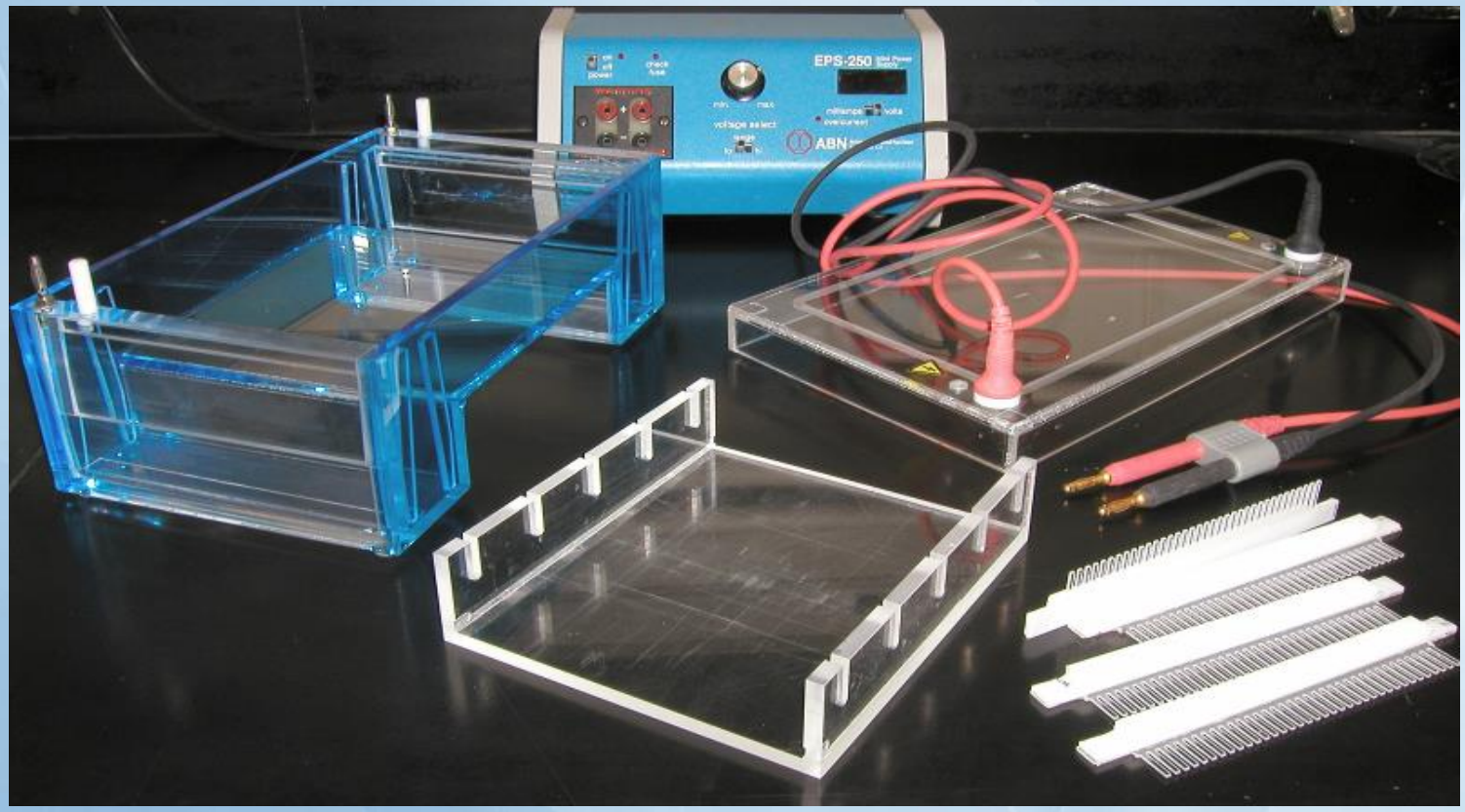
4. Separate fragments by electrophoresis.

