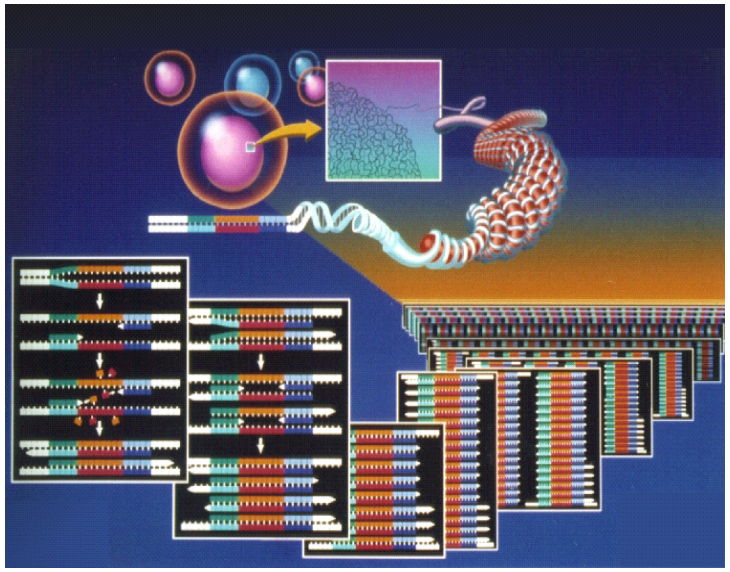
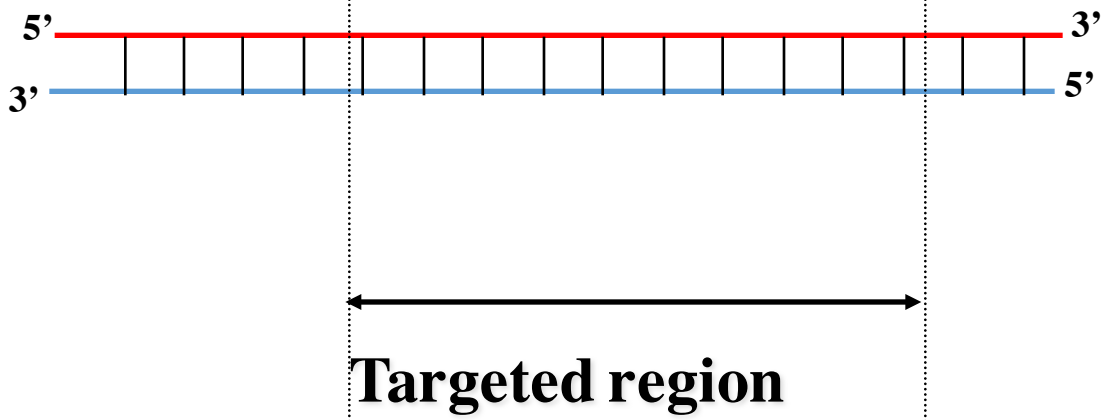


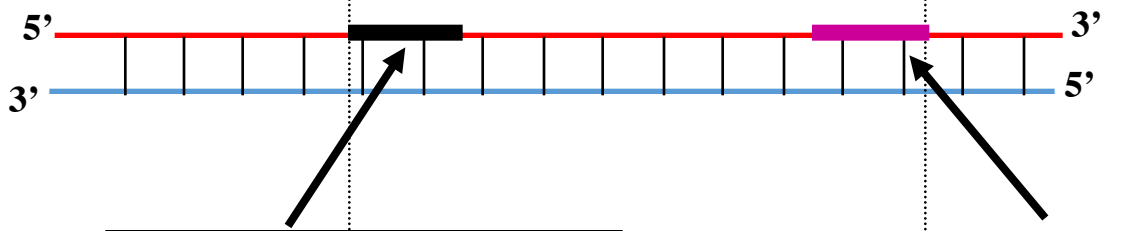
Polymerase Chain Reaction2

Double stranded template DNA



Double stranded template DNA

Synthesize the primers!



