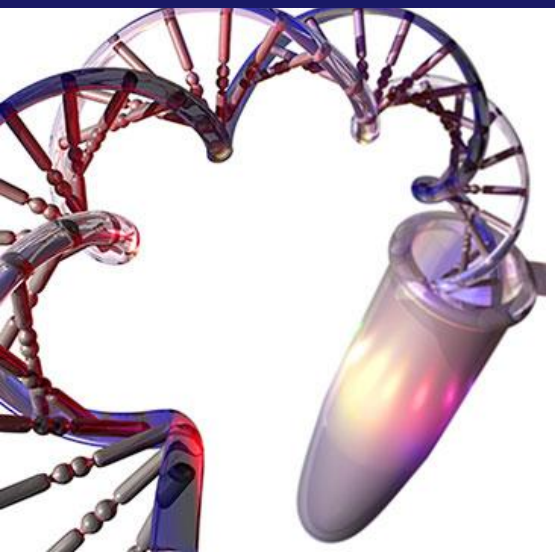
Connective Tissue Diseases Research Center

CTDRC

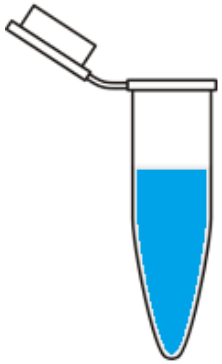
Principle and procedure of PCR



Azam Safary

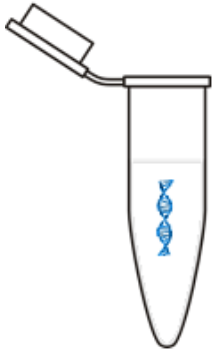
Assistant Professor of Medical Biotechnology

PCR Reaction Components

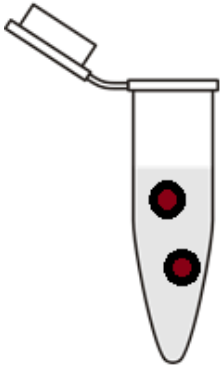


Water

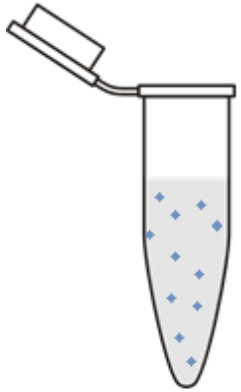
Buffer



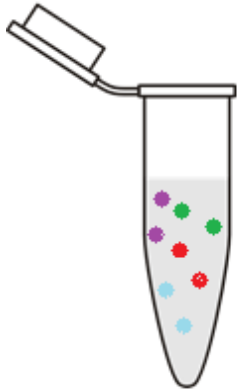
DNA template



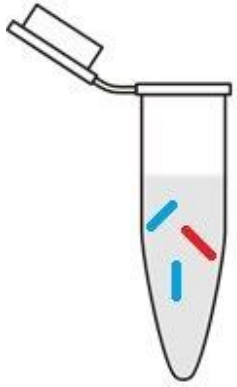
DNA Polymerase



Mg^{++} ions

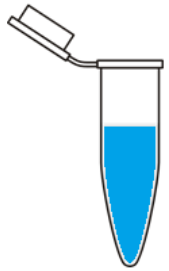


Nucleotides



Primers

PCR Reaction: Water and Buffer

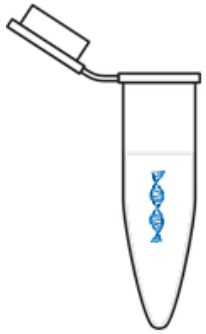


- Water
  - ✓ The medium for all other components.

- Buffer
  - ✓ Stabilizes the DNA polymerase, DNA, and nucleotides
  - ✓ 500 mM KCl
  - ✓ 100 mM Tris-HCl, pH 8.3
  - ✓ Triton X-100 or Tween

PCR Reaction: DNA Template

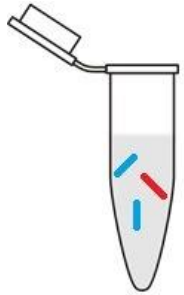
- Water
- Buffer



DNA template

- ✓ Contains region to be amplified
- ✓ Use high quality, purified DNA templates
- ✓ Use 1 pg–1 ng of plasmid or viral templates.
- ✓ Use 1 ng–1 µg of genomic templates.