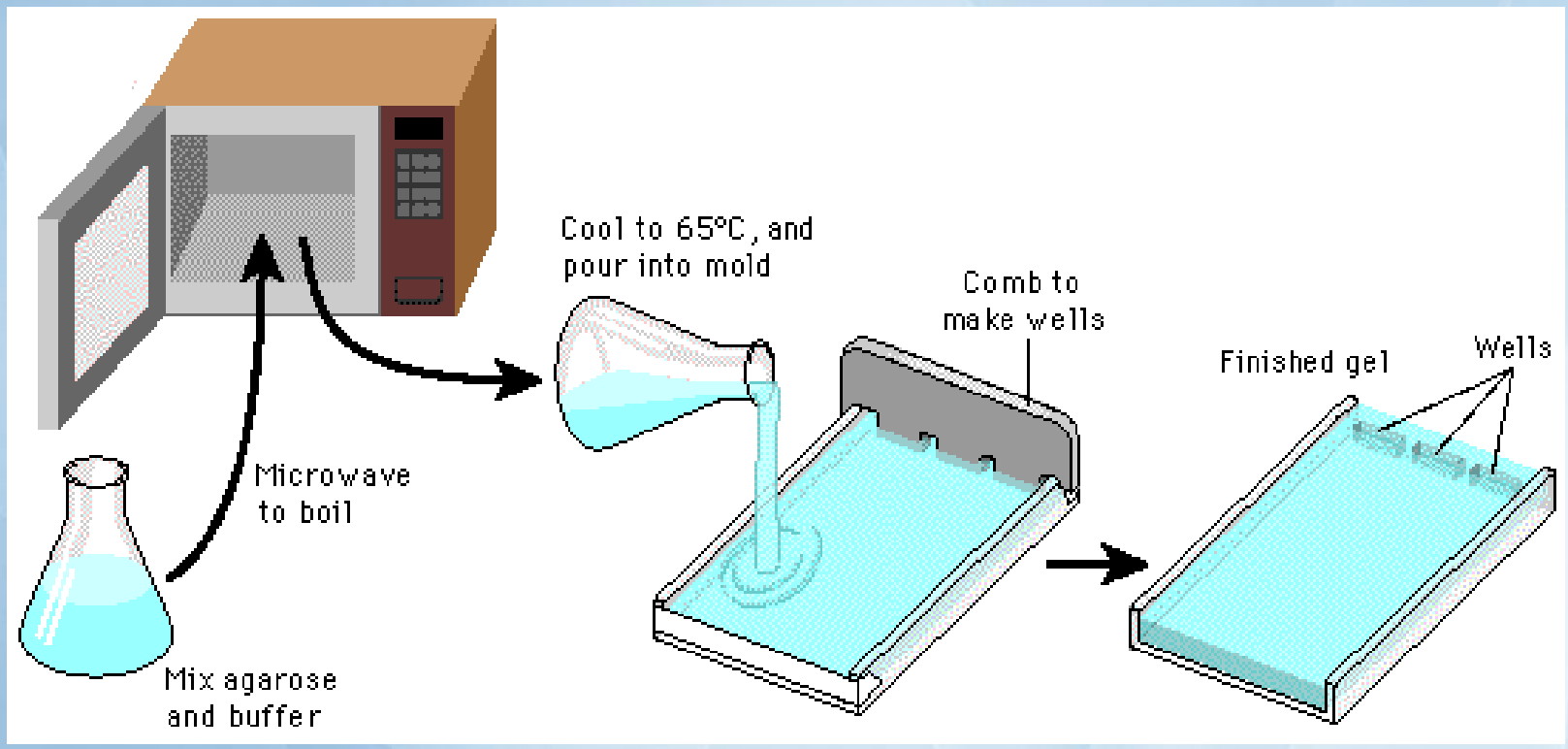


5. Stain DNA fragments and measure distances.

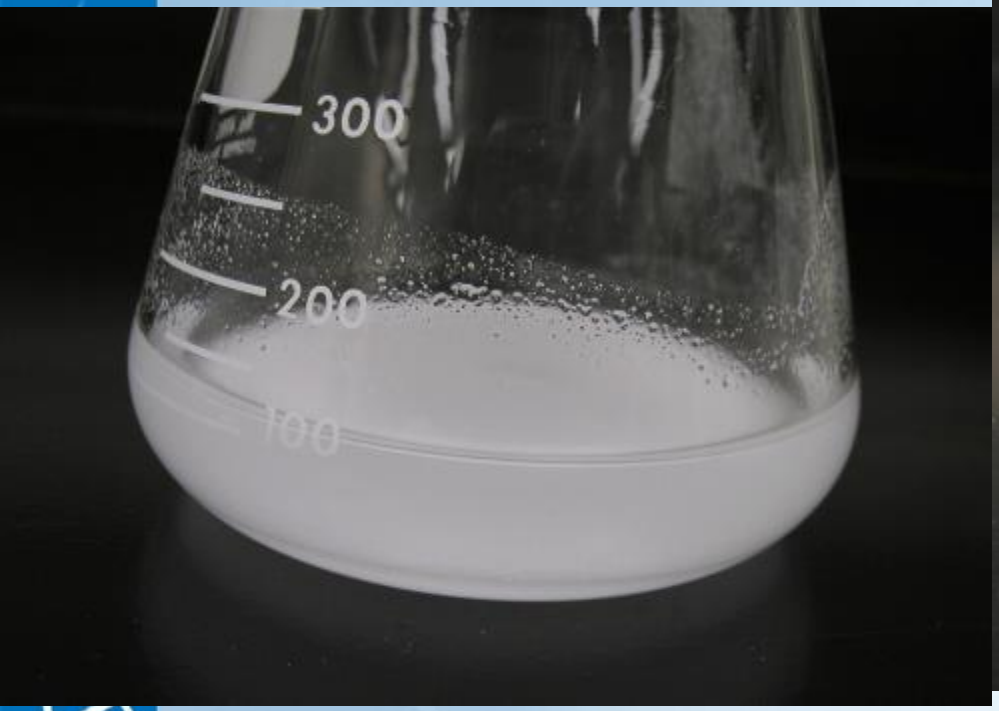


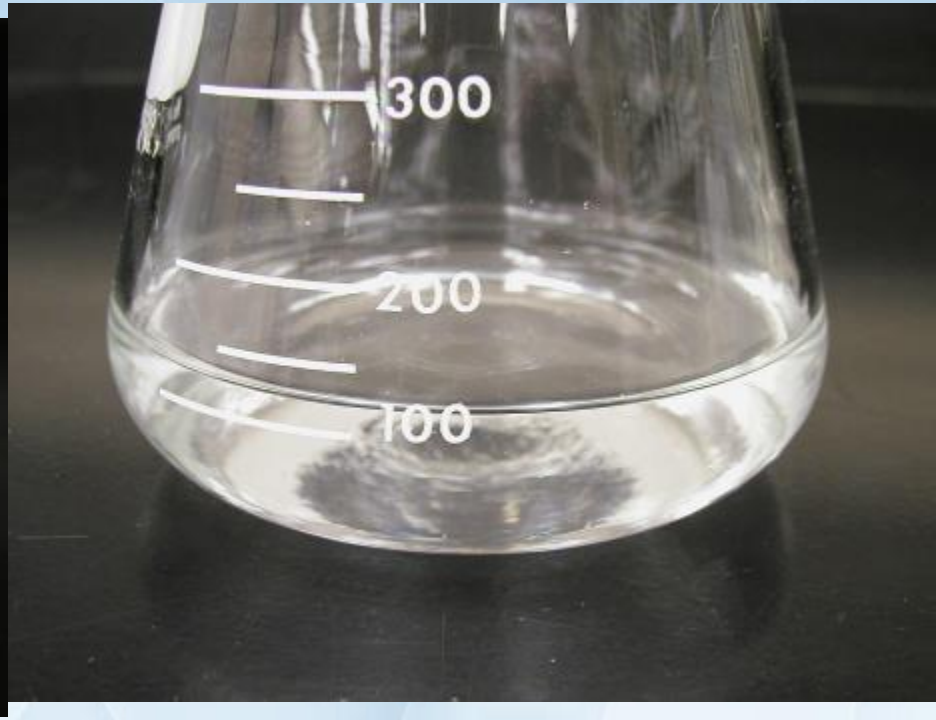
Agarose Gel Preparation




Cool to 65°C, and
pour into mold
Comb to
make wells
Finished gel
Wells
Microwave
to boil
Mix agarose
and buffer

