SAWA UNIVERSITY

VIROLOGY

PCR
The Polymerase Chain Reaction

Sawa University — جامعـــــة ســـــــاوة الاهلية

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. Three :Stage — المرحلـــــــــــة الثالثة

الجانب العملي — محاضرة رقم 10...

Practical — Lecture No.10..

تدريسي المادة : م. د. سيف علي

Polymerase Chain Reaction

Is a technique used in molecular biology to create several copies of a certain DNA segment. This technique was developed in 1983 by Kary Mullis, an American biochemist. PCR has made it possible to generate millions of copies of a small segment of DNA.

Nobel Prize in Chemistry

Principle of PCR

The PCR technique is based on the enzymatic replication of DNA. In PCR, a short segment of DNA is amplified using primer mediated enzymes. DNA Polymerase synthesises new strands of DNA complementary to the template DNA. The DNA polymerase can add a nucleotide to the pre-existing 3'-OH group only. Therefore, a primer is required. Thus, more nucleotides are added to the 3' prime end of the DNA polymerase

Basic Components of PCR

1- Reagents.

2-Instruments:

A)Thermocycler.

B)Electrophoresis Apparartus

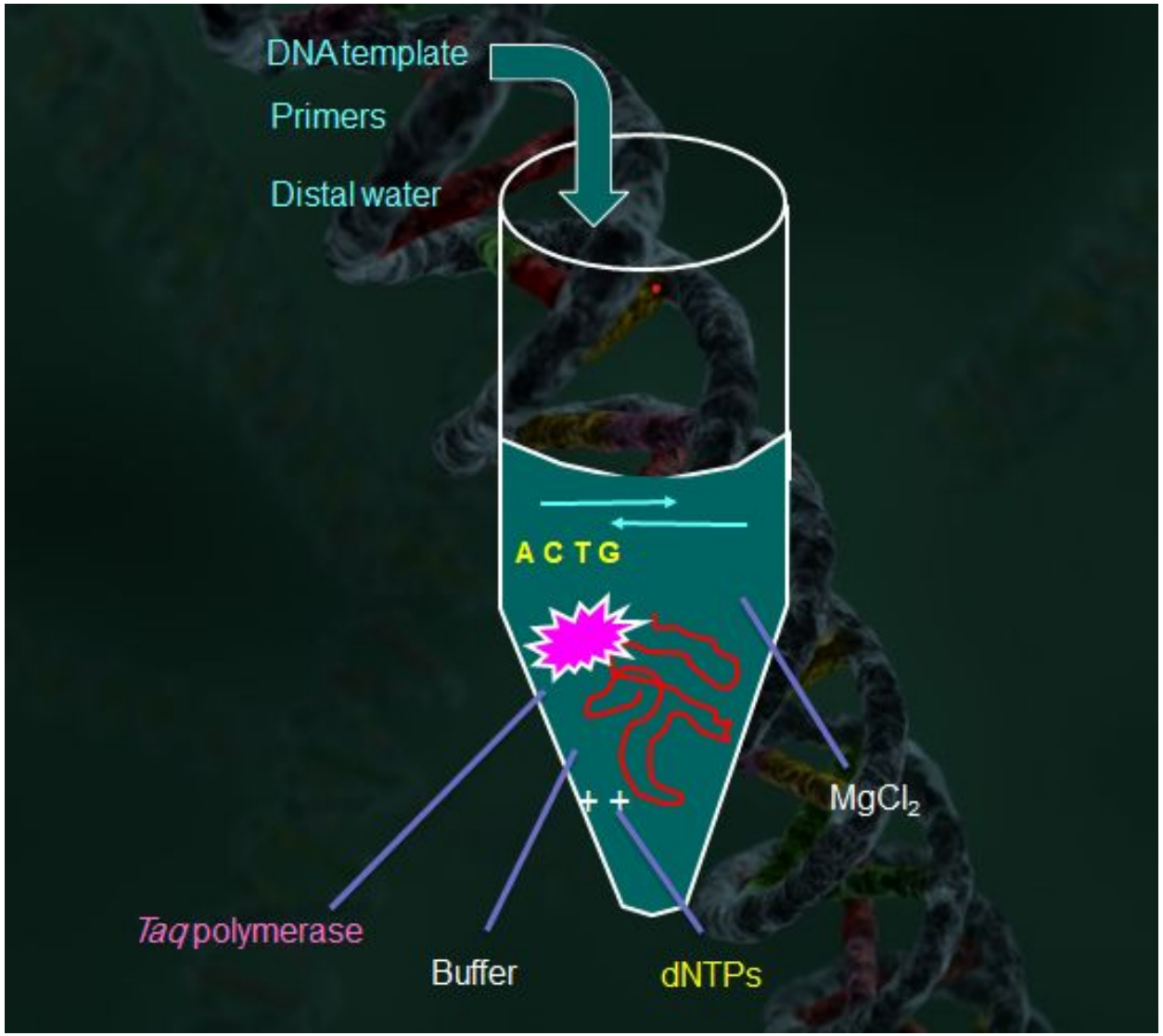
Components Of PCR

Components Of PCR constitutes the following:

1. **DNA Template**– The DNA of interest from the sample.
2. **DNA Polymerase**– Taq Polymerase is used. It is thermostable and does not denature at very high temperatures.
3. **Oligonucleotide Primers-** These are the short stretches of single-stranded DNA complementary to the 3’ ends of sense and anti-sense strands.

4-Deoxyribonucleotide triphosphate(dNTPs)– These provide energy for polymerization and are the building blocks for the synthesis of DNA. These are single units of bases.

5- Buffer System– Magnesium and Potassium provide optimum conditions for DNA denaturation and renaturation. It is also important for fidelity, polymerase activity, and stability


DNA template
Primers
Distal water
A C T G
+ +
$MgCl_2$
Taq polymerase
Buffer
dNTPs

Thermocyclers

Electrophoresis Apparartus

95º C
5 min

55º C
3 min

72º C
5 min

35 times

8 BORING hours per PCR!

PCR Steps

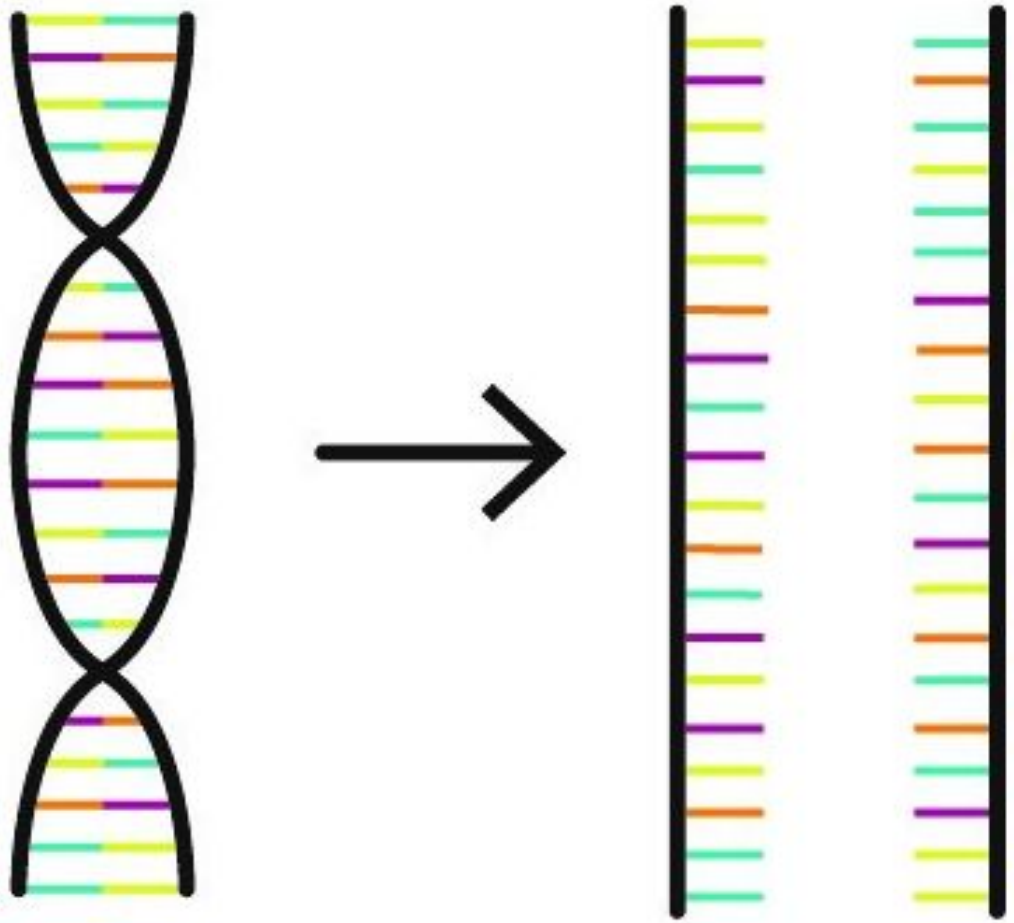
The PCR involves three major cyclic reactions:

Denaturation

Denaturation occurs when the reaction mixture is heated to 94℃ for about 0.5 to 2 minutes. This breaks the hydrogen bonds between the two strands of DNA and converts it into a single-stranded DNA.

The single strands now act as a template for the production of new strands of DNA. The temperature should be provided for a longer time to ensure the separation of the two strands.

Denaturation of DNA



Annealing:-

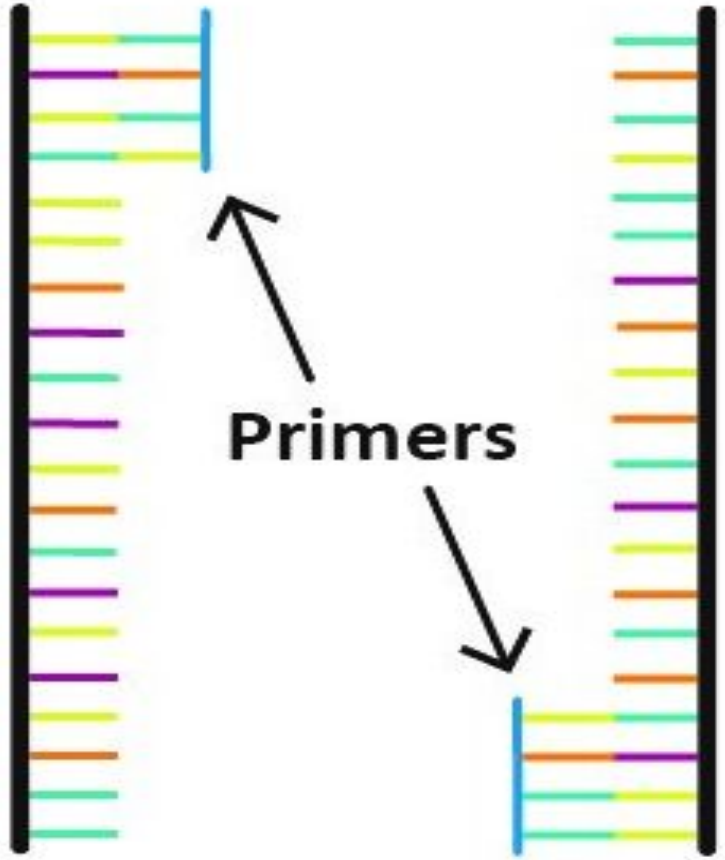
The reaction temperature is lowered to 54-60℃ for around 20-40 seconds. Here, the primers bind to their complementary sequences on the template DNA.