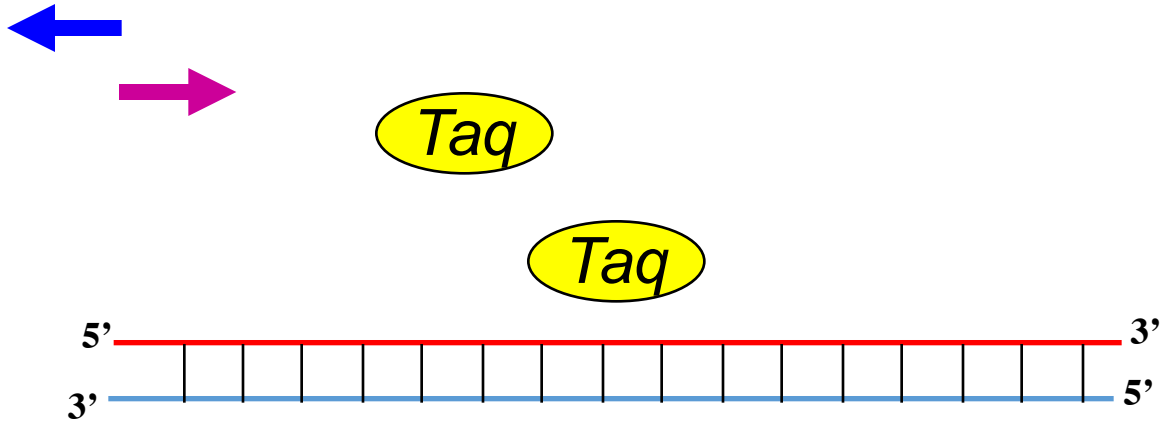
Similar to this sequence

Complementer to this sequence

PCR CYCLE 1

Double stranded template DNA

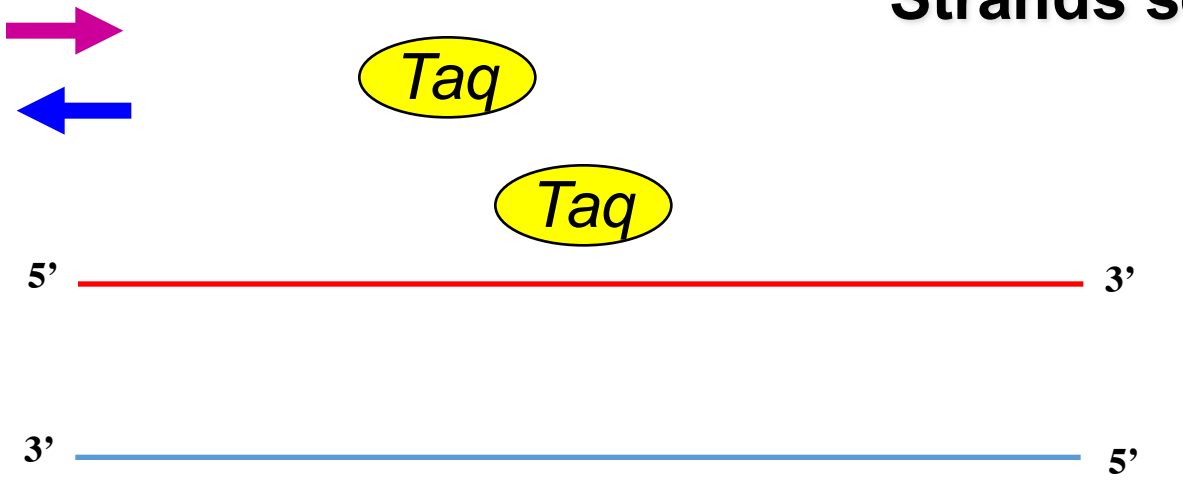
Primers



PCR CYCLE 1

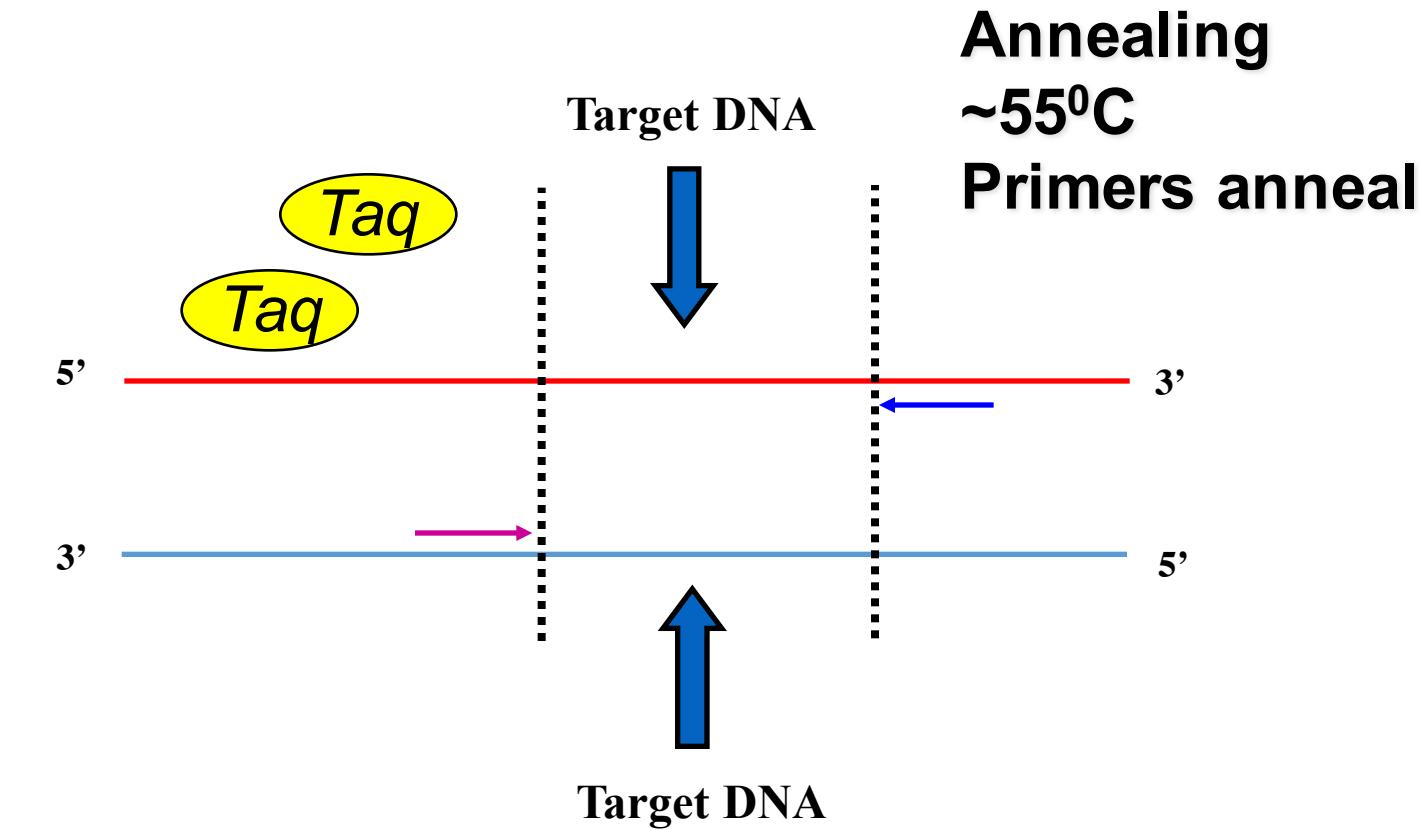
Denaturation at 95^0C
Strands separated

Primers



***Taq* polymerase has a termostabile characteristic**

PCR CYCLE 1

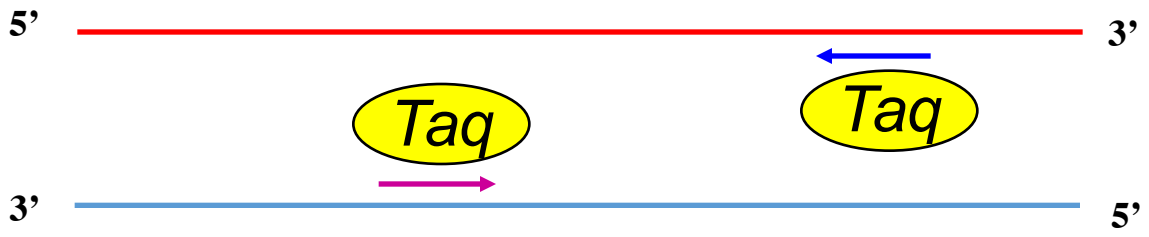


Forward primer anneals to upper strand

Reverse primer anneals to lower strand

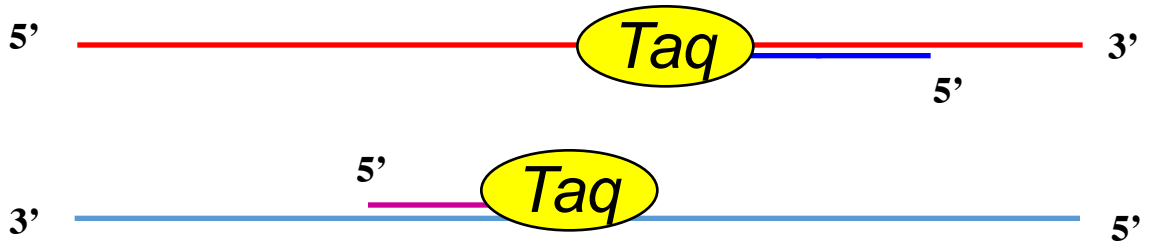
PCR CYCLE 1

Annealing
~55^{0}C
***Taq* anneals to primer-strand complexes**



PCR CYCLE 1

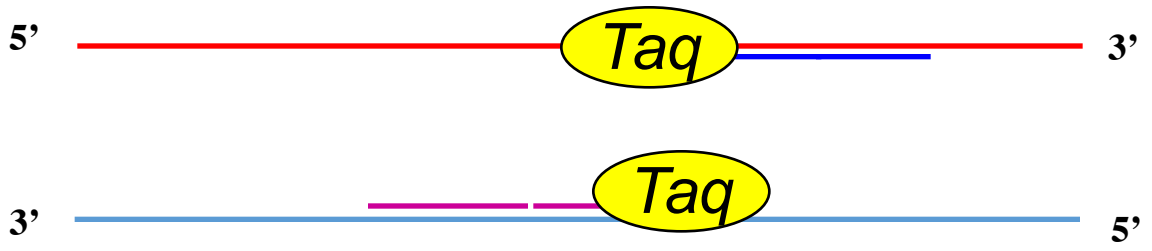
Extension
72°C
By the help of dNTPs
Taq copies DNA



***Taq synthesizes DNA in the direction* 5’ to 3’**

PCR CYCLE 1

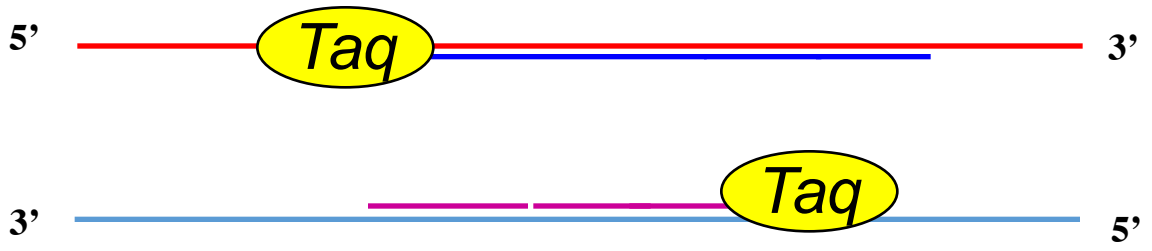
Extension
72°C
By the help of dNTPs
Taq copies DNA



***Taq synthesizes DNA in the direction* 5’ to 3’**

PCR CYCLE 1

Extension
72°C
By the help of dNTPs
Taq copies DNA



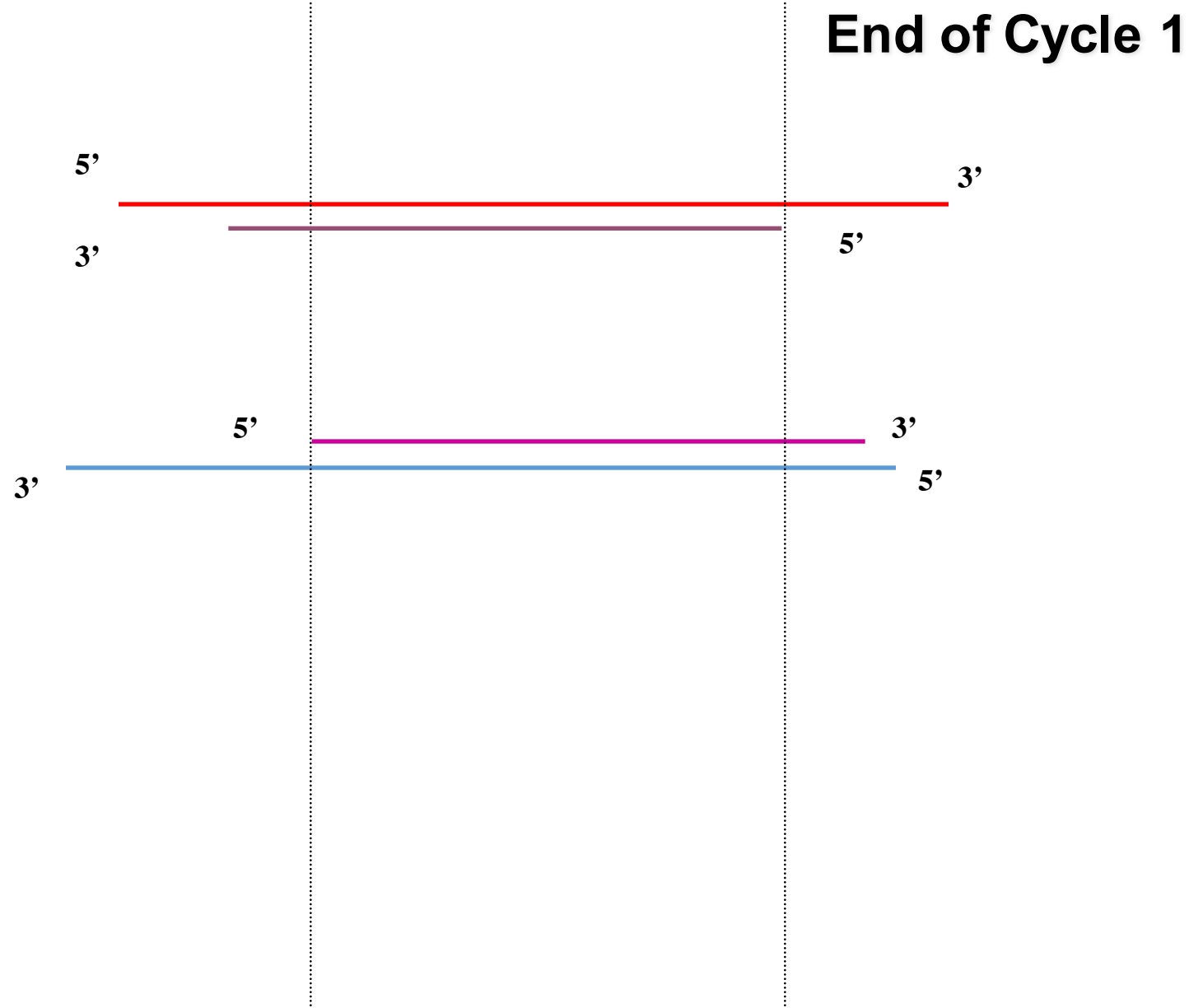
***Taq synthesizes DNA in the direction* 5’ to 3’**