Melting the Agarose





Differences between agarose and polyacrylamide gels

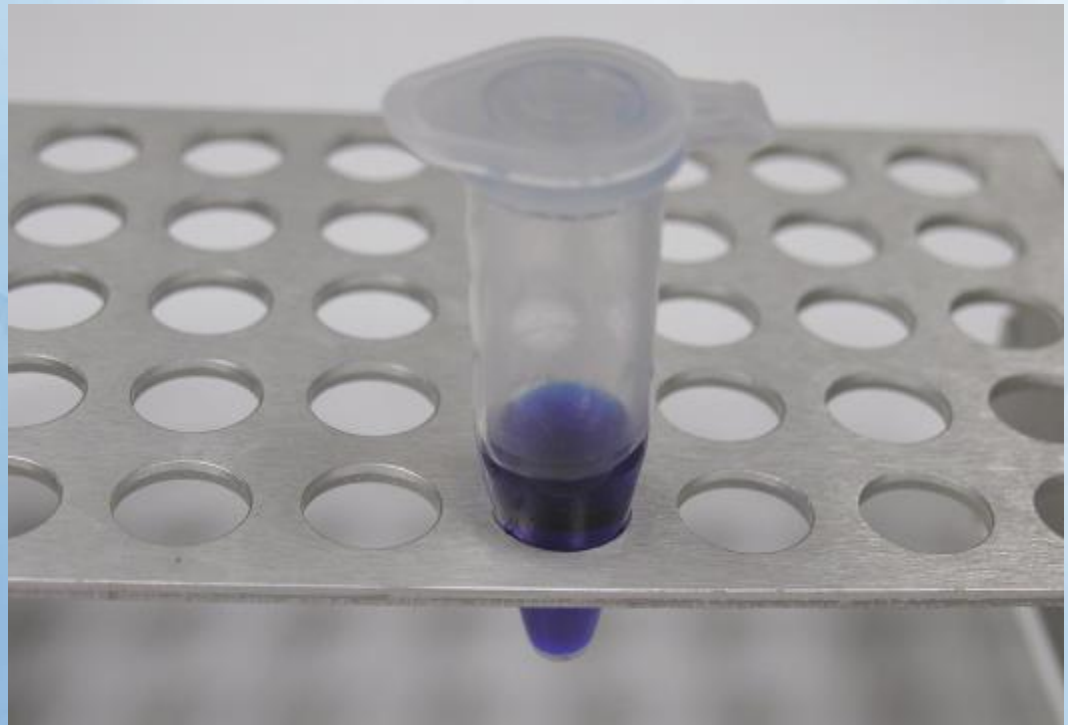
Agarose gel	Polyacrylamide gel
A polysaccharide extracted from sea weed.	A cross-linked polymer of acrylamide.
Gel casted horizontally	Gel casted vertically
Non-toxic	Potent neuro-toxic
Separate large molecules	Separate small molecules
Commonly used for DNA separations	Used for DNA or protein separations.
Staining can be done before or pouring the gel	Staining can be done after pouring the gel.

Sample Preparation for Loading

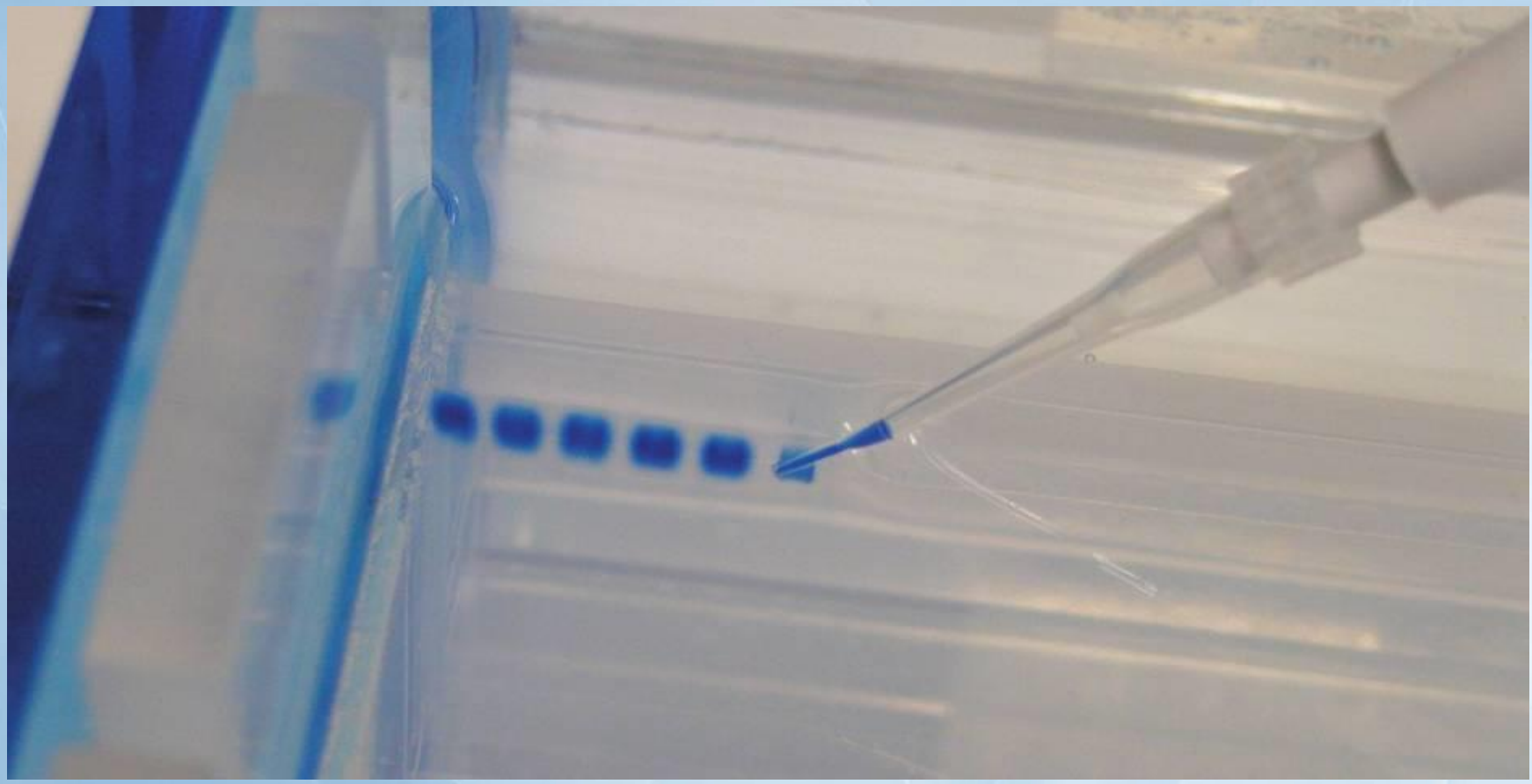
Mix the samples of DNA with the 6X sample loading buffer (w/ tracking dye). This allows the samples to be seen when loading onto the gel, and increases the density of the samples, causing them to sink into the gel wells.