Primers are single-strand sequences of DNA or RNA around 20 to 30 bases in length.

They serve as the starting point for the synthesis of DNA.

The two separated strands run in the opposite direction and consequently there are two primers- a forward primer and a reverse primer.

Annealing of Primers



Elongation(Extension)

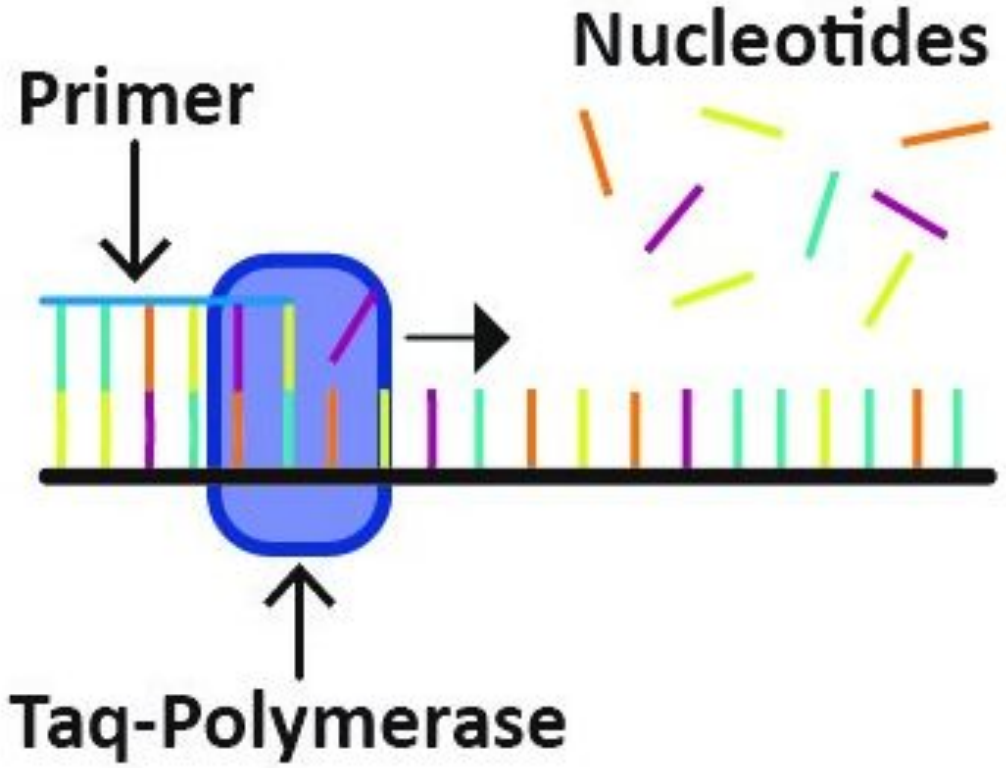
At this step, the temperature is raised to 72-80℃. The bases are added to the 3’ end of the primer by the Taq polymerase enzyme.

This elongates the DNA in the 5’ to 3’ direction. The DNA polymerase adds about 1000bp/minute under optimum conditions.

Taq Polymerase can tolerate very high temperatures. It attaches to the primer and adds DNA bases to the single strand. As a result, a double-stranded DNA molecule is obtained.

These three steps are repeated 20-40 times in order to obtain a number of sequences of DNA of interest in a very short time period.