PCR Reaction: Primers

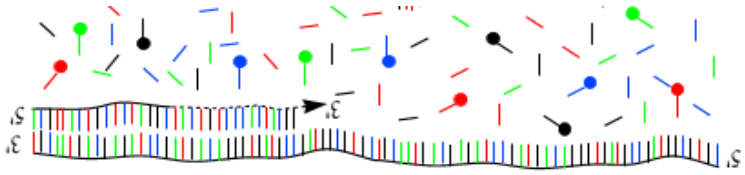


- Water
- Buffer
- DNA template

- **Primers**
  - Specific for ends of amplified region
  - Forward and Reverse
  - Annealing temps should be known
    - Depends on primer length, GC content, etc.
  - Length 18-30 nt
  - Conc 0.1 – 1.0 µM (pMol/ul)

PCR Reaction: Nucleotides

- Water
- Buffer
- DNA template
- Primers



Nucleotides

- Added to the growing chain
- dATP, dGTP, dCTP, dTTP
- Stored at 10 mM, pH 7.0
- Add to 20-200 µM in reaction

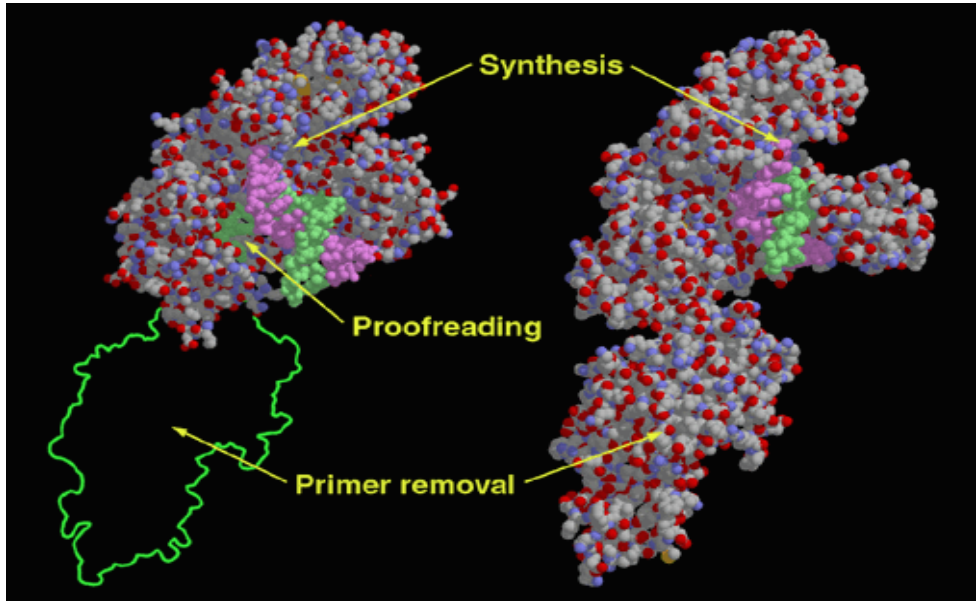
PCR Reaction: Mg^{++}

- Water
- Buffer
- DNA template
- Primers
- Nucleotides

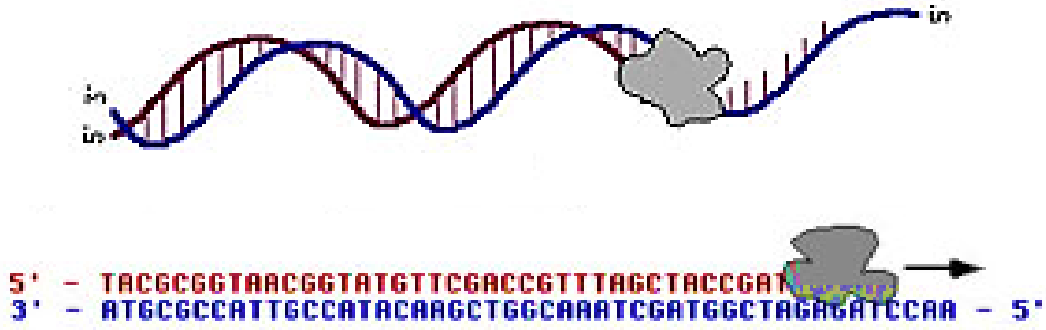
- **Mg^{++} ions**
  - Essential co-factor of DNA polymerase.
  - Stabilizes the DNA double-helix.
  - Too little: Enzyme don’t work.
  - Too much: DNA extra stable, non-specific priming, band smearing.
  - Used at 0.5 to 1.5 mM in the reaction.

PCR Reaction: DNA Polymerase

- Water
- Buffer
- DNA template
- Primers
- Nucleotides
- Mg^{++} ions



- **DNA Polymerase**
  - The enzyme that does the extension
  - Taq or similar
  - Heat-stable



A Typical PCR Reaction

Components	µl
Sterile Water	38.0
10X PCR Buffer	5.0
$MgCl_2$ (50mM)	2.5
dNTP's (10mM each)	1.0
Primer (F) (25 pmol/ml)	1.0
Primer (R)	1.0
DNA Polymerase	0.5
DNA Template	1.0
Total Volume	50.0

Preparation a PCR Master-mix

Component	1X(µl)	20X(µl)
Sterile Water	38.0	760
10X PCR Buffer	5.0	100
MgCl2 (50mM)	2.5	50
dNTP's (10mM each)	1.0	20
Primer (F) (25 pmol/ul)	1.0	20
Primer (R)	1.0	20
DNA Polymerase	0.5	10
DNA Template	1.0	--
Total Volume	50.0	980