6X Loading Buffer: →

- Bromophenol Blue (for color and marker)
- Glycerol (for weight)

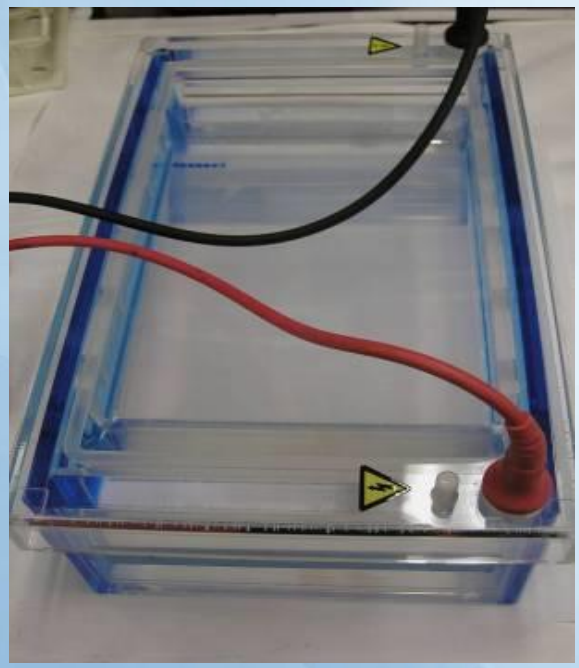


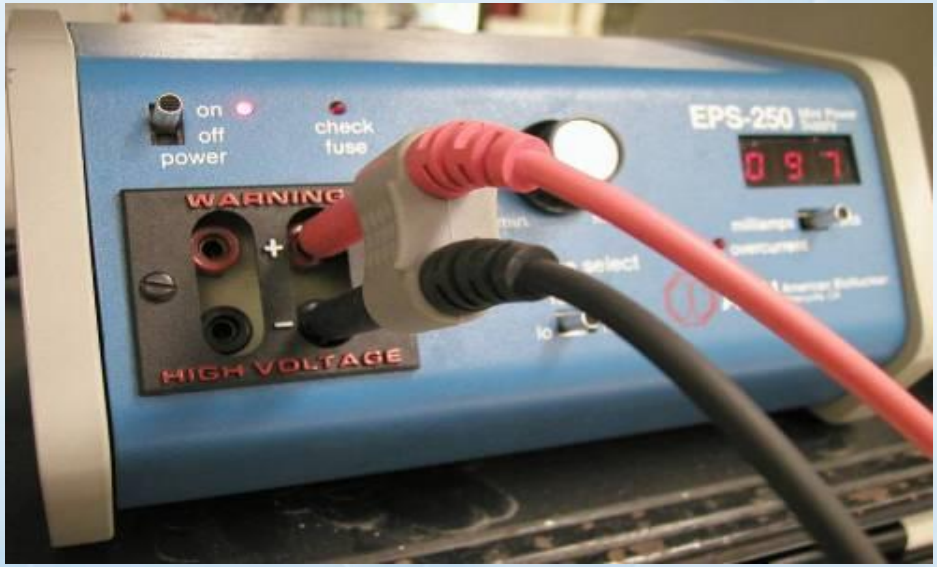
Loading the Gel

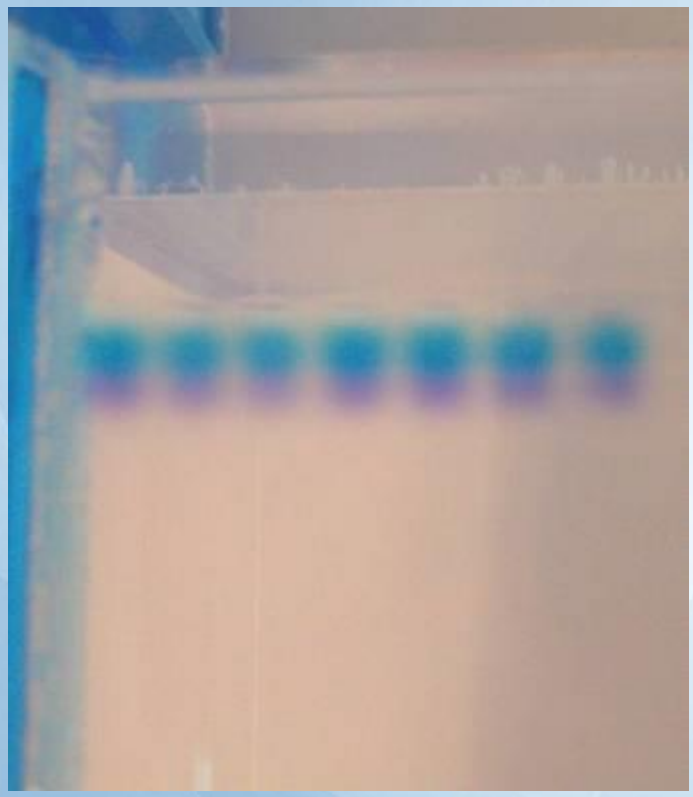