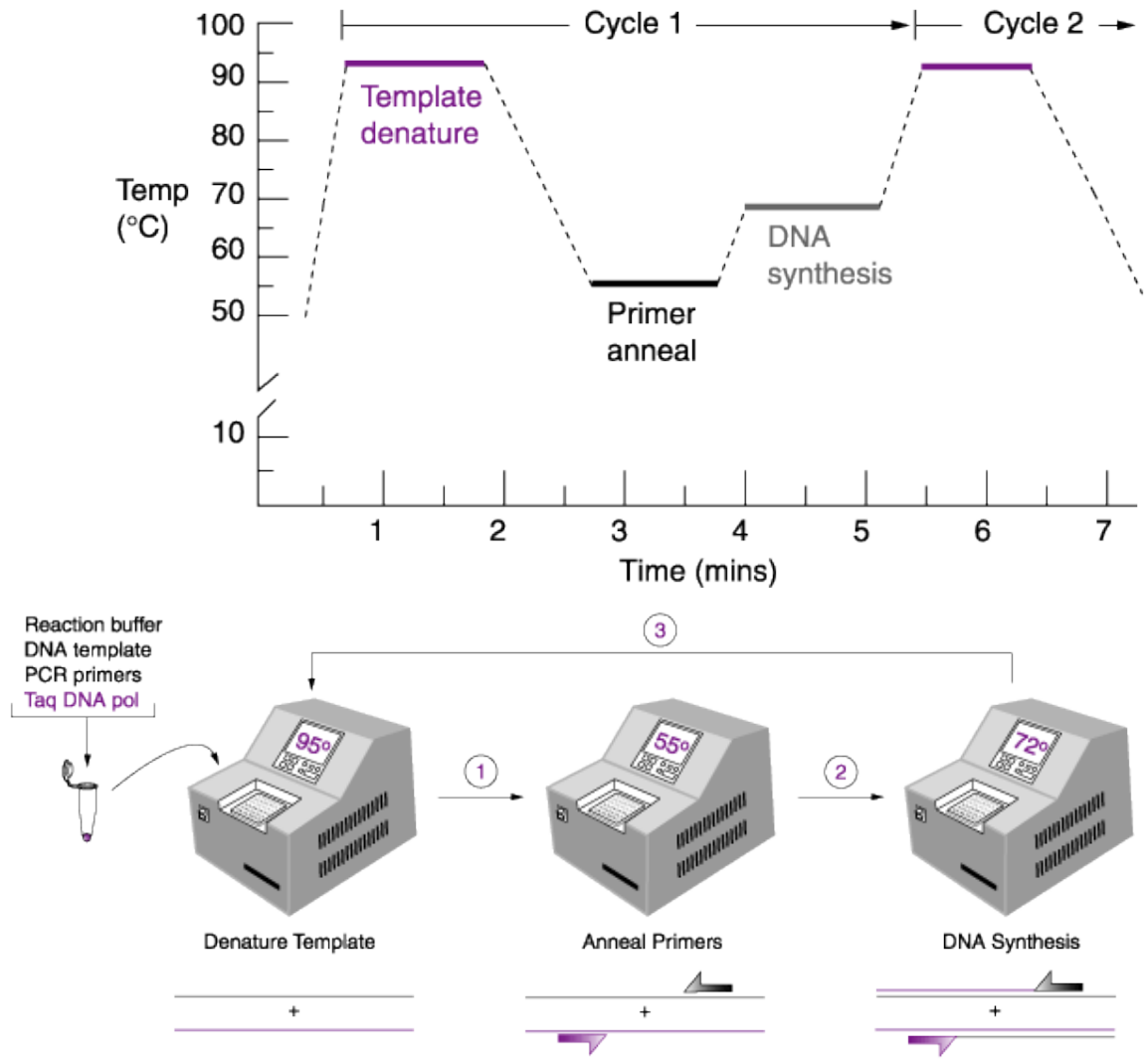
PCR CYCLE 1

Extension
72°C
By the help of dNTPs
Taq copies DNA

***Taq synthesizes DNA in the direction* 5' to 3'**

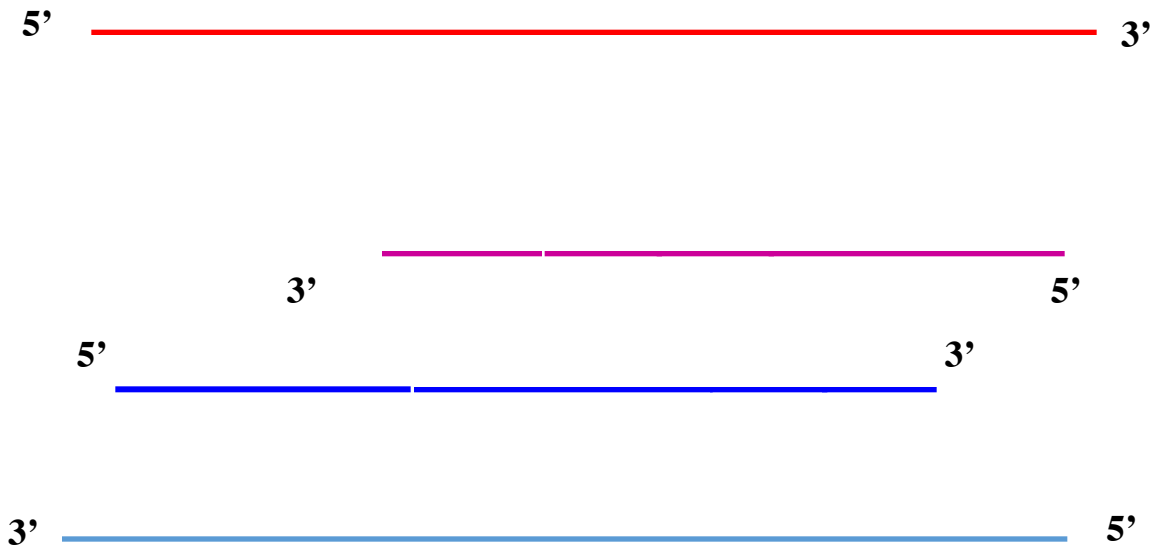
PCR CYCLE 1




100
90
80
70
60
50
10
Temp
(°C)
Cycle 1
Cycle 2
Template
denature
Primer
anneal
DNA
synthesis
1
2
3
4
5
6
7
Time (mins)
Reaction buffer
DNA template
PCR primers
Taq DNA pol
95°
55°
72°
1
2
3
Denature Template
Anneal Primers
DNA Synthesis
+
+
+