Aliquot
49 µl

Add 1 µl DNA

Premixes for PCR Reaction

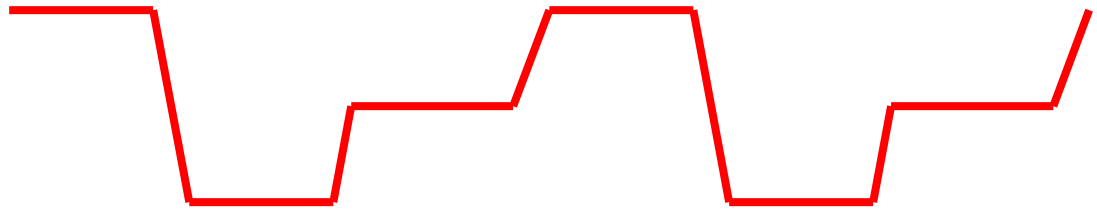
Component	µl
PREMIX	**24.0**
Buffer	
MgCl2	
dNTP's	
DNA Polymerase	
"Enhancers	
Primers (F+R)	**1.0**
DNA Template	**2.0**
Sterile Water	**23.0**
Total Volume	**50.0**

Premixes

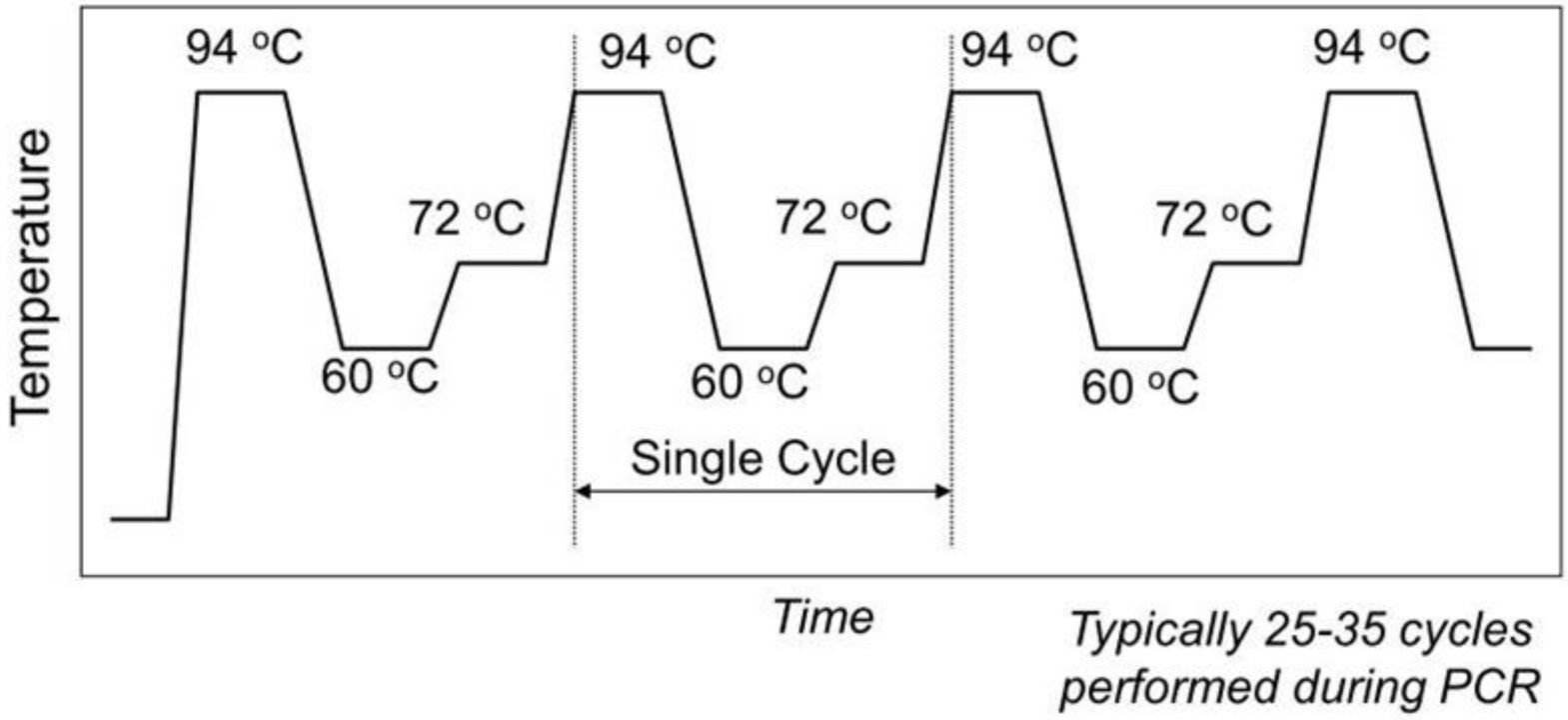
can reduce the number of items added to the mix

Typical Thermal Cycler Program

a. Initial Denaturation	95° C	2-4 min
b1. DNA Denaturation	95° C	45 s
b2. Primer Annealing	55-65° C	1 min
b3. Extension	68- 72° C	1 min
Go to step #2,	Repeat 30-35 more times	
c. Final Extension	72° C	4 min



Thermal Cycling temperature profile for PCR



منابع

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Thank you