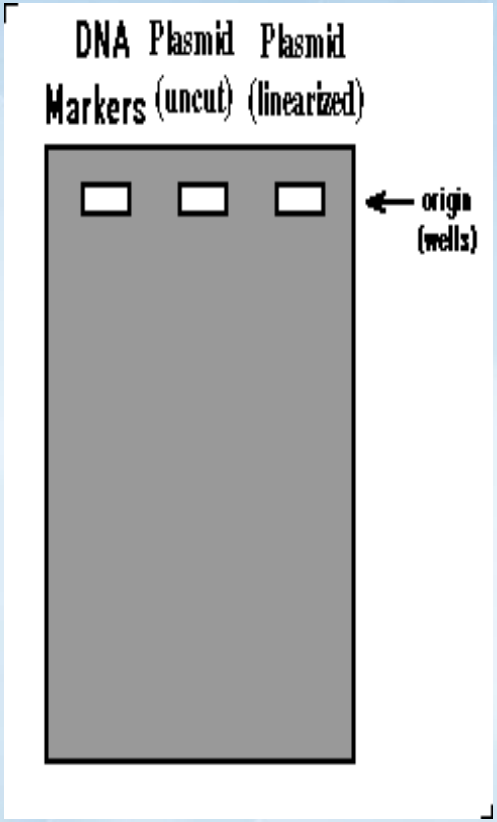
Running the Gel

Voltage (75, 100, 150 ……)



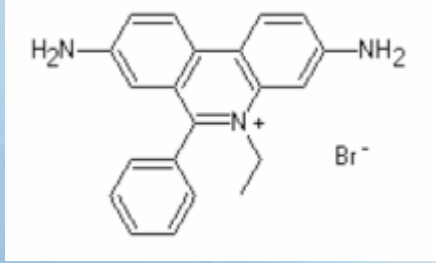


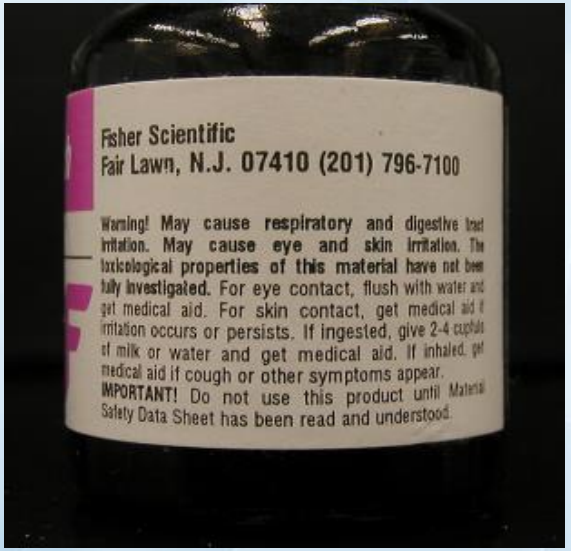



DNA Markers
Plasmid (uncut)
Plasmid (linearized)
origin (wells)

