

# How PCR Works

By

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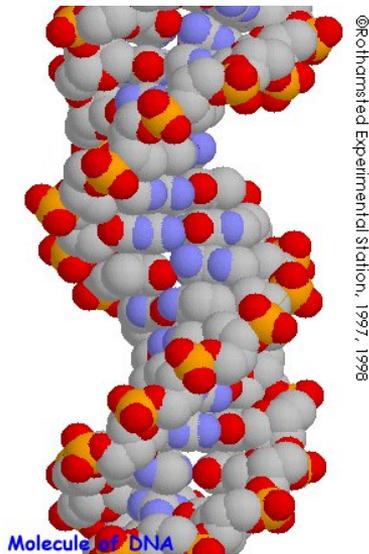
Guest faculty

Zoology department

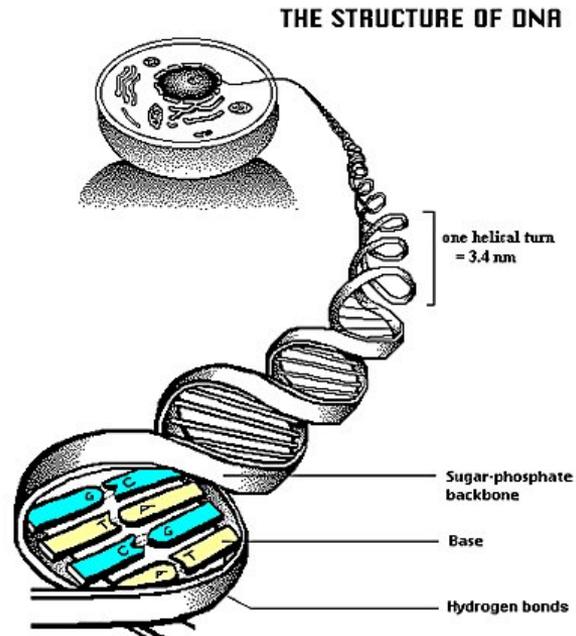
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**B.Sc 2<sup>nd</sup> yr gen/sub**

# Structure of DNA



Double  
Helix



Complementary Base Pairing



Back one Step



Repeat Step

# First Cycle

## Extend Primers

72° C



Next Step

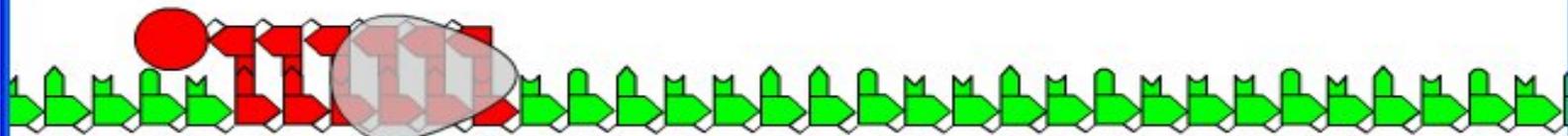
The temperature is raised again to provide the optimum temperature for a special heat resistant type of DNA polymerase called *Thermus aquaticus*®. This extends the primers to make a copy of the DNA.



DNA Polymerase  
*Thermus aquaticus*®



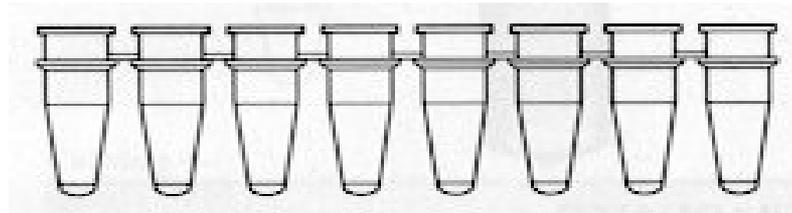
Continue



# The PCR Reaction Chemistry

## PCR Reaction Components

- Water
- Buffer
- DNA template
- Primers
- Nucleotides
- Mg<sup>++</sup> ions
- DNA Polymerase



## Water

The medium for all other components.

## Buffer

Stabilizes the DNA polymerase,  
DNA, and nucleotides

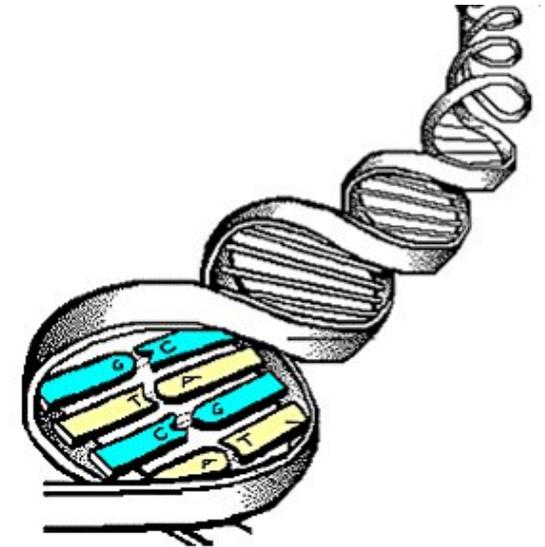
500 mM KCl

100 mM Tris-HCl, pH 8.3

Triton X-100 or Tween

# PCR Reaction: Template DNA

- Water
- Buffer
- DNA template
  - Contains region to be amplified
  - Any DNA desired
  - Purity not required
  - Should be free of polymerase inhibitors



