FACULTY OF ENGINEERING &TECHNOLOGY
DEPARTMENT OF BIOTECHNOLOGY

PCR (POLYMERASE CHAIN REACTION)

- PCR was developed by Kary Mullis in 1985 and was awarded with the nobel prize in 1993.
- PCR machine also known as Thermocycler.
- PCR is a invitro technique to amplify a specific region of a DNA strand from a small amount of DNA.
- Small amount may be as sample like a drop of blood, semen strains, single hair, vaginal swabs etc.

There are two methods to amplify DNA

1. Cloning
2. PCR

Requirements to prepare reaction mixture for the PCR

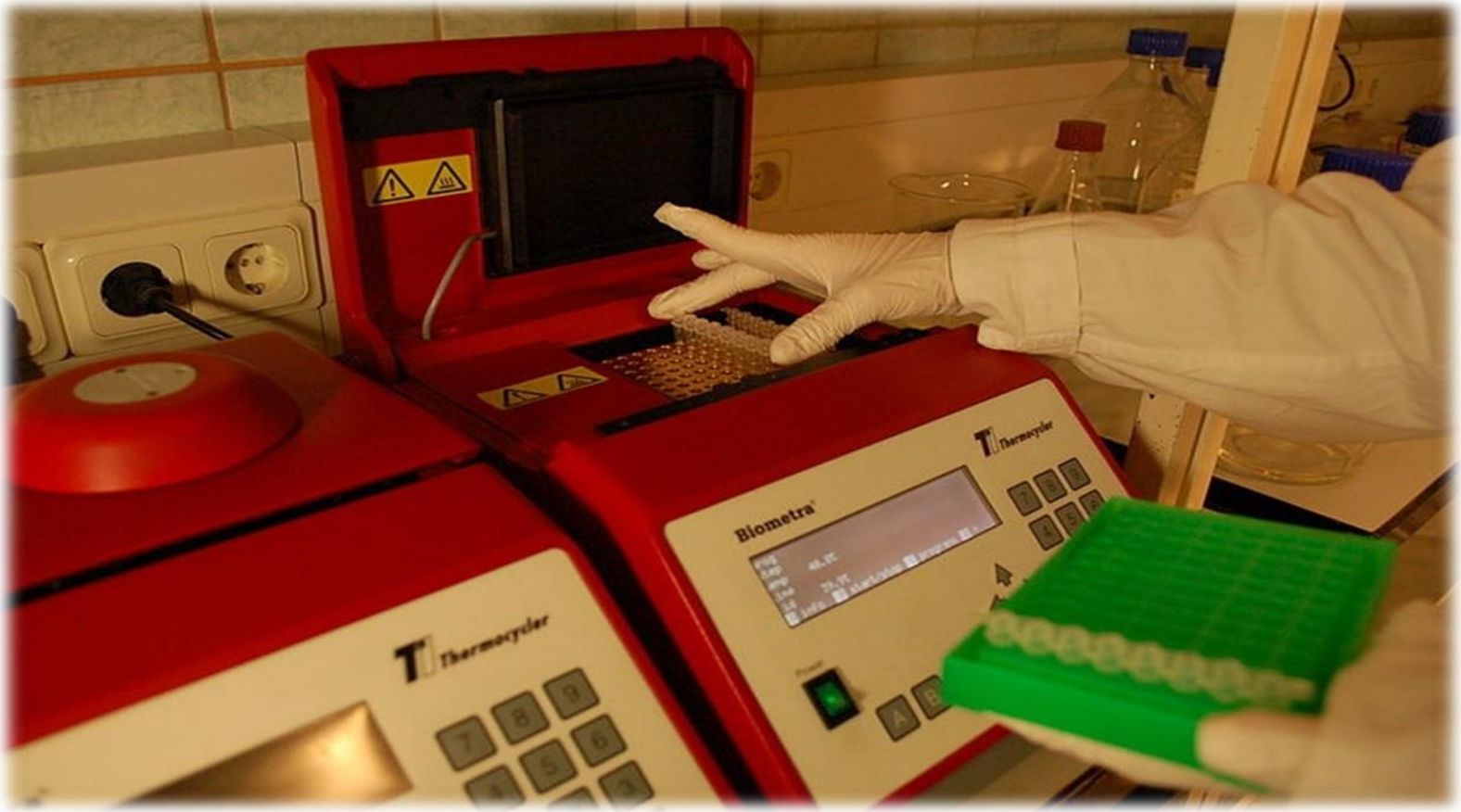
1. DNA Template
2. Primers
3. Taq polymerase
4. Deoxynucleoside triphosphates(dNTPs)
5. Buffer solution
6. Divalent cations(eg.Mg2+)
7. Nuclease free water