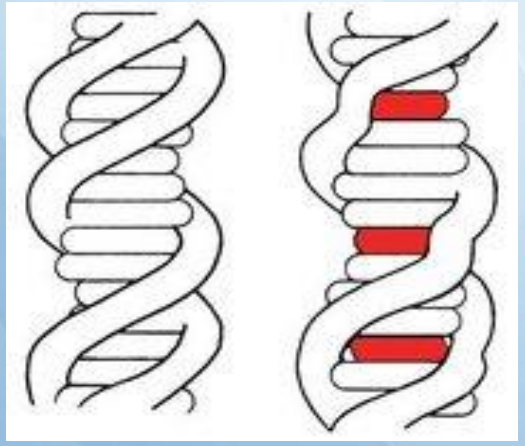
Gel Staining

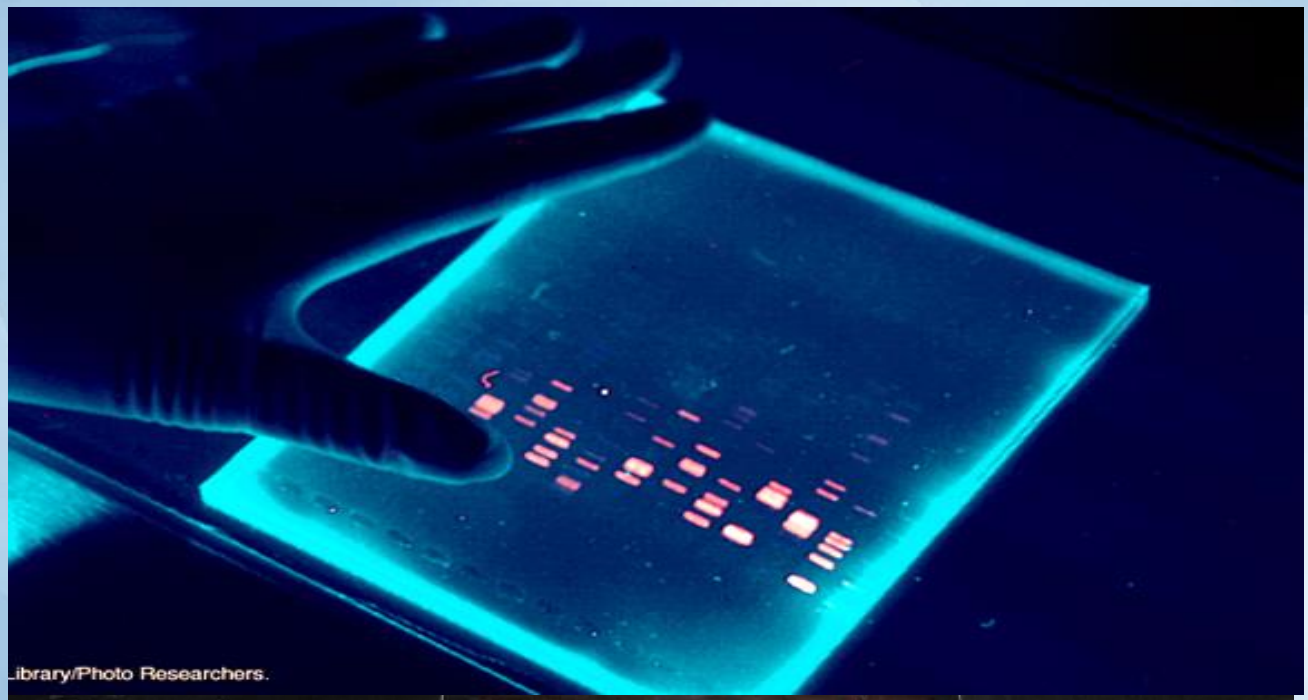
- Ethidium bromide binds to DNA and fluoresces under UV light, allowing the visualization of DNA on a Gel.
- Ethidium bromide can be added to the gel and/or running buffer before the gel is run or the gel can be stained after it has run.