# PCR Reaction:

- Water
- Buffer
- DNA template
- Primers

## Primers

- Specific for ends of amplified region
- Forward and Reverse
- Annealing temps should be known
  - Depends on primer length, GC content, etc.
- Length 15-30 nt
- Conc 0.1 – 1.0  $\mu\text{M}$  (pMol/ul)

TACGCGGTACGGTATGTTGACCGTTTAGCTACCGAT•  
TACGCGGTACGGTATGTTGACCGTTTAGCT•  
TACGCGGTACGGTATGTTGACCGTTT•  
TACGCGGTACGGTATGTTGACCGTT•  
TACGCGGTACGGTATGTTGACCGT•  
TACGCGGTACGGTATGTT•  
TACGCGGTACGGTATG•  
TACGCGGTACGGTAT•  
TACGCGGTACGGT•  
TACGCGGT•

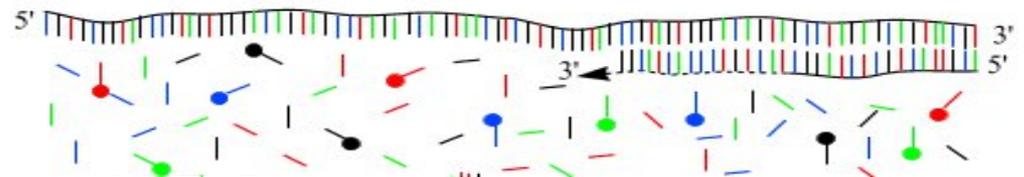
# PCR Reaction:

- Water
- Buffer
- DNA template
- Primers

## Nucleotides

- **Nucleotides**

- Added to the growing chain
- Activated NTP's
- dATP, dGTP,



pH 7.0

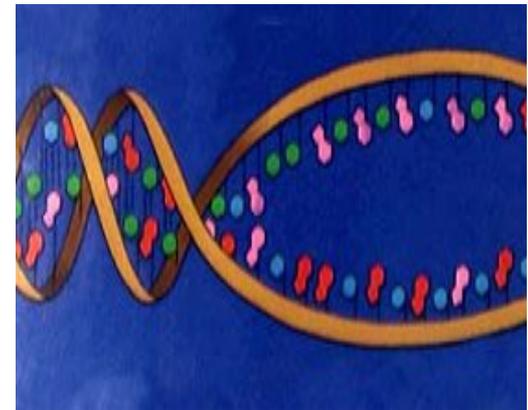
- Add to 20-200 uM in assay

# PCR

## Reaction:

## Magnesium

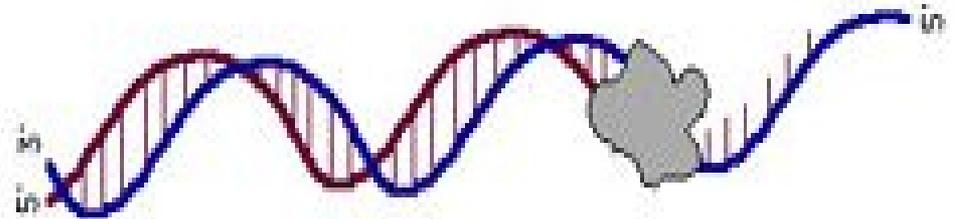
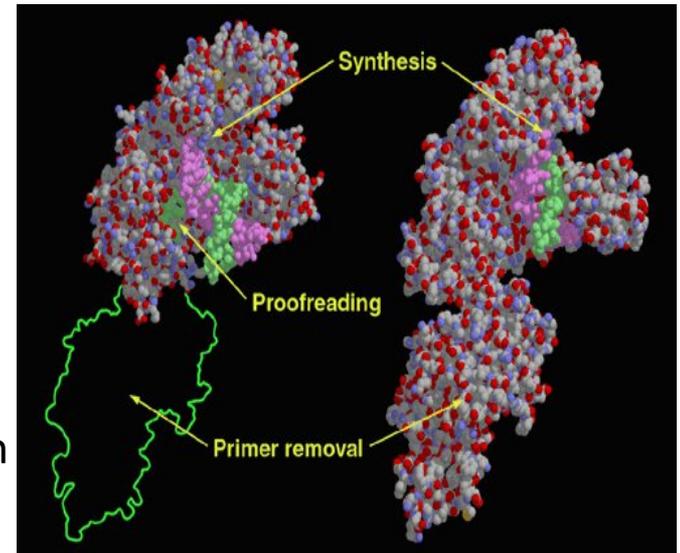
- Water
- Buffer
- DNA template
- Primers
- Nucleotides
- **Mg<sup>++</sup> ions**
  - Essential co-factor of DNA polymerase
  - Too little: Enzyme won't work.
  - Stabilizes the DNA double-helix
  - Too much: DNA extra stable, non-specific priming, band smearing
  - Used at 0.5 to 3.5  $\mu\text{M}$  in the assay



PCR Reaction:

Polymerase

- Water
- Buffer
- DNA template
- Primers
- Nucleotides
- Mg<sup>++</sup> ions
- DNA Polymerase
  - The enzyme that does the extension
  - TAQ or similar
  - Heat-stable
  - Approx 1 U / rxn



5' - TACGCGGTACGGTATGTTTCGACCGTTTAGCTACCGAT  
3' - ATGCCCATTTGCCATACAGCTGGCAATCGATGGCTAGAGATCCAA - 5'

# PCR Reaction Components

## Summary:

- Water
- Buffer
- DNA template
- Primers
- Nucleotides
- Mg<sup>++</sup> ions
- DNA Polymerase