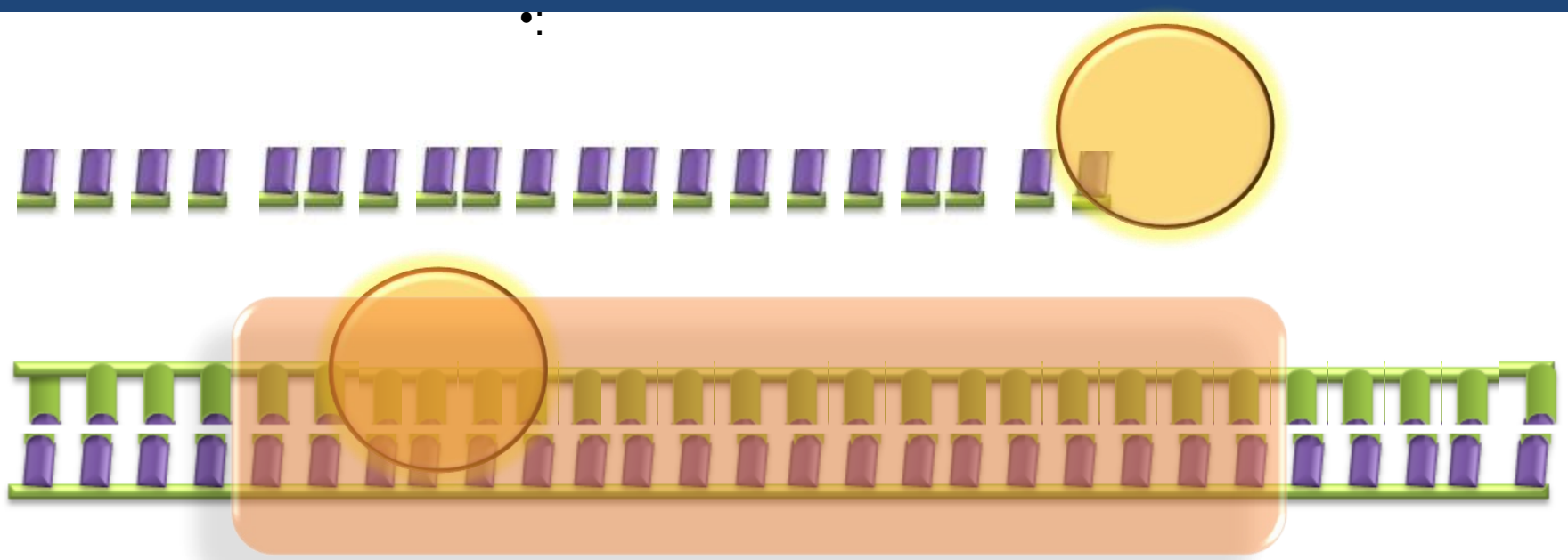
Stages for the PCR reaction: There are three main stages to perform PCR.