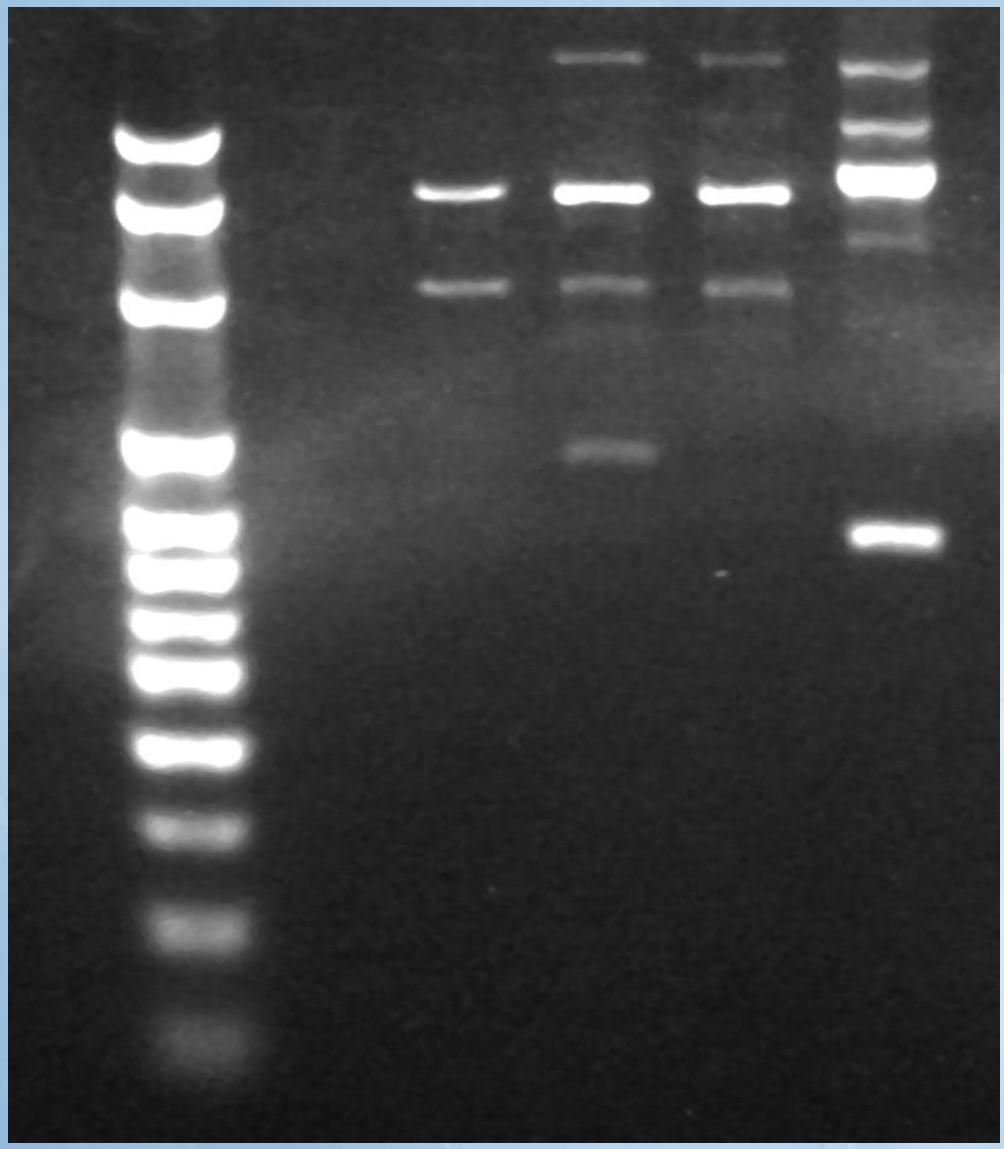


***CAUTION! Ethidium bromide is a powerful mutagen and is moderately toxic. Gloves should be worn at all times.




ibrary/Photo Researchers.

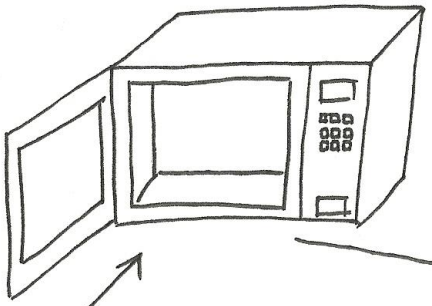

High Performance
Ultraviolet Transilluminator
UVP
HI
LOW
ON
OFF



AGAROSE GEL ELECTROPHORESIS

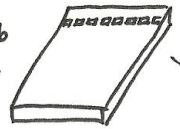
Weigh out the correct amount of agarose

Mix agarose powder with buffer

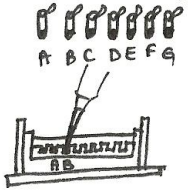


Boil the mixture in a microwave oven

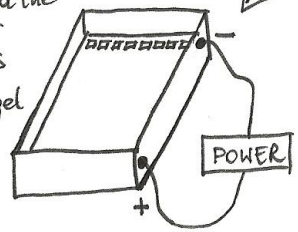
After cooling the solution pour it into a casting tray



Once agarose solidifies remove a comb from the gel

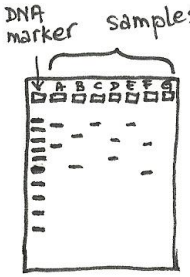


Place the gel in an electrophoresis apparatus filled with buffer and load your samples into wells of gel



Turn on the power supply. The DNA has a net negative charge and moves toward the "+" (positive) pole

Once the DNA is separated (the smallest fragments are farthest from the well and the heavier molecular weight fragments are closer to the well), stain the gel and analyse it



Determine the size of DNA bands of your samples by comparing with the size of the DNA bands in the DNA marker