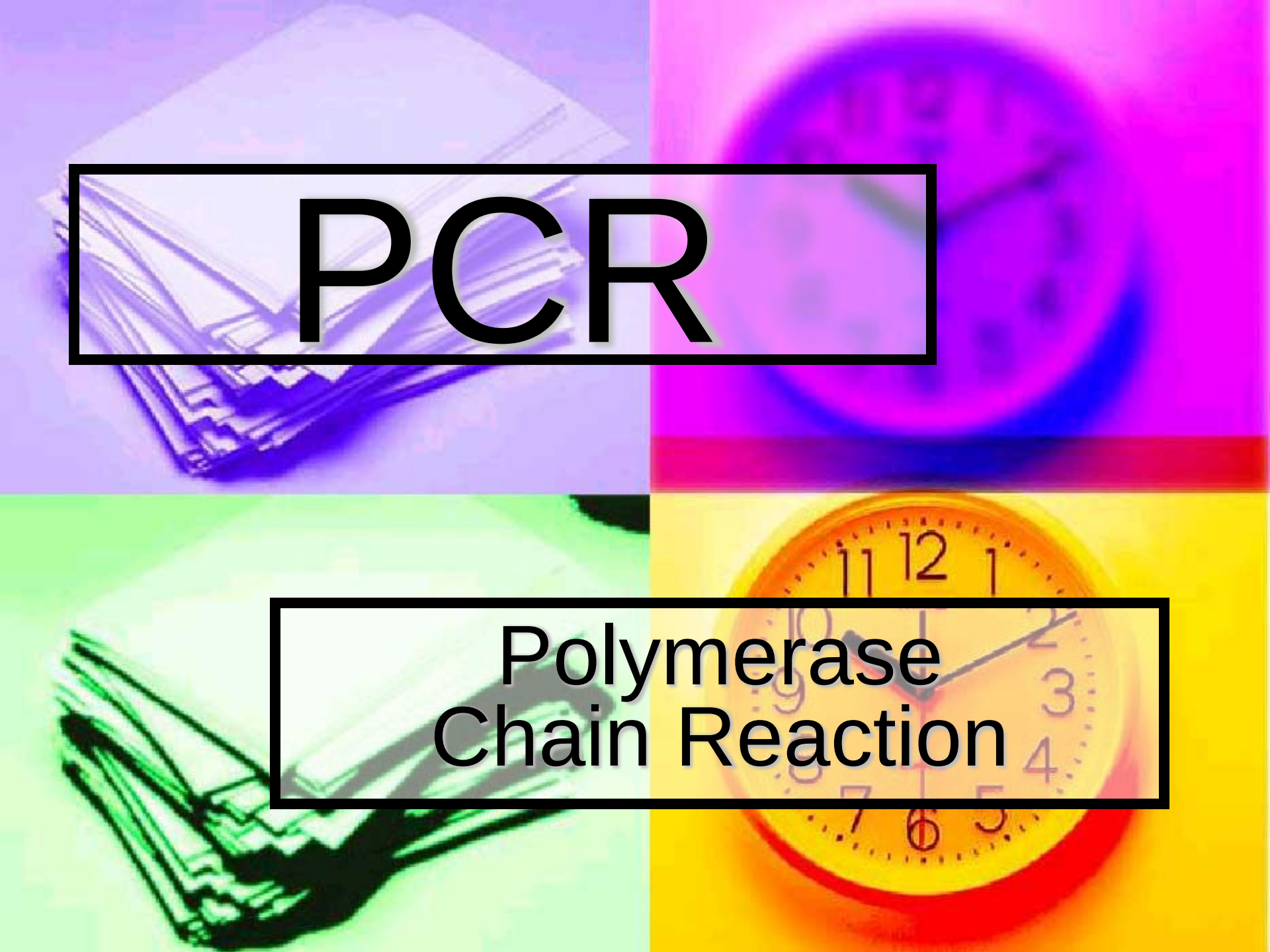

PCR
Polymerase
Chain Reaction

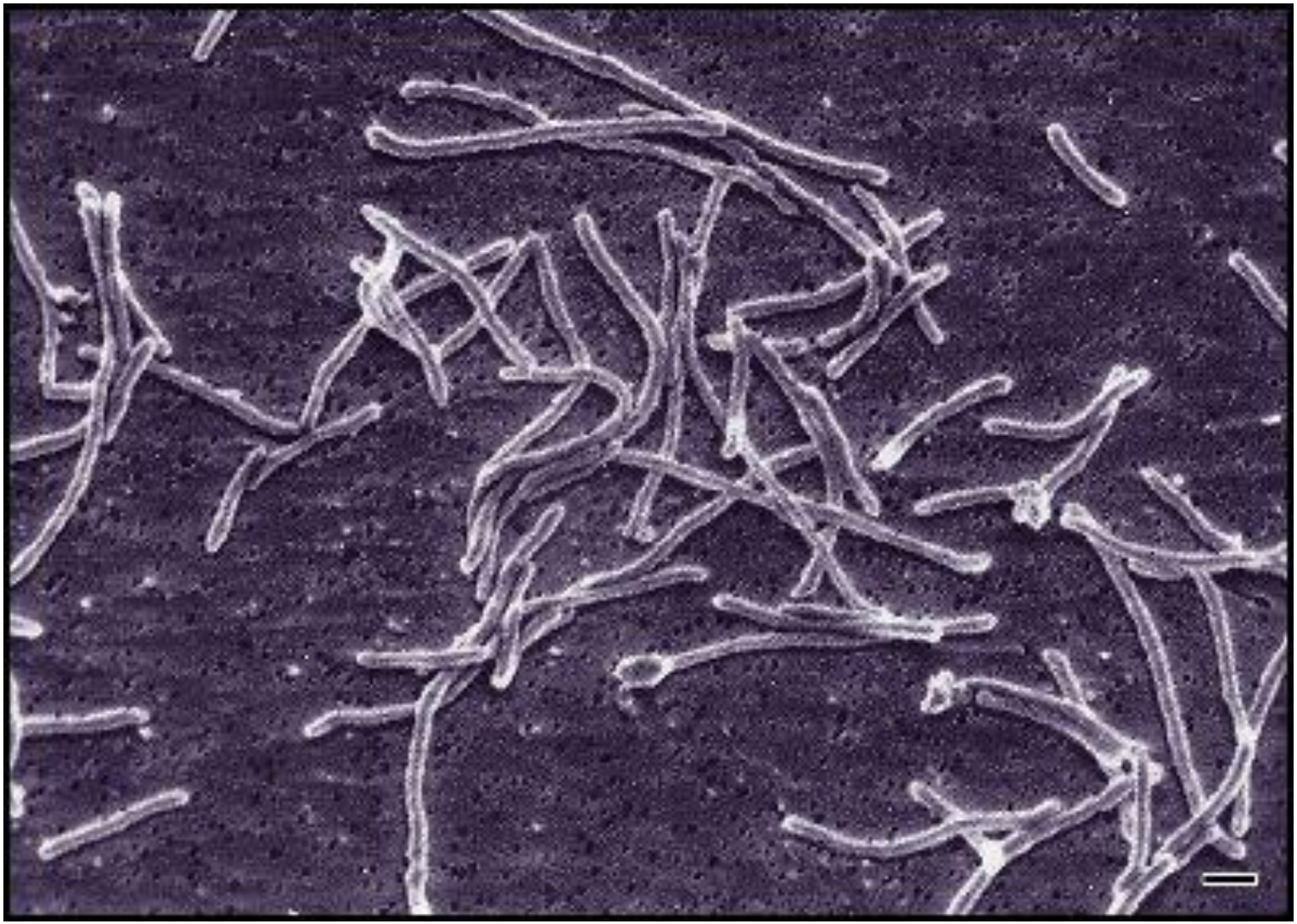
The Nobel Prize in Chemistry 1993

Kary B. Mullis

"for contributions to the developments of methods within DNA-based chemistry" "for his invention of the polymerase chain reaction (PCR) method”

Taq polymerase

Thermus aquaticus

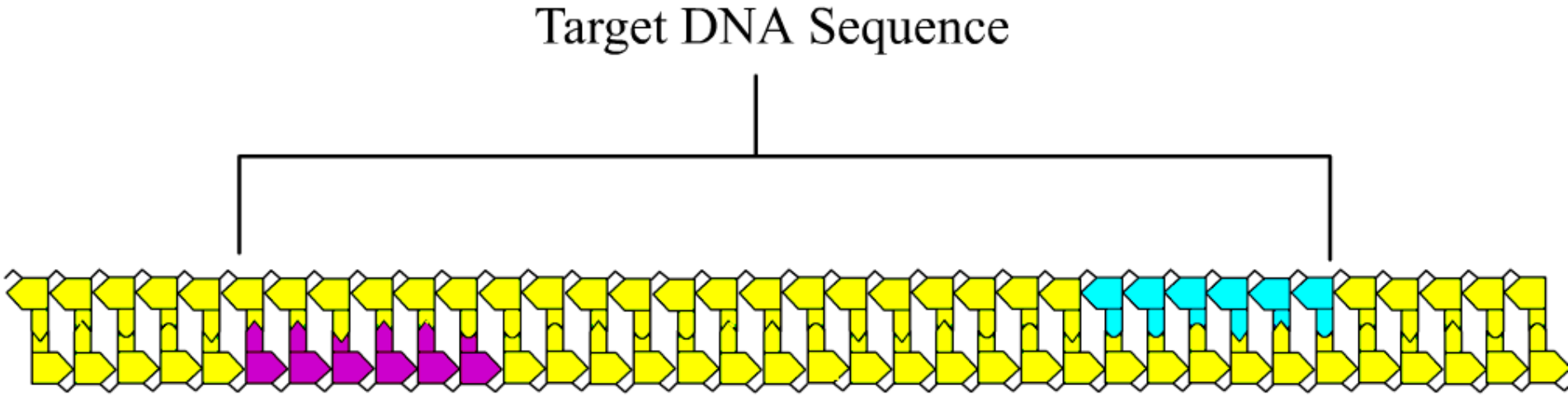


Thermal Cycler

Materials of PCR

- target DNA
- Taq DNA polymerase
- 2 Primers
  - ~20 nucleotides in length
  - Forward and reverse
- the four DNTP'S
  - Adenine
  - Thymine
  - Cytosine
  - Guanine
- cofactor $MgCl_2$.

Locate the Target Sequence

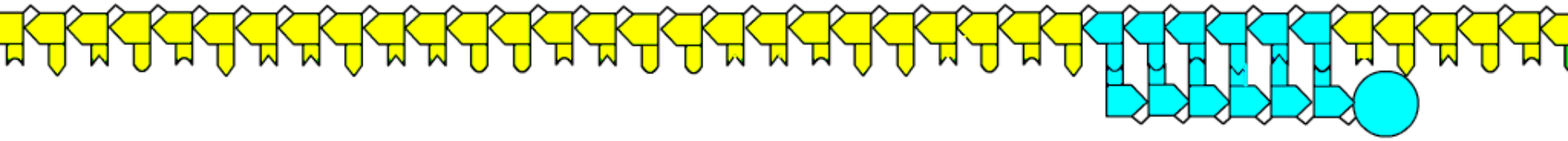


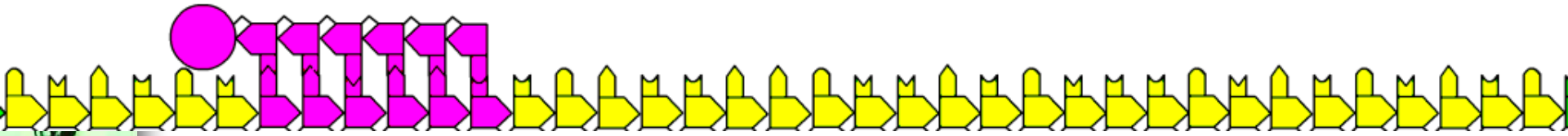
- Scientists determine which GENE they are interested in studying
- Locate Primers Upstream and Down stream of gene

Step 1 Denaturing

60 seconds @ 94°C

Step 2 Annealing

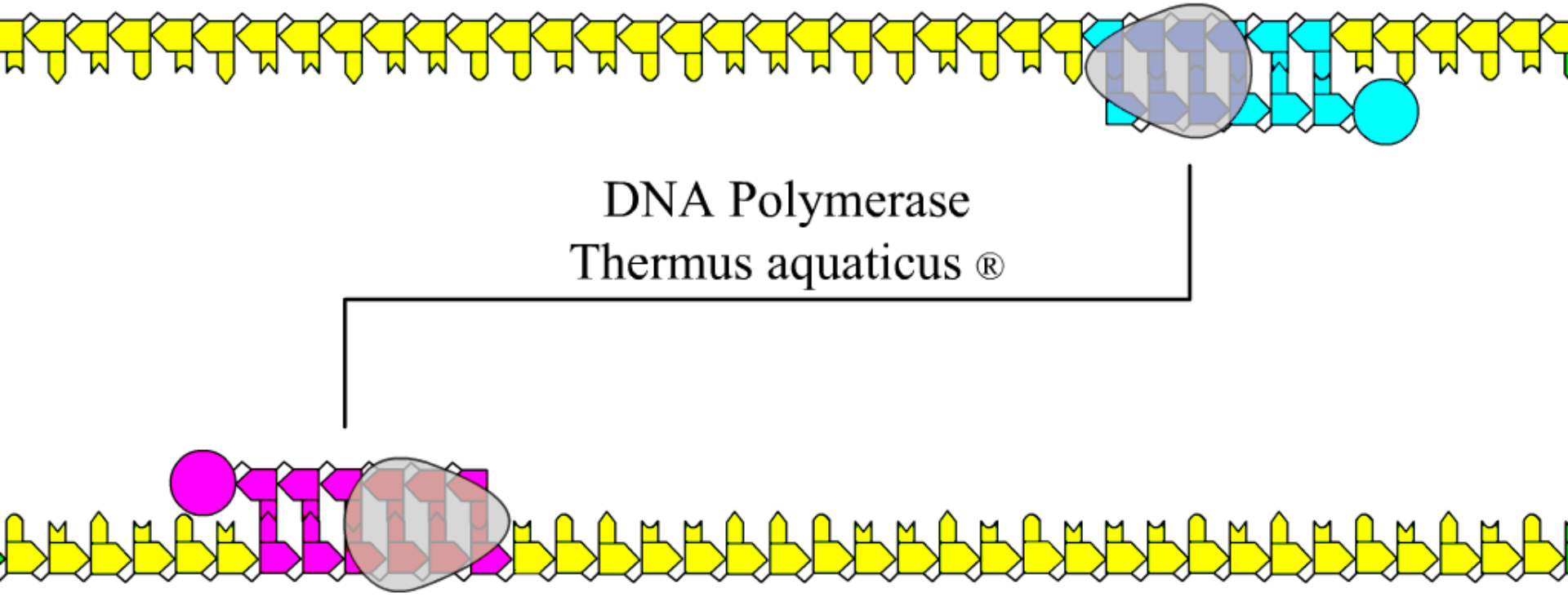




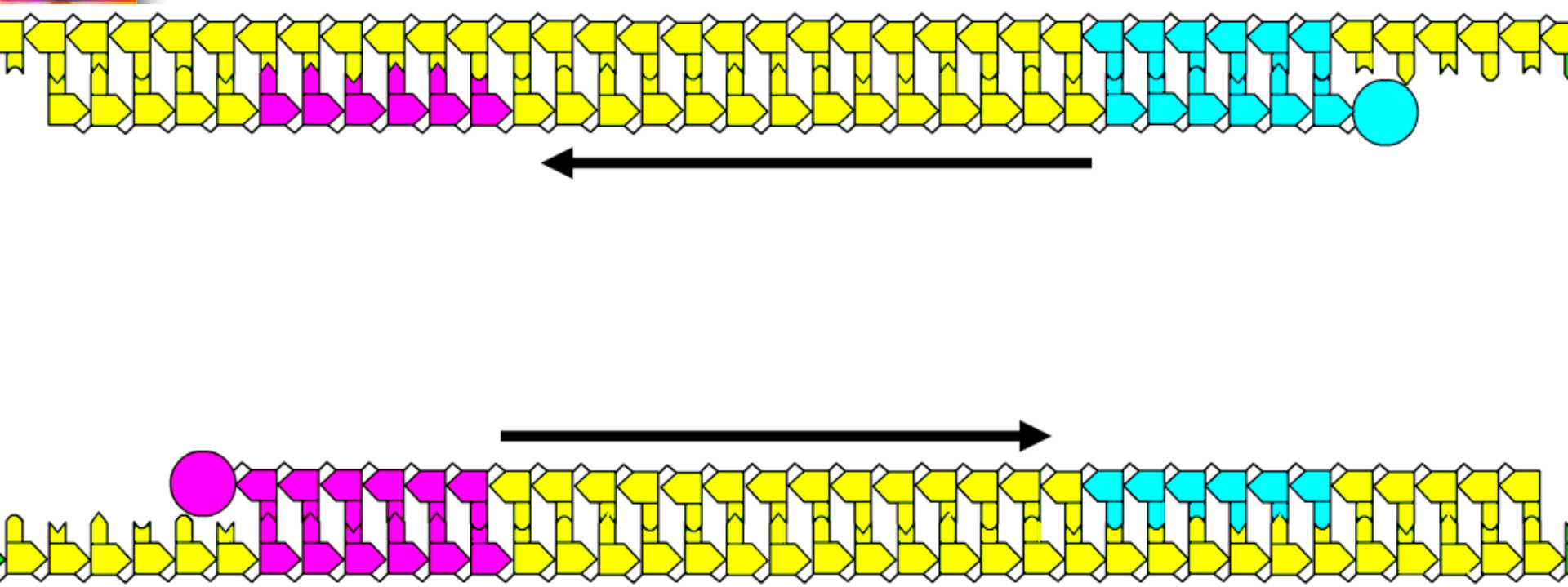
60 seconds @ 60°C

Forward and Reverse Primers

Taq Polymerase Binds