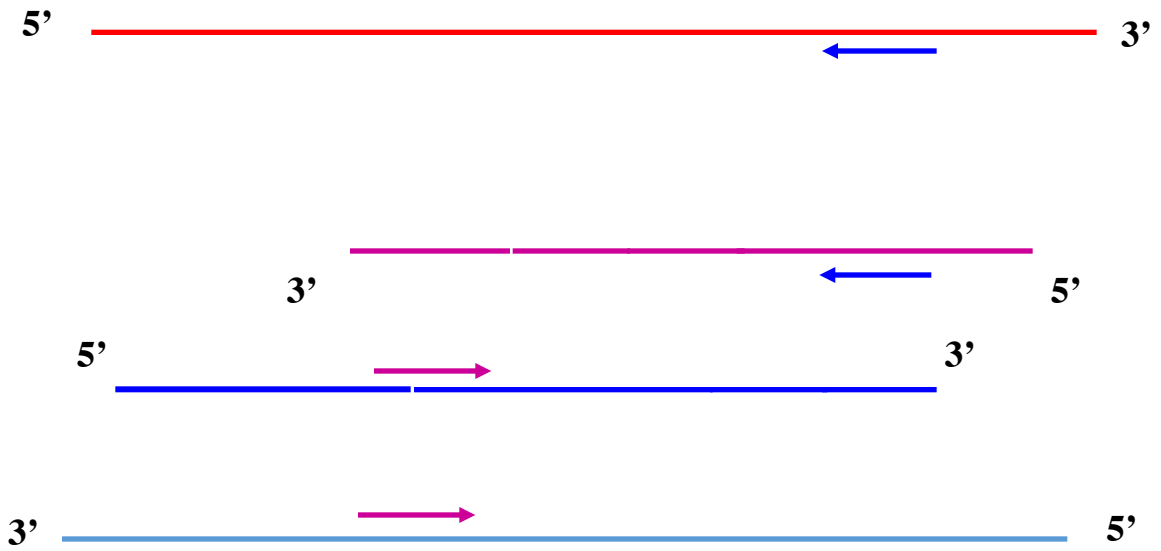
PCR CYCLE 2

Denaturation at 95^0C
Strands separated



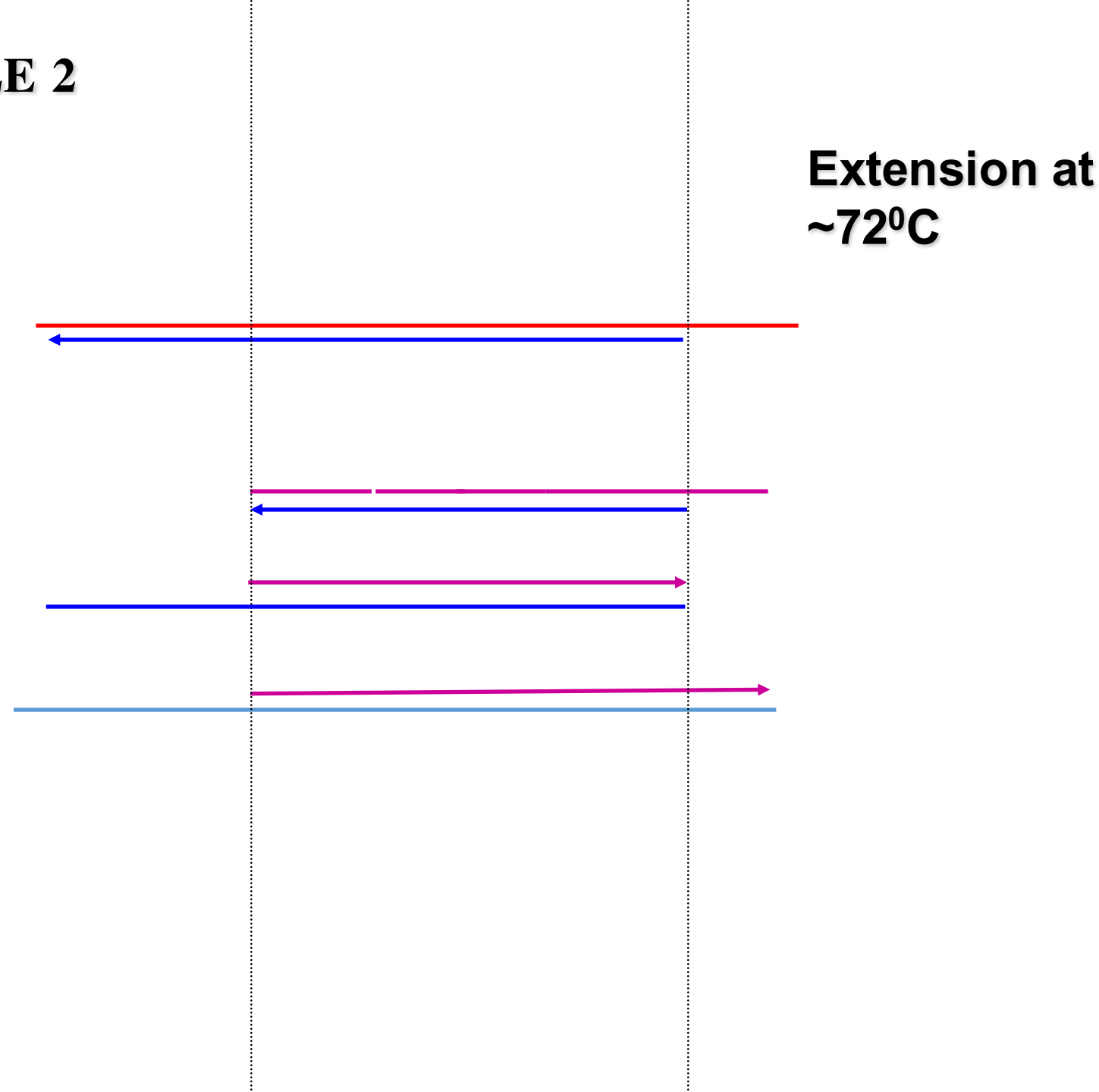
PCR CYCLE 2

Binding of primers at ~55^{0}C

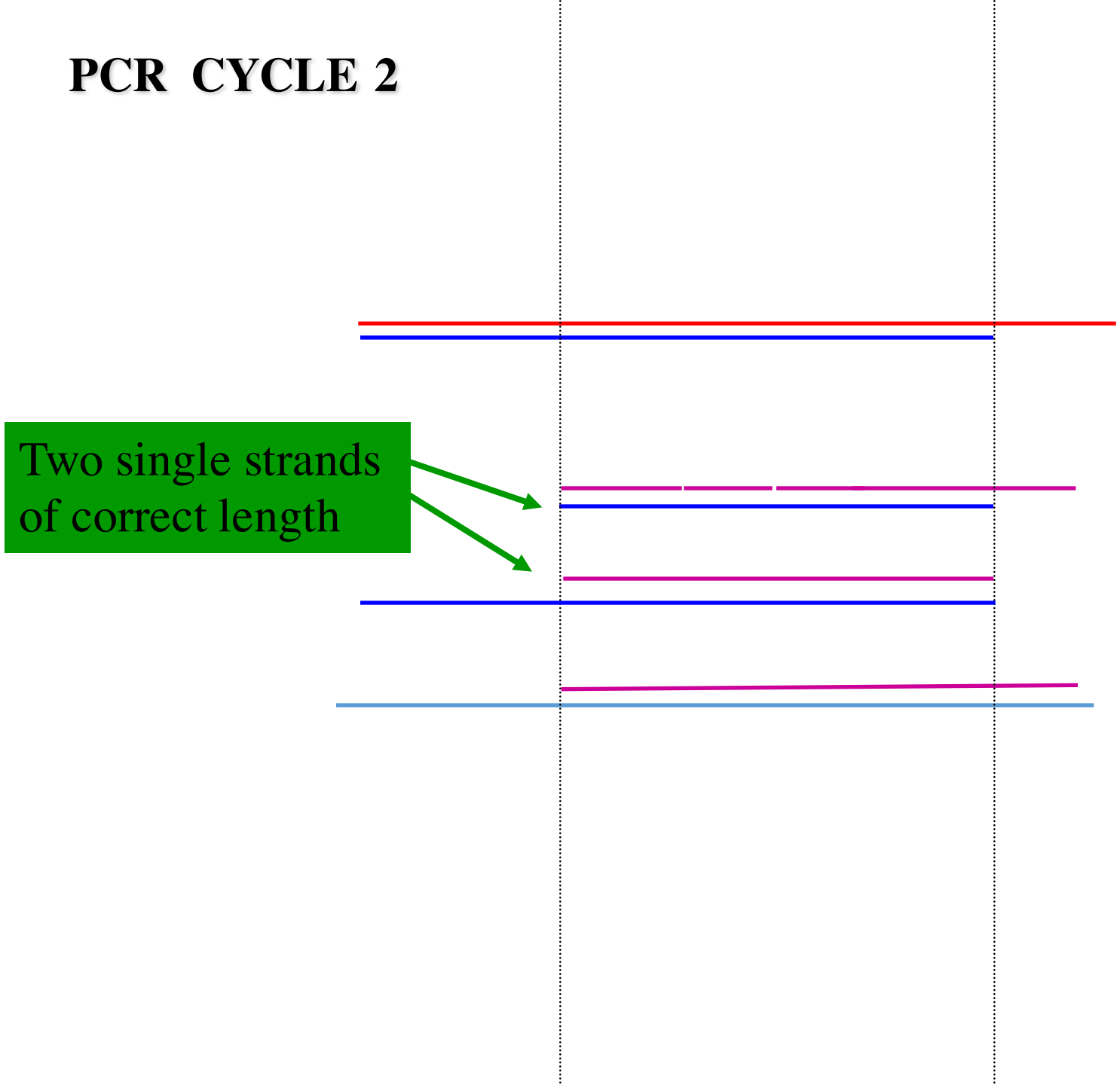


PCR CYCLE 2

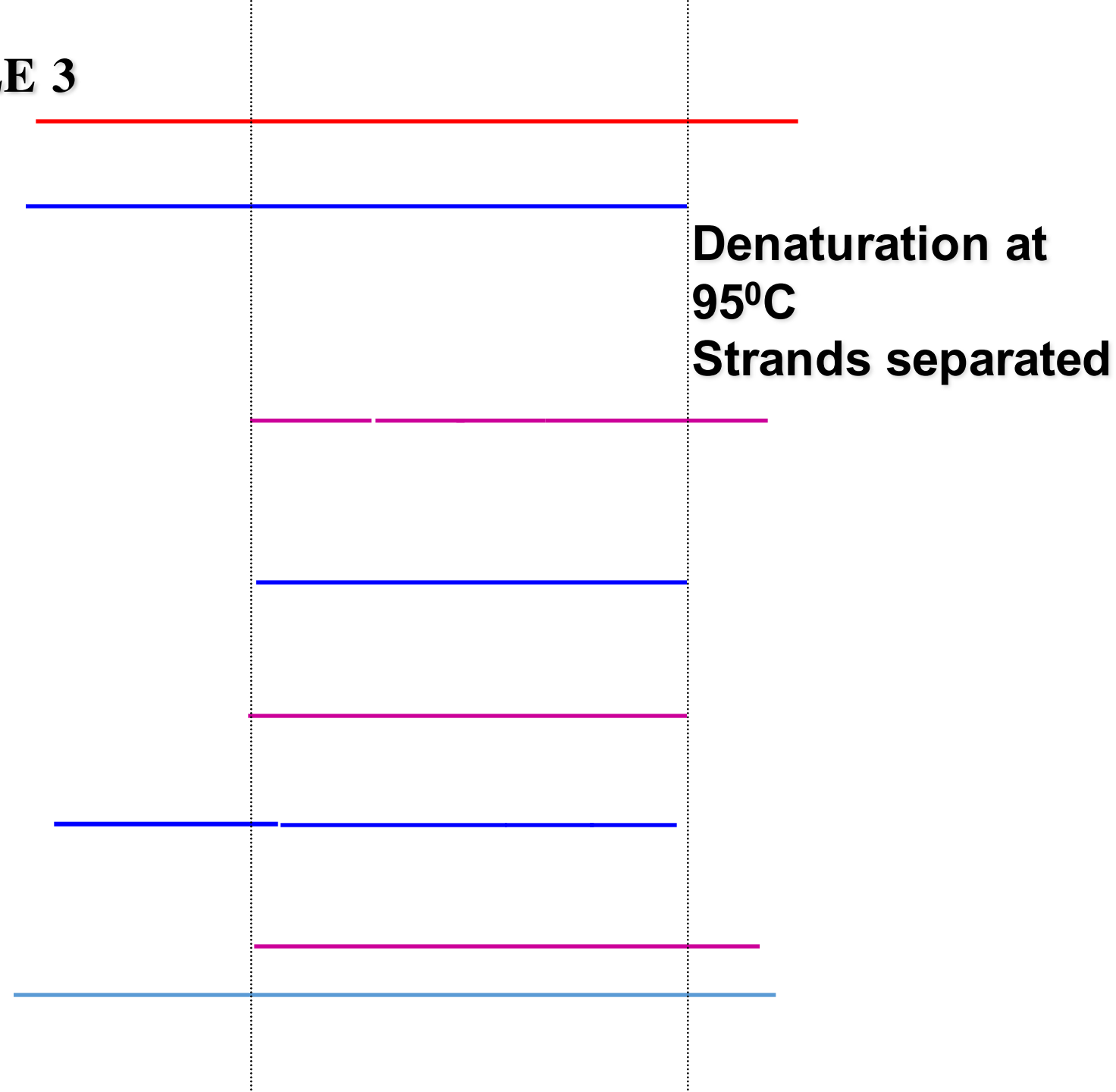
Extension at ~72^{0}C



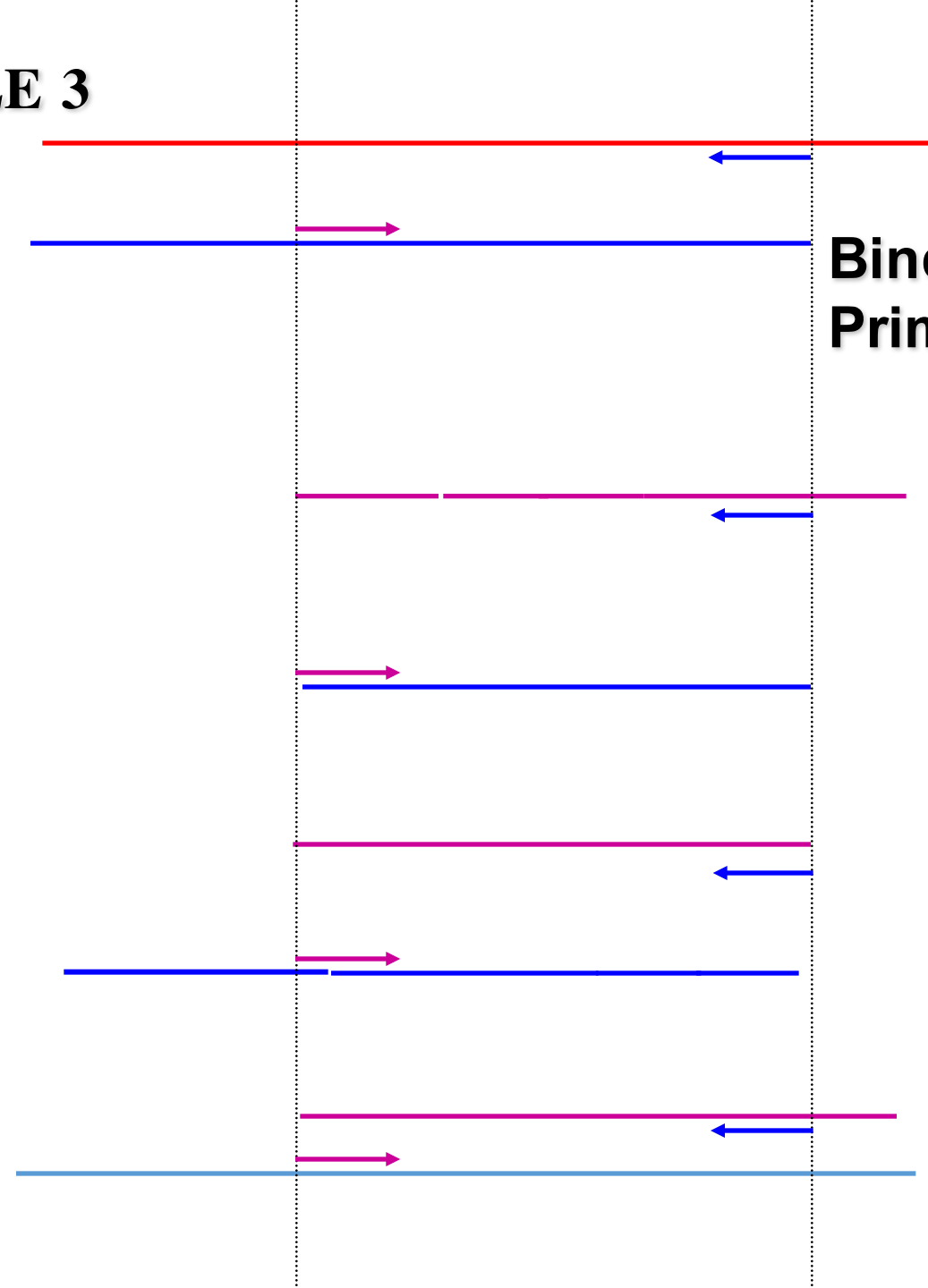