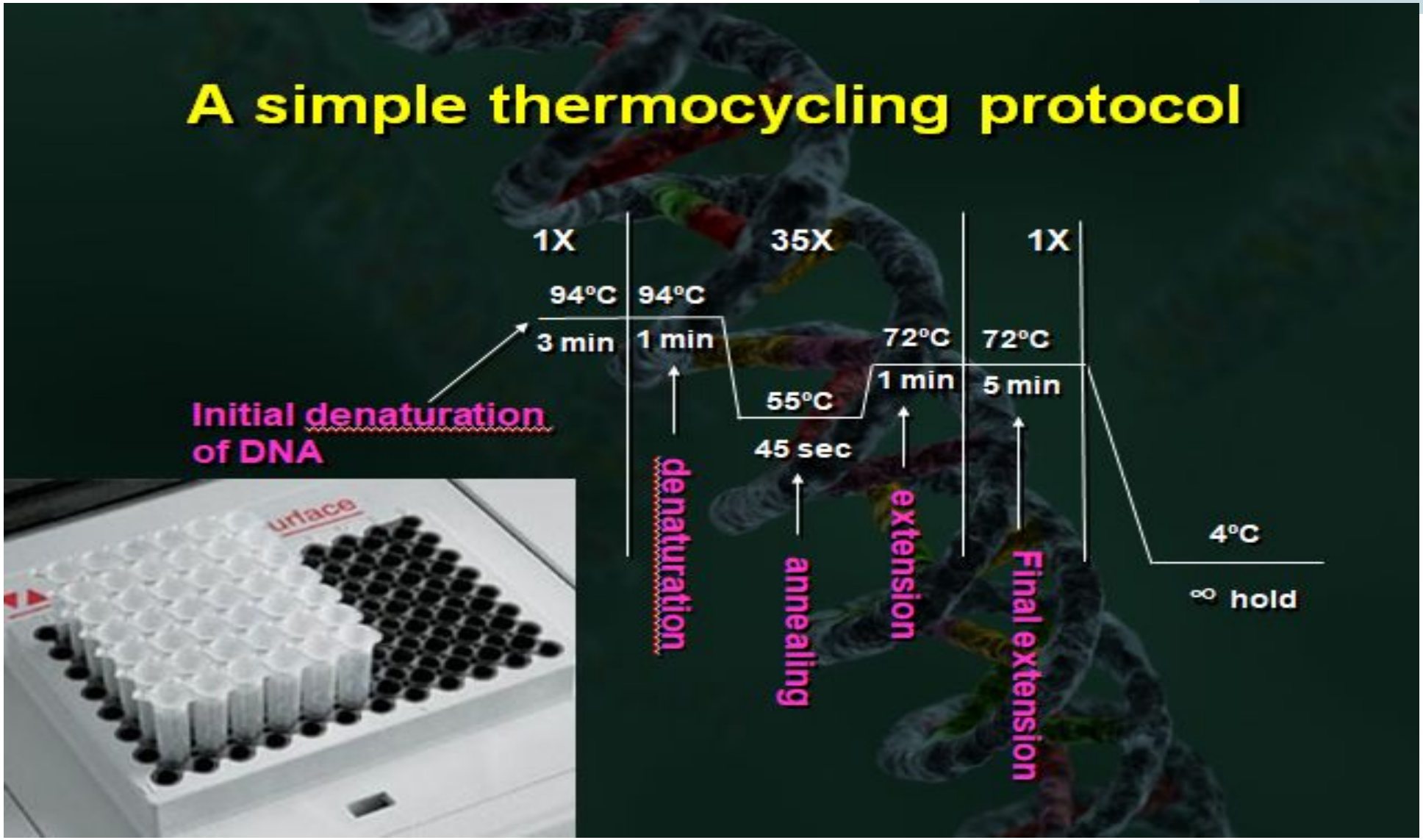
Extension

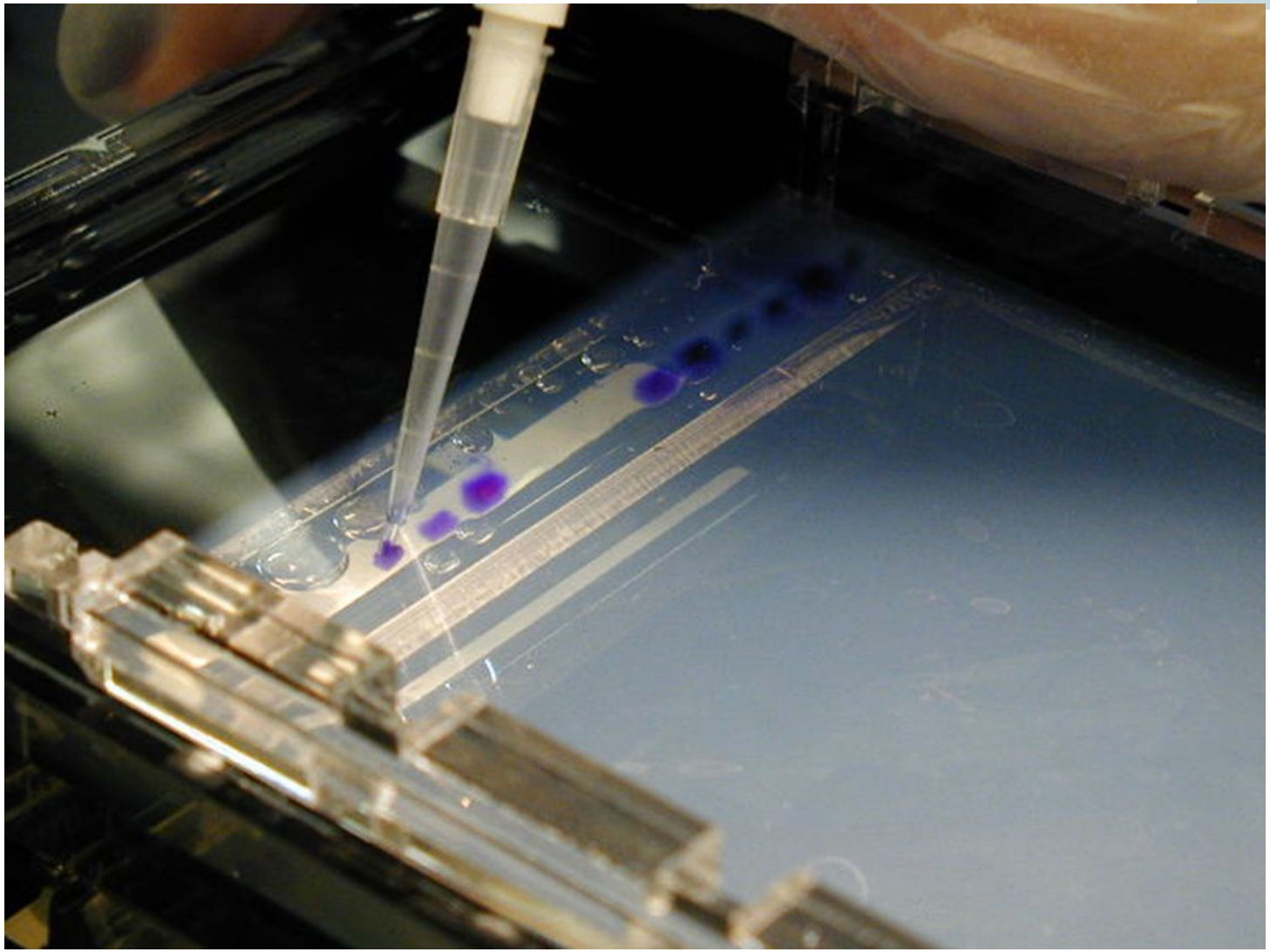


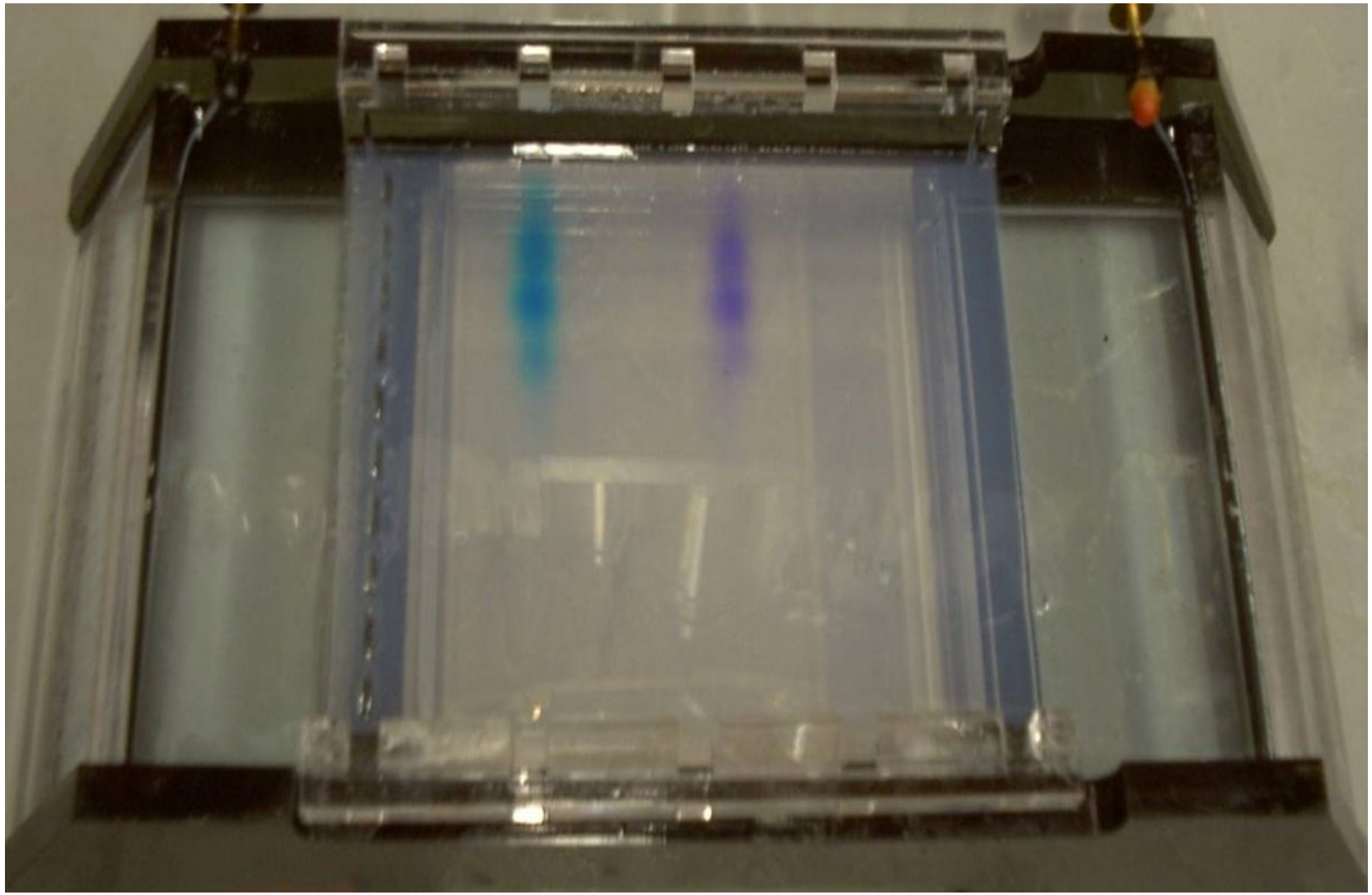
Gel electrophoresis

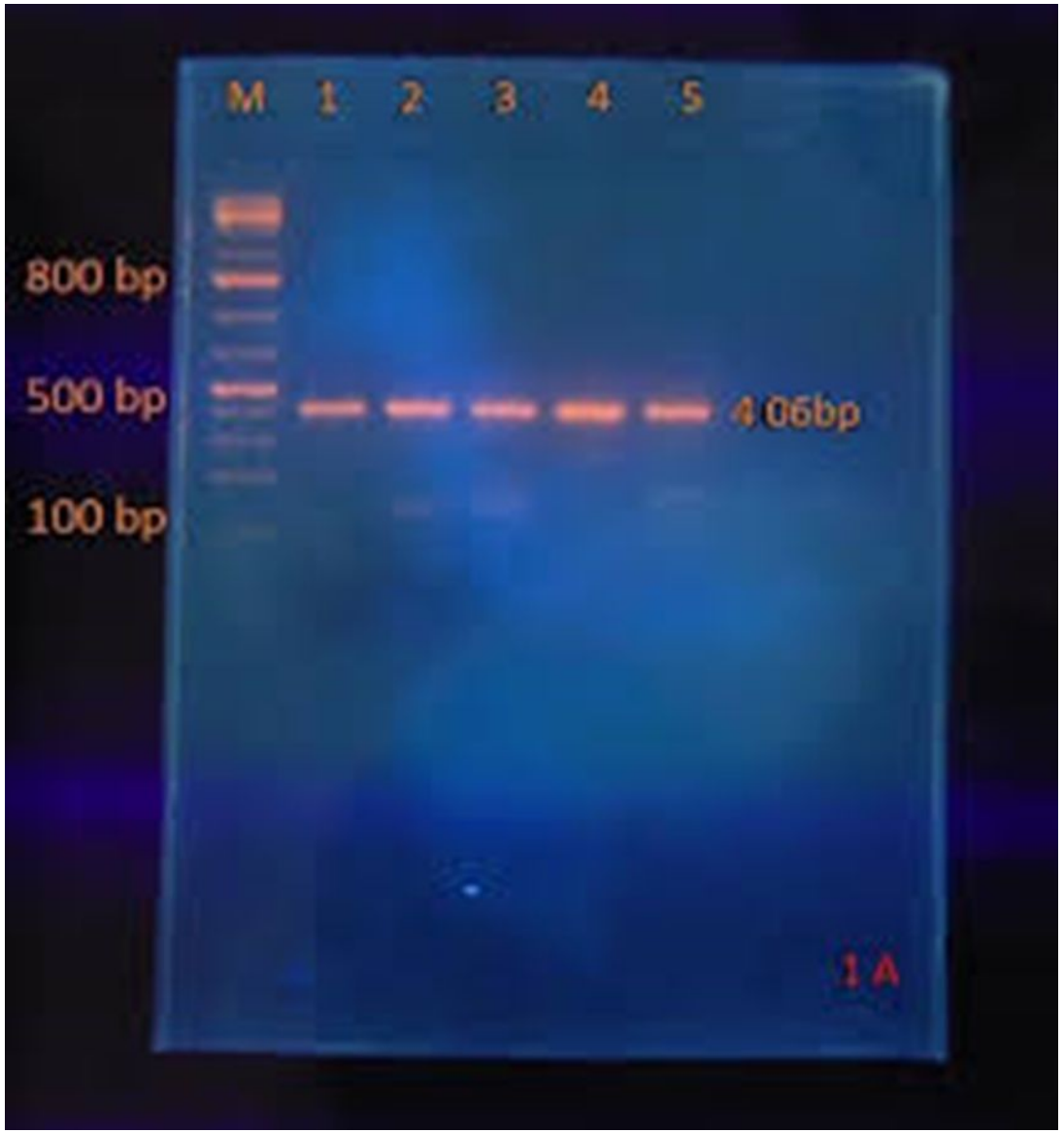
is a laboratory technique used to separate and analyze molecules like DNA, RNA, and proteins based on their size and charge. It works by applying an electric current to a porous gel matrix, causing molecules to migrate through the gel. Shorter molecules travel faster and farther, while longer ones move slower and cover less distance. This creates visible "bands" of separated molecules that can be visualized and analyzed.

A simple thermocycling protocol








M
1
2
3
4
5
800 bp
500 bp
100 bp
406bp
1A

Any questions?