1. Denaturation
2. Annealing
3. Extension


Eppendorf's tube
Mix DNA, primers,
dNTPs, Taq, buffer, Mg2

Thermal cycler





Denaturation at 94°C which lasts for 1 min

Annealing at 54°C which lasts for 1 min

extension at 72°C which lasts for 2 min

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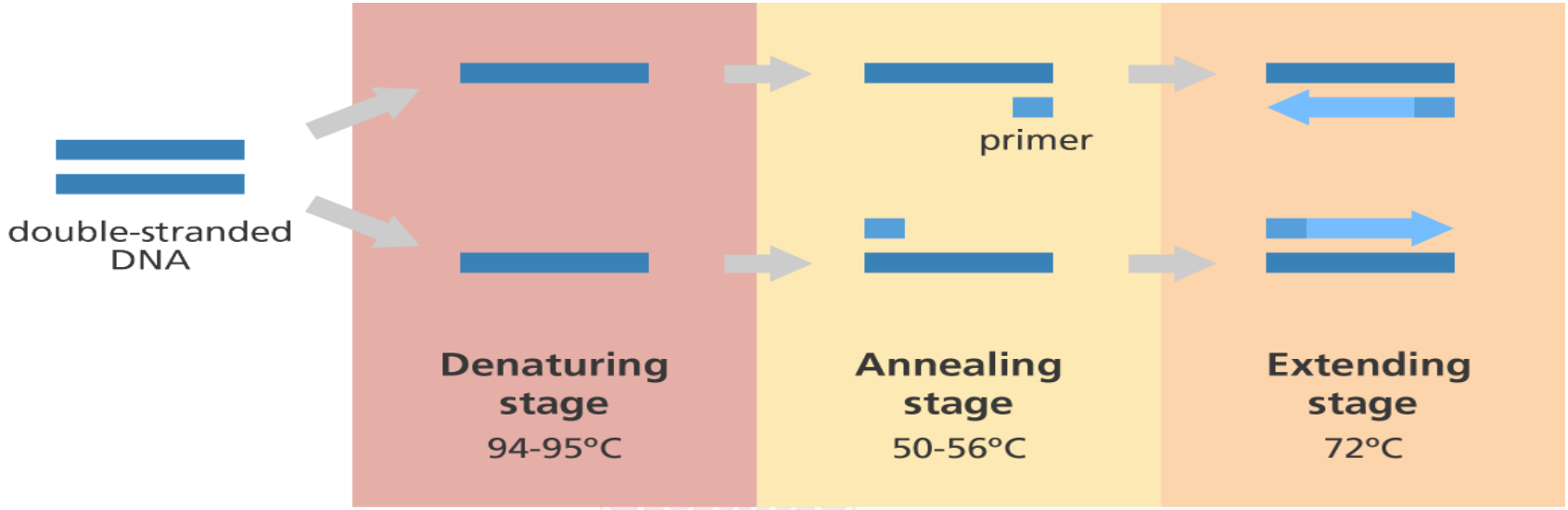

Image taken from yourgenome.org

Types of PCR

- ❖Overlap extension PCR
- ❖Reverse Transcription PCR (for the c-DNA synthesis)
- ❖Real Tme PCR
- ❖Assemble PCR
- ❖Helicase dependent amplication
- ❖Intersequence-specific PCR(ISSR)
- ❖Ligation-mediated PCR
- ❖Methylation –specifin PCR
- ❖Miniprimer PCR
- ❖Multiplex PCR
- ❖Nested PCR
- ❖Solid phase PCR
- ❖Touch down PCR

PCR (POLYMERASE CHAIN REACTION)

Applications of PCR:

- In clinical diagnosis
- In DNA sequencing
- In forensenic medicine
- In Gene manipulation and expression studies
- In comparative study of genomics
- In comparison with gene cloning
- In gene detection
- In pathogen detection
- In inherited genetic disorder

Limitations of PCR:

- Sequence Information can not be obtained.
- Can not get the information regarding amplicon
- Error rate during amplification
- Sensitivity to inhibitors
- Contamination
- Artefacts