

Medical Biology

Lab : 4 Polymerase Chain Reaction (PCR)

DNA polymerase duplicates DNA

Chain Reaction: The product of a reaction is used to amplify the same reaction

Results in rapid increase in the product

Setting up a PCR Reaction

Add template DNA and primers

Add dNTPs

Add DNA polymerase

Taq DNA polymerase

Derived from *Thermus aquaticus*

Heat stable DNA polymerase

Ideal temperature 72C

Thermal Cycling

A PCR machine controls temperature

Typical PCR go through three steps

1-Denaturation

2-Annealing

3-Extension

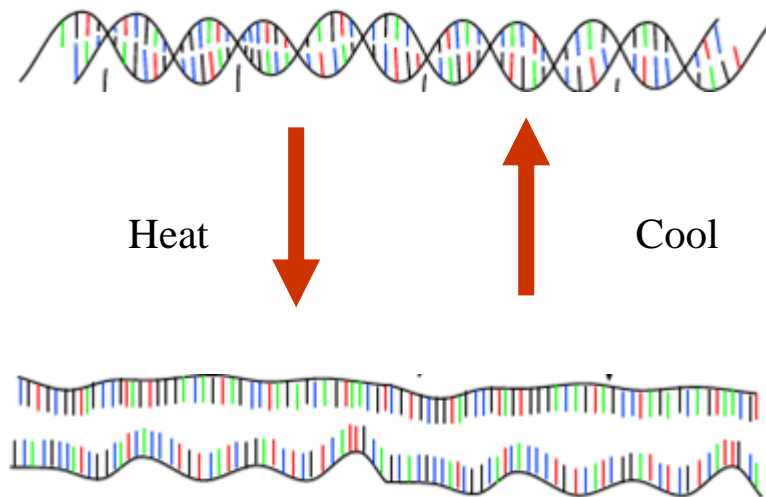
Denaturation

Heating separates the double stranded DNA

Denaturation

Slow cooling anneals the two strands

Renaturation



Annealing

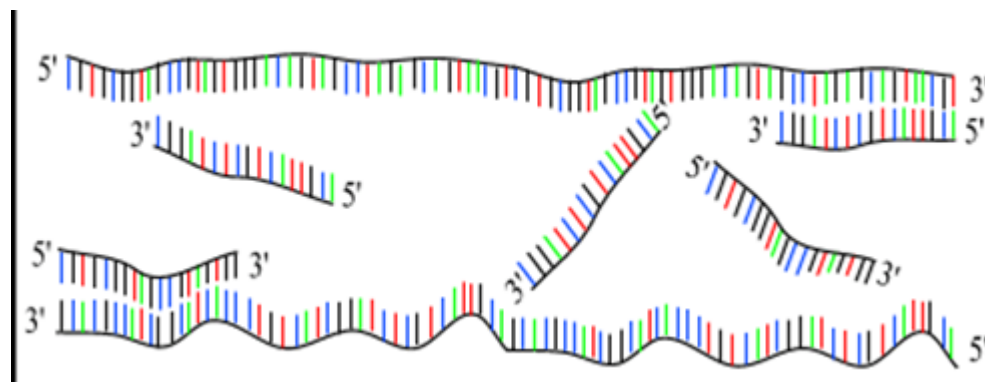
Two primers are supplied in molar excess

They bind to the complementary region

As the DNA cools, they wedge between two template strands

Optimal temperature varies based on primer length etc.

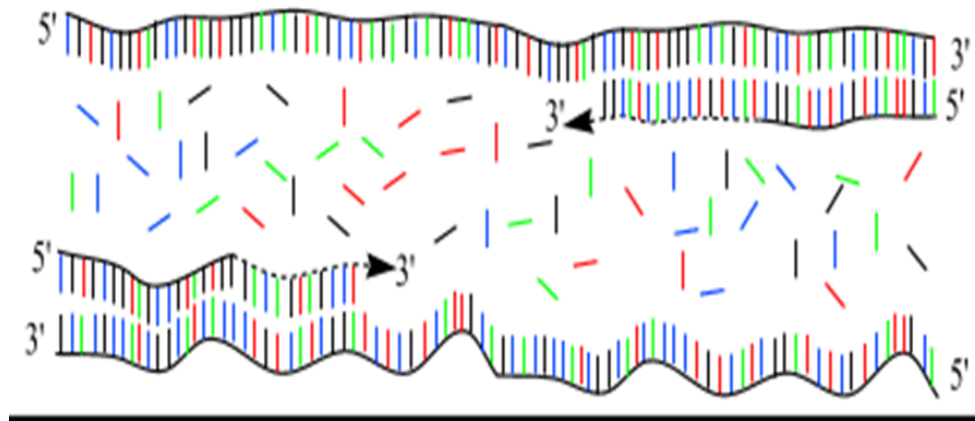
Typical temperature from 40 to 60 C



Extension

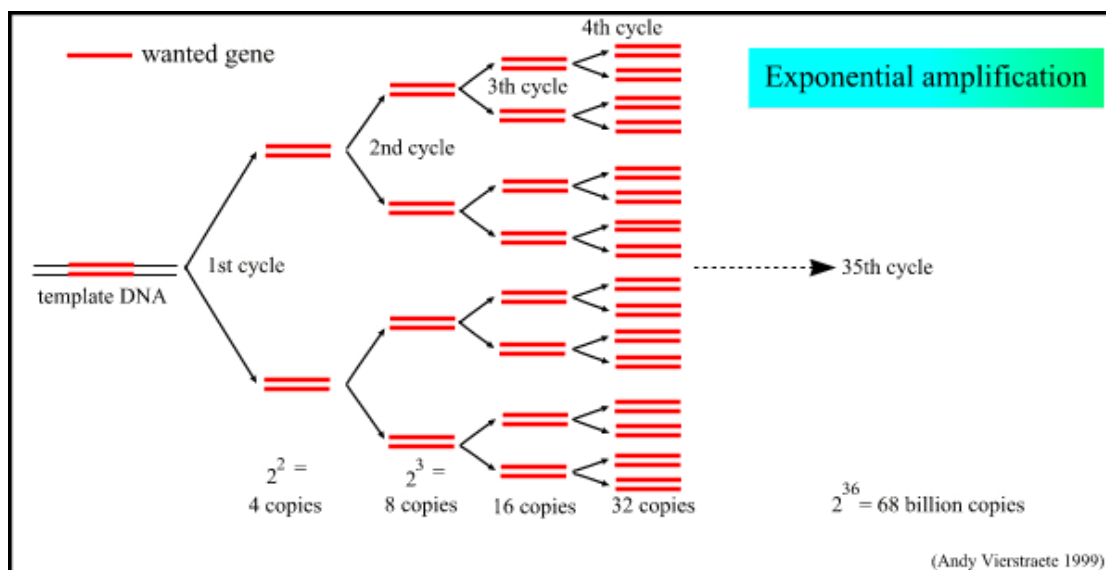
DNA polymerase duplicates DNA

Optimal temperature 72C



PCR Amplification

Exponential Amplification of template DNA



Typical PCR mix

In a thin wall Eppendorf tube assemble the following

PCR components	Amount
Template DNA (5- 200 ng)	Variable
1 mM dNTPs (200 uM final)	10 uL
10 ^x PCR buffer	5 uL
25 mM MgCl ₂ (1.5 mM final)	3 uL
20 uM forward primer (20 pmoles final)	1 uL
20 uM revers primer (20 pmoles final)	1uL
5 units/uL Taq DNA polymerase (1.5 units)	0.3 uL
Water	Variable
Final Volume	50 uL