PCR CYCLE 2



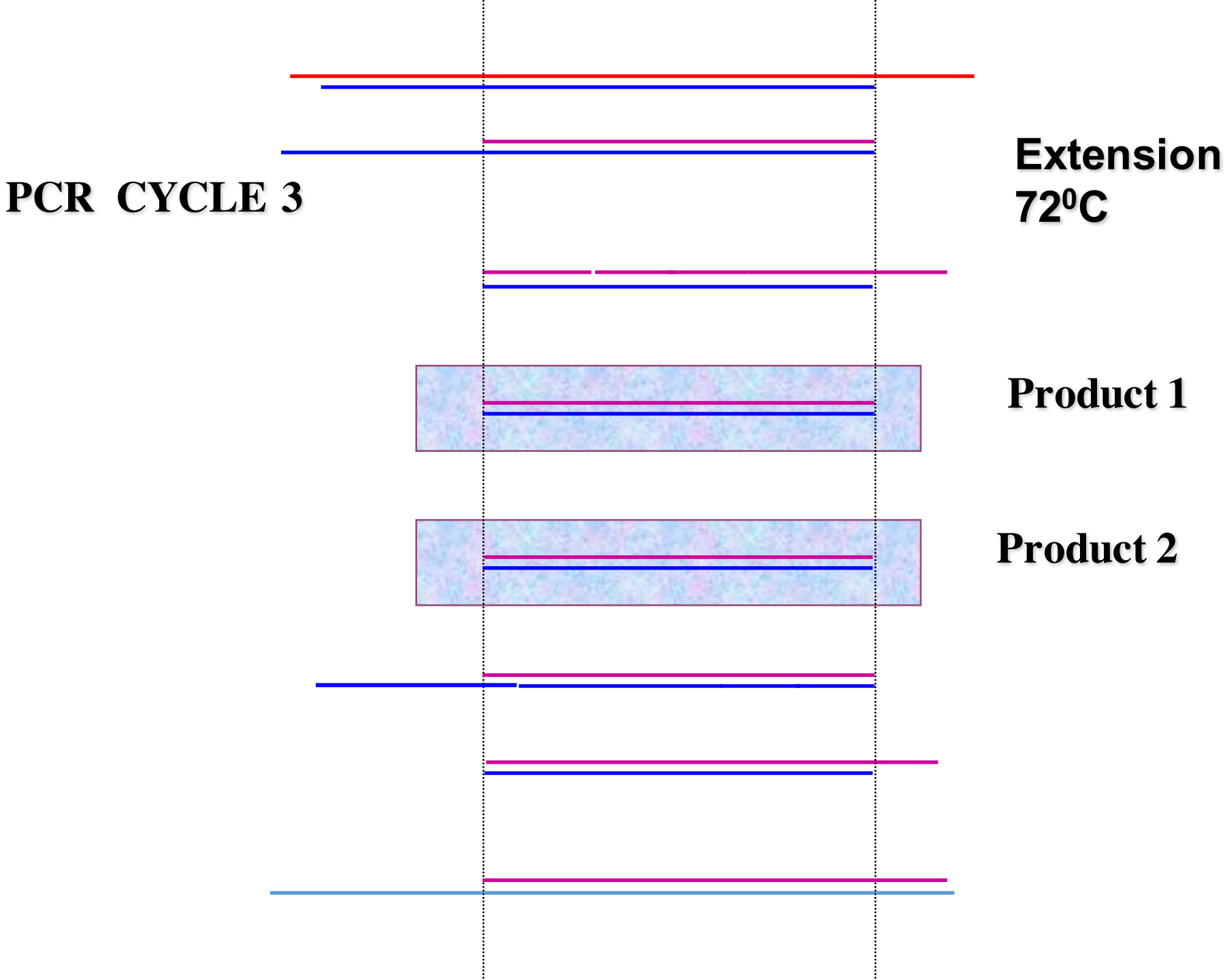
PCR CYCLE 3



PCR CYCLE 3



Binding of
Primers at ~55^{0}C


PCR CYCLE 3
Extension
72⁰C
Product 1
Product 2

3. Cycle is the first cycle when the first products of targeted length

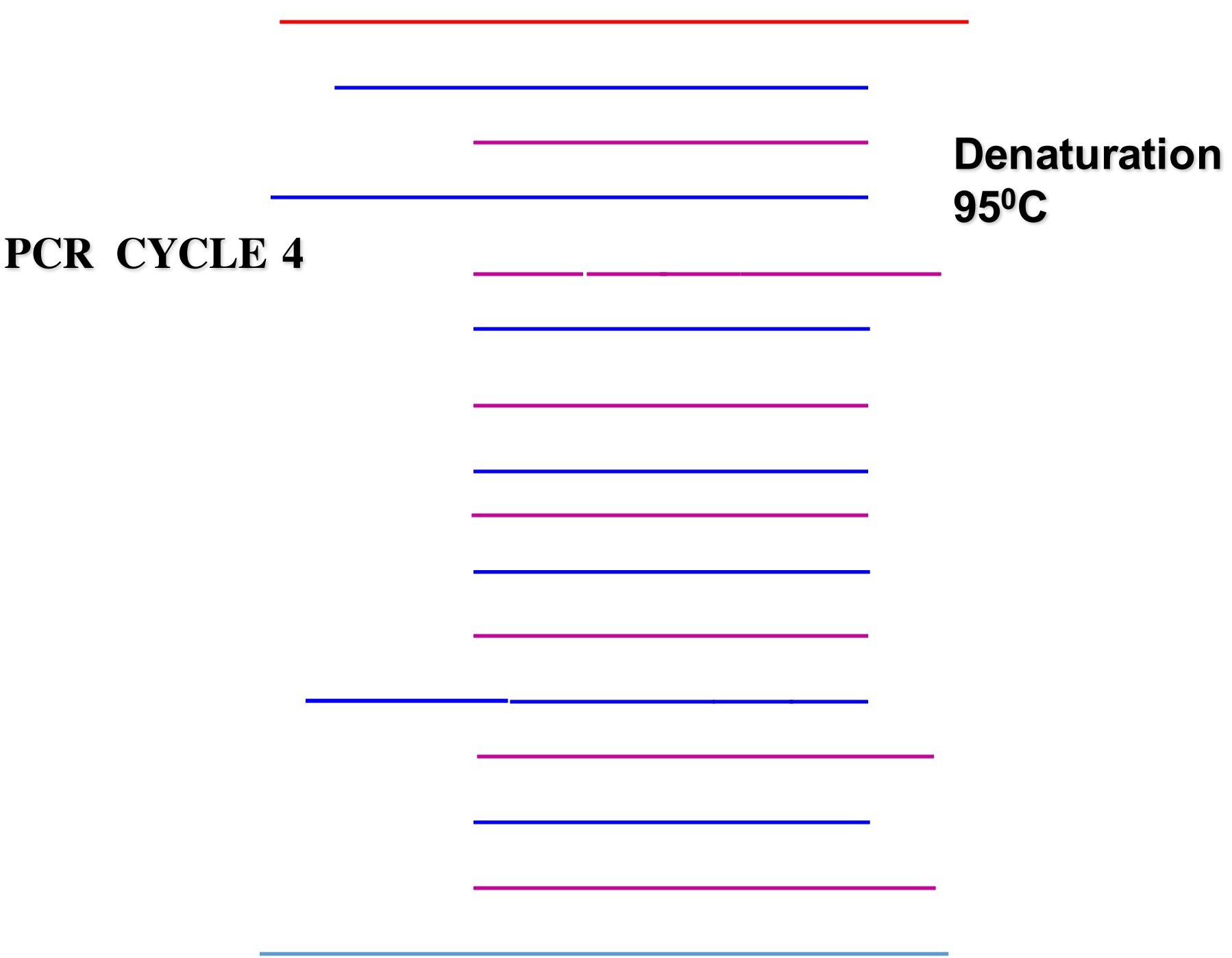
From this cycle on amplication proceeds exponentially

Amplimers synthesized according to equation mentioned below:

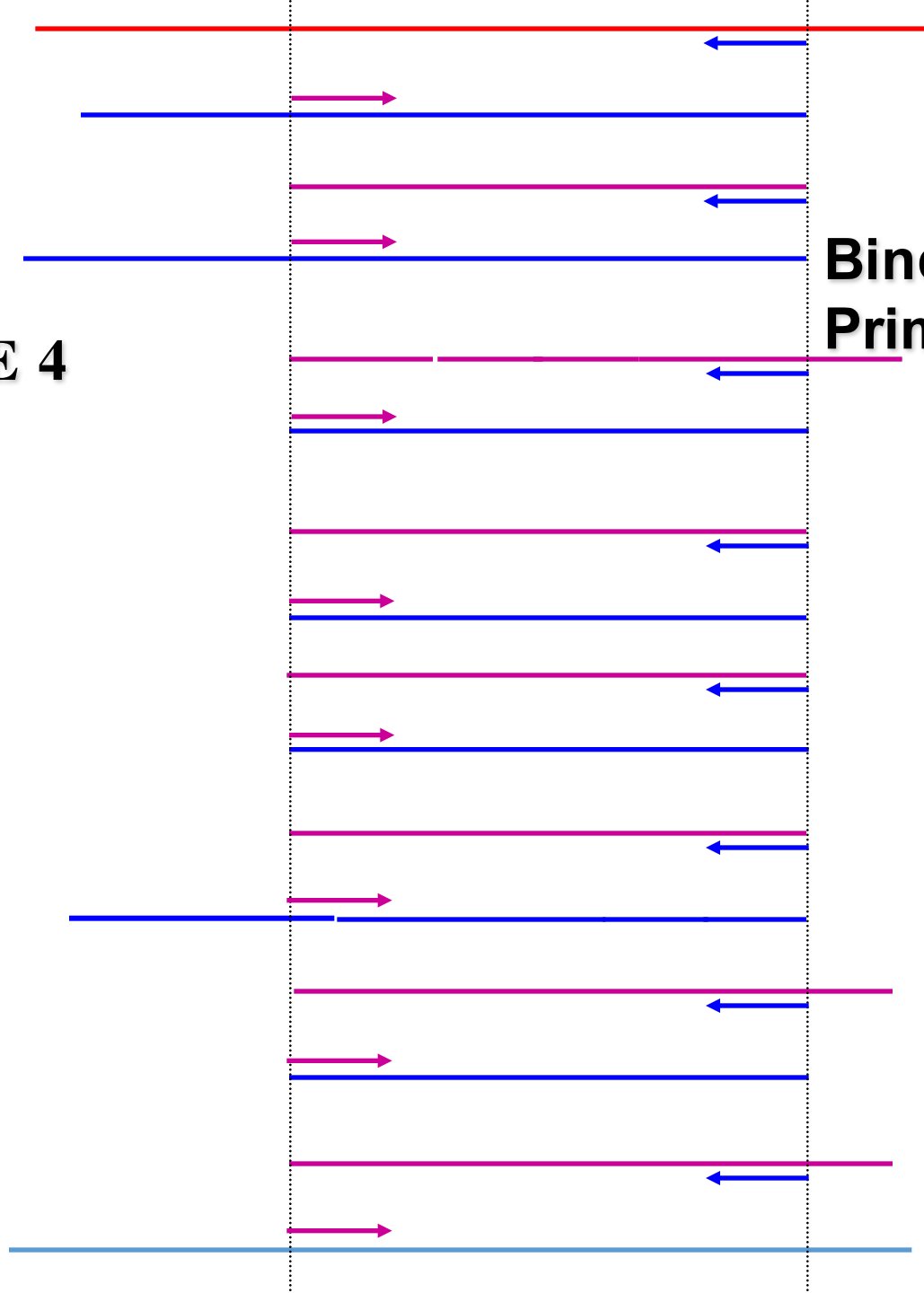
After n number of PCR cycles where exponential amplification $\boldsymbol{No(1+Y)^{n-1}}$ target copy will be formed!

No starting number of DNA targets
Y Efficiency of PCR reaction
n cycle number

END OF
PCR CYCLE 3

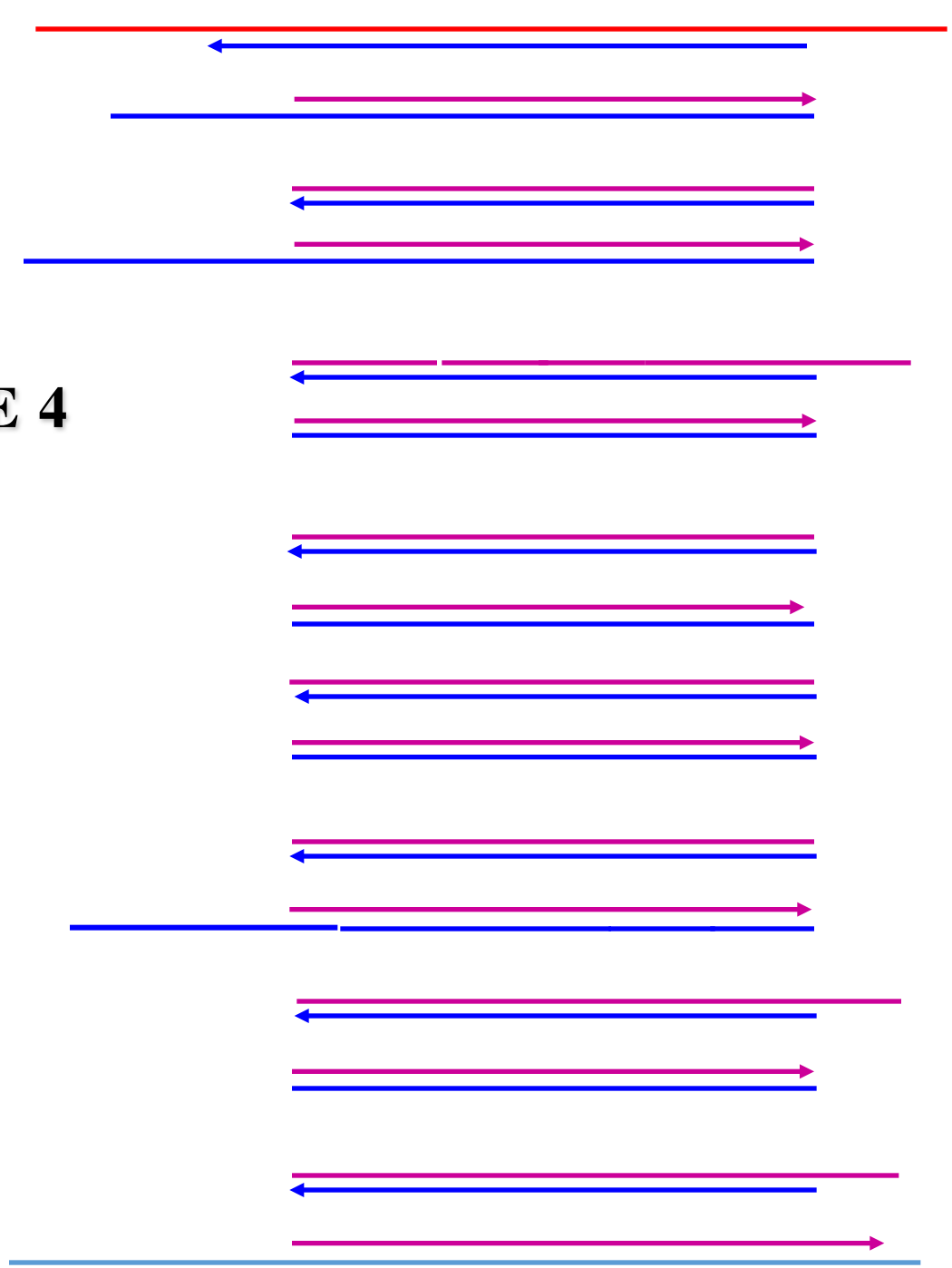

PCR CYCLE 4
Denaturation
95⁰C

PCR CYCLE 4



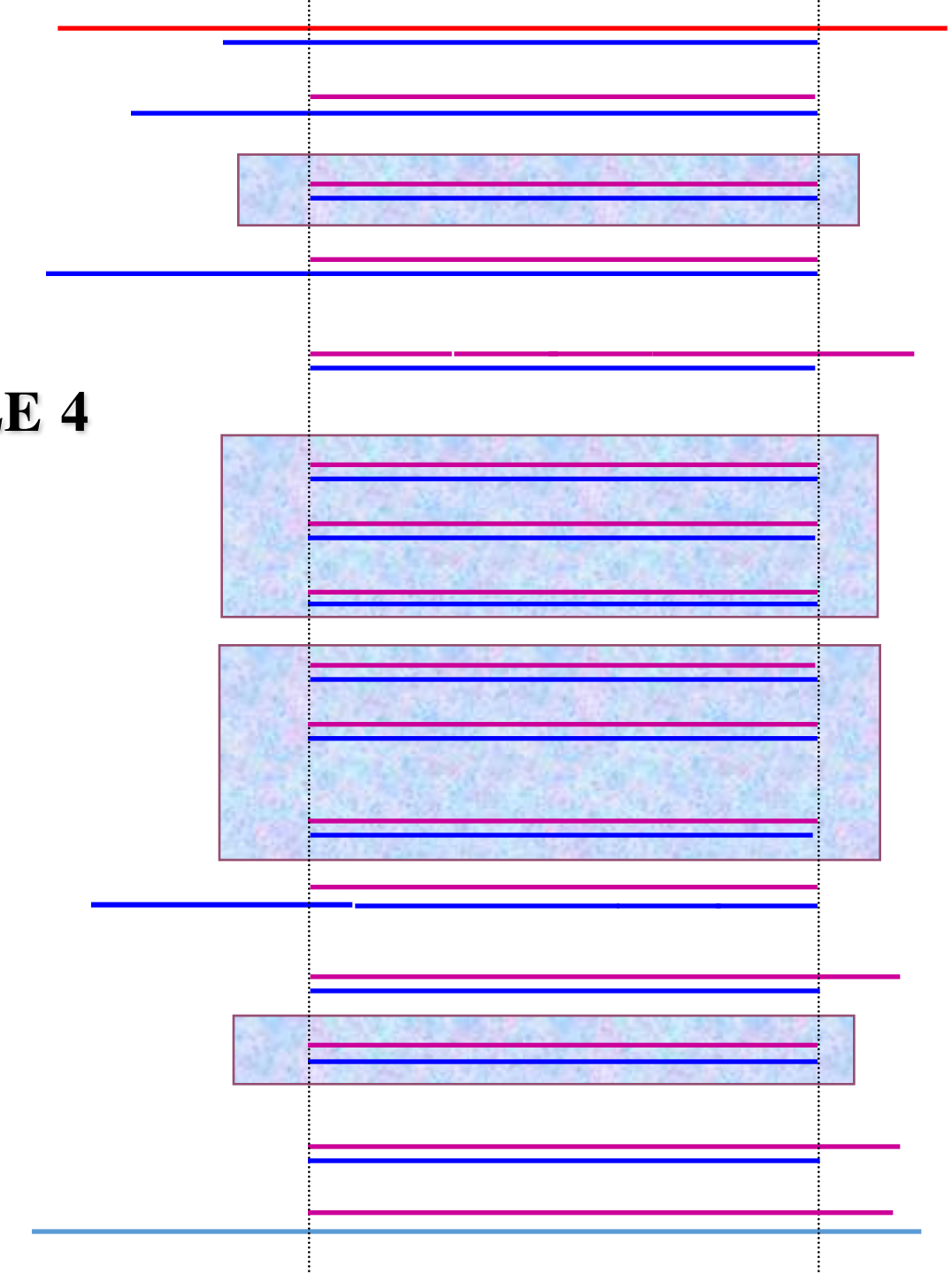
Binding of
Primers at ~55^{0}C

PCR CYCLE 4



Extension
72^0C

PCR CYCLE 4



1

2,3,4

5,6,7

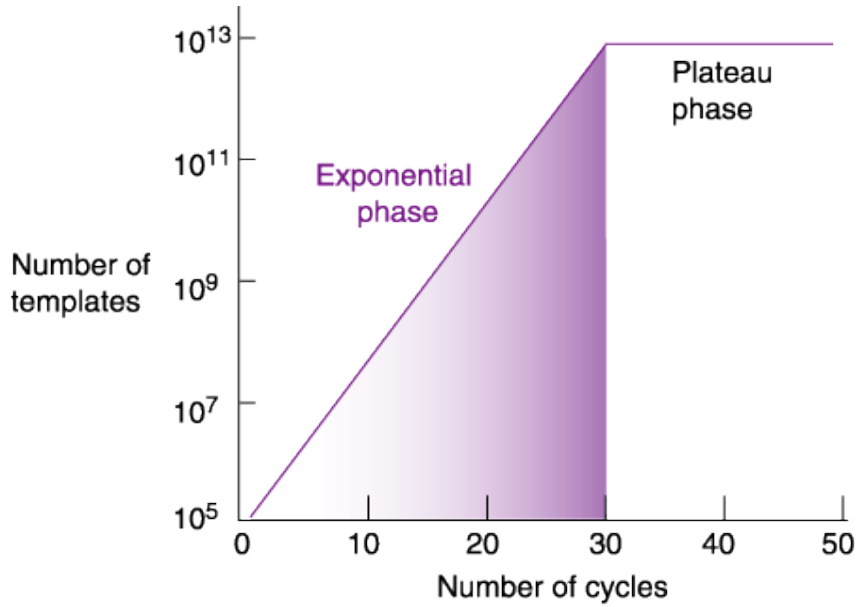
8

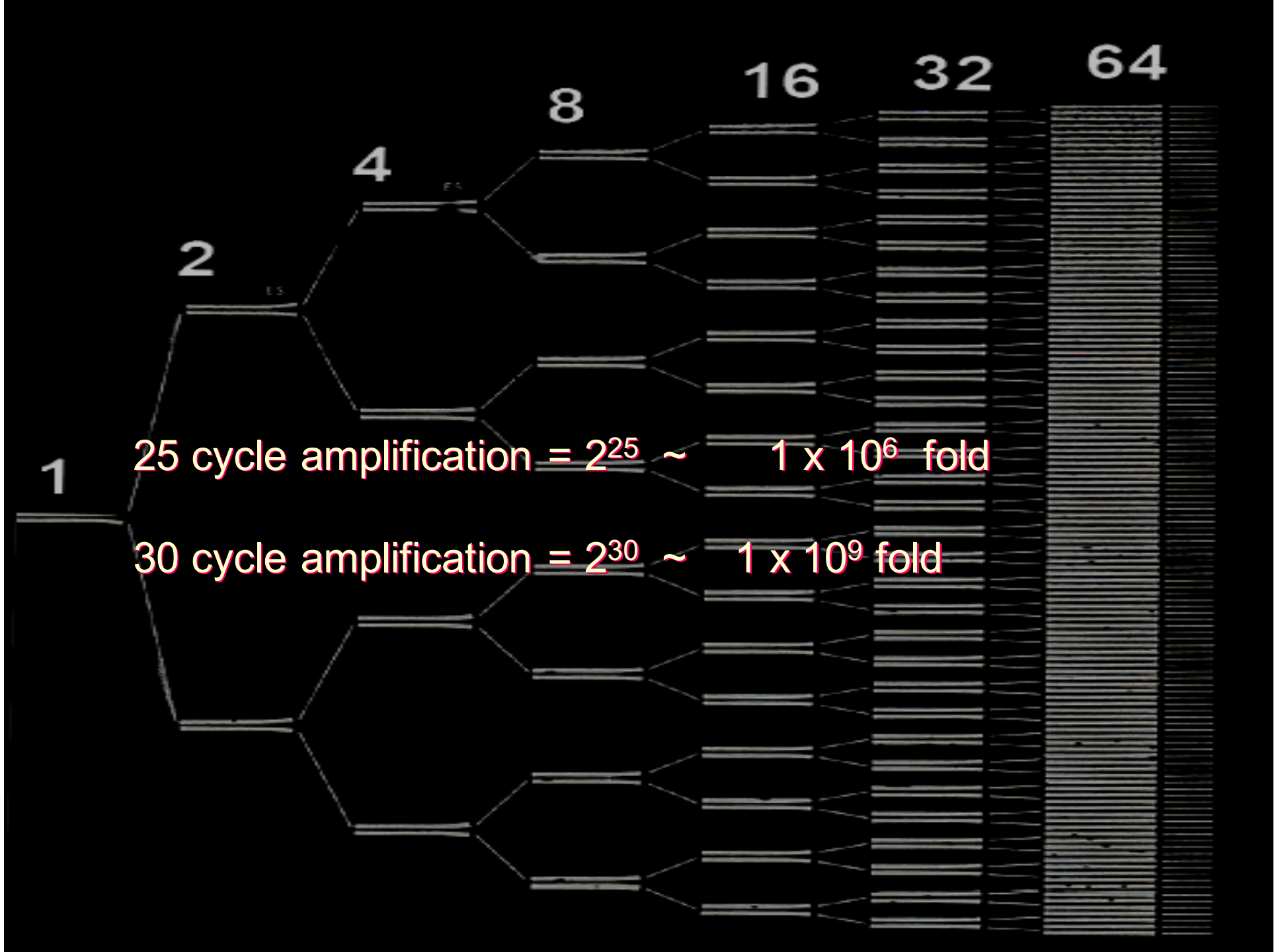
Lets presume that only 1 DNA target exists

- 4. cycle $1(1+1)^3=8$
- 5. cycle $1(1+1)^4=16$
- 6. cycle $1(1+1)^5=32$

$$No(1+Y)^{n-1}$$

- After a definite number of cycles PCR efficiency decreases.

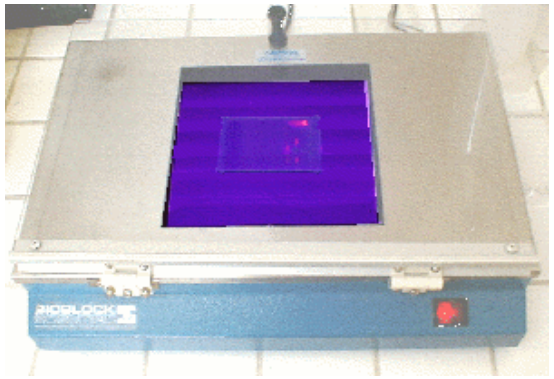



1
2
4
8
16
32
64
25 cycle amplification = 2^{25} ~ 1 x 10^6 fold
30 cycle amplification = 2^{30} ~ 1 x 10^9 fold

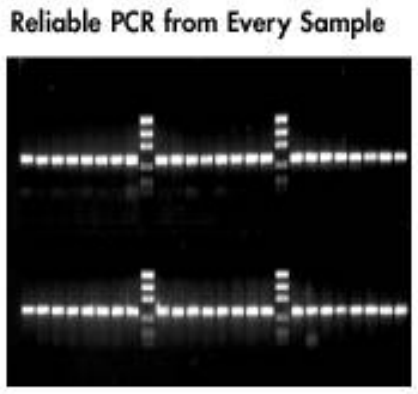
PCR

Agarose gel electrophoresis

3-4 hours



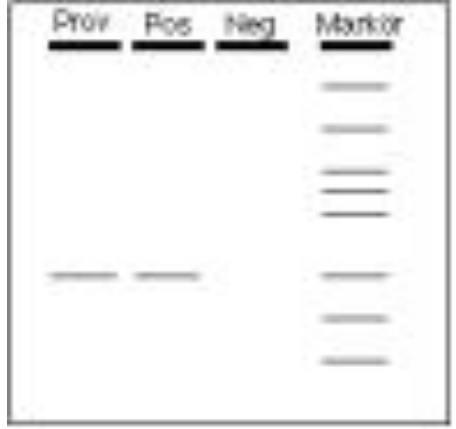
UV imaging



Final product

ALWAYS SHOULD BE

REMEMBERED!



PCR is a very sensitive technique– DNA contamination with an unwanted DNA could be significant!

Always add negative controls to the reaction!

Always add positive controls to the reaction!

Use appropriate filtered pipets and pippet tipds

Perform PCR in separate units

Use laminar flows with UV lambs

Polymerisation

- **Nucleofilic effect**
- **phosphodiester bonds cathelysis**

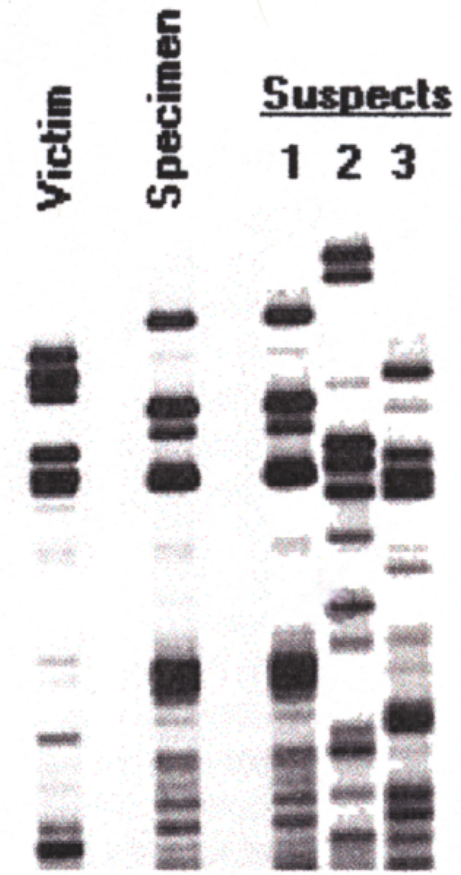
PCR is used for;

- Cloning of gene or gene fragments
- Genetic diagnosis – Detection mutations
- Maternity-Paternity Tests
- DNA sequence analysis
- Forensic identification
- Determination of quality control of industrial products
- Determination of appropriate tissue type for tissue transplantation
- Determination of polymorphism in between species
- Molecular typing
- Detection of pathogens

PCR for forensic identification:

Example 3: Multilocus Fingerprinting

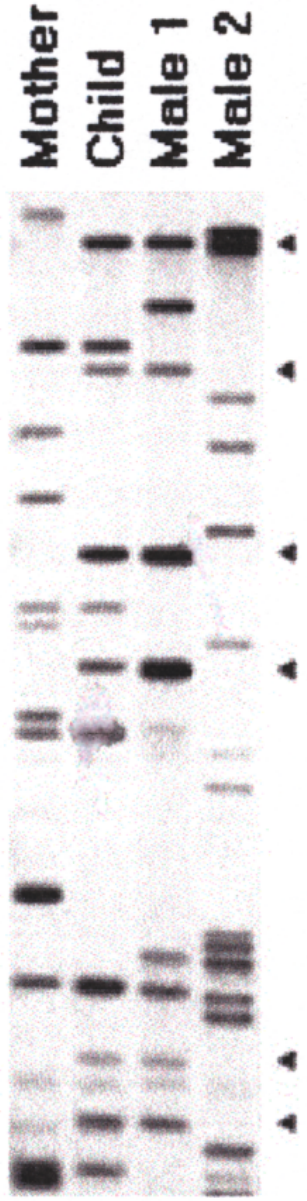
Multilocus fingerprinting to match trace evidence from a crime with suspects. Which suspect matches the specimen?



Maternity/Paternity Testing:

Example 2: Multilocus Fingerprinting

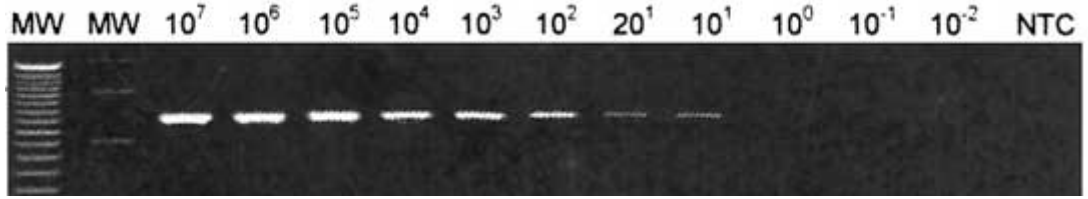
Microsatellite fingerprinting to establish parentage. The probe, $(CAG)_5$, recognizes a large number of loci. Examine the bands detected in DNA from the child that are not detected with DNA from the mother. Which male is the biologic father of the child?



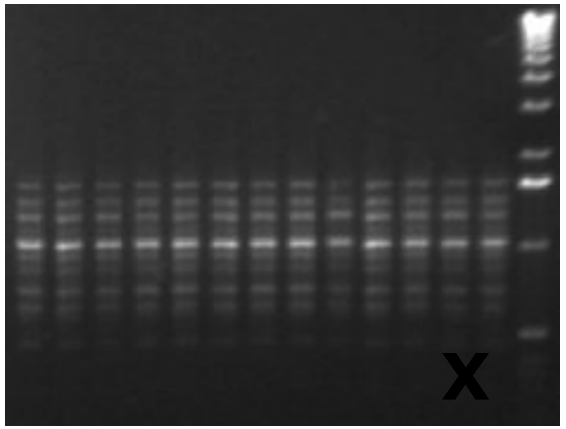
Advantages and Disadvantages of PCR!

- High sensitivity and specificity!
- Fast detection and identification!
- Could detect inanimate (dead) agents!
- Could detect acid-fast and environment fragile agents!
- Could detect slow growing bacteria
- Provide the probability of later sophisticated studies (typing, sequence analysis, clonning) olanak sağlaması

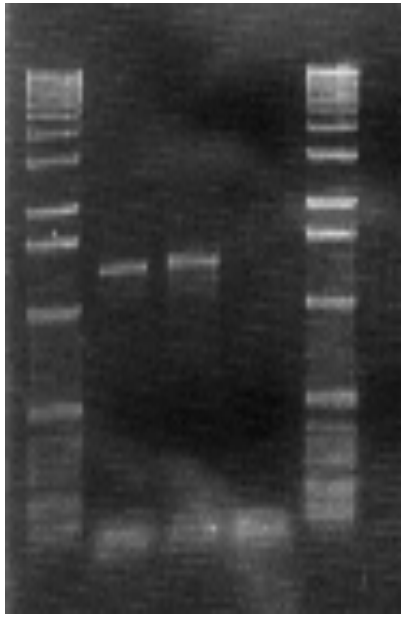
- Still cannot replace isolation in definitive diagnosis!
- False-positiveness due to cross-contamination!
- Requires lab infrastructure!
- Requires well trained personnel!
- High expendature costs!



template DNA concentration

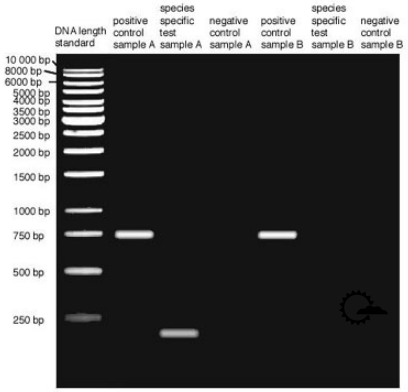


M 1 2 T M

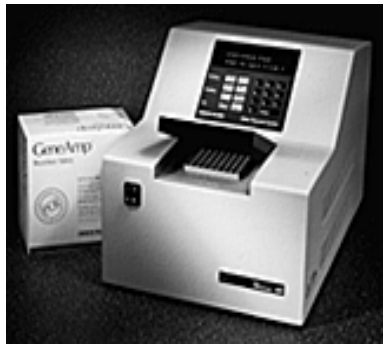


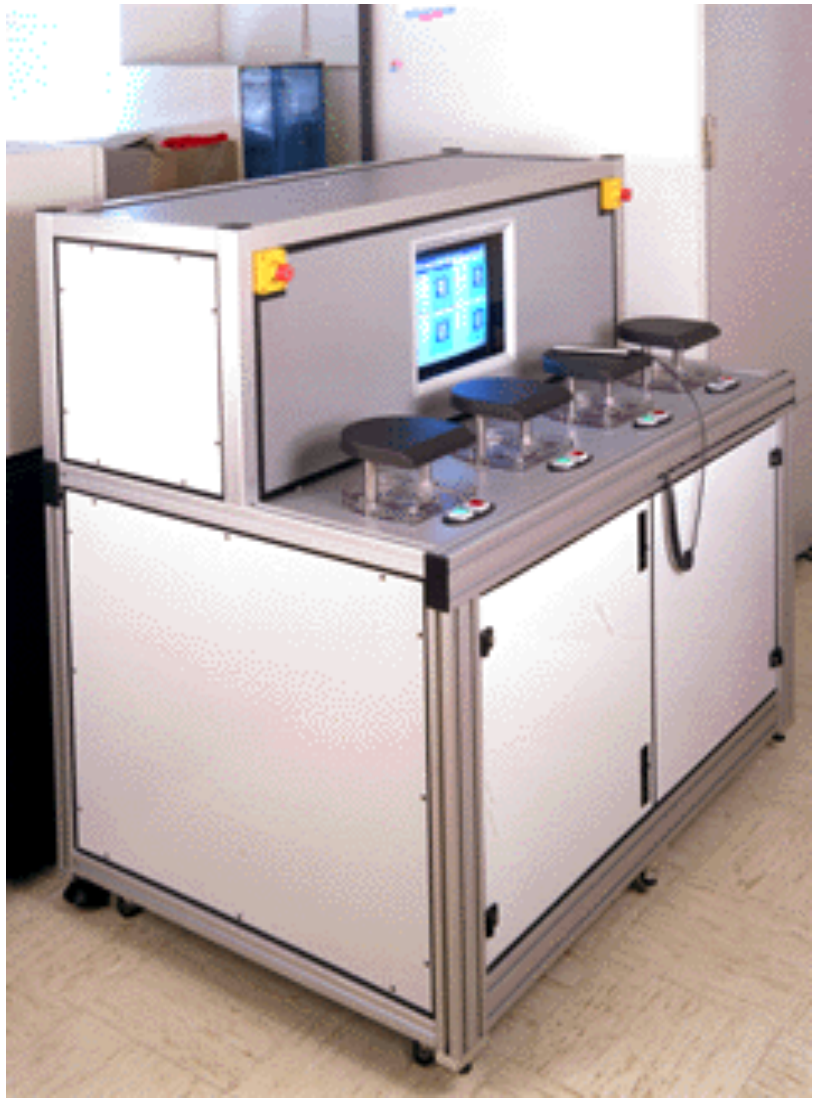
← PCR products

← Artefactual products



Thermal Cycler Models





Portative Molecular Biology Laboratories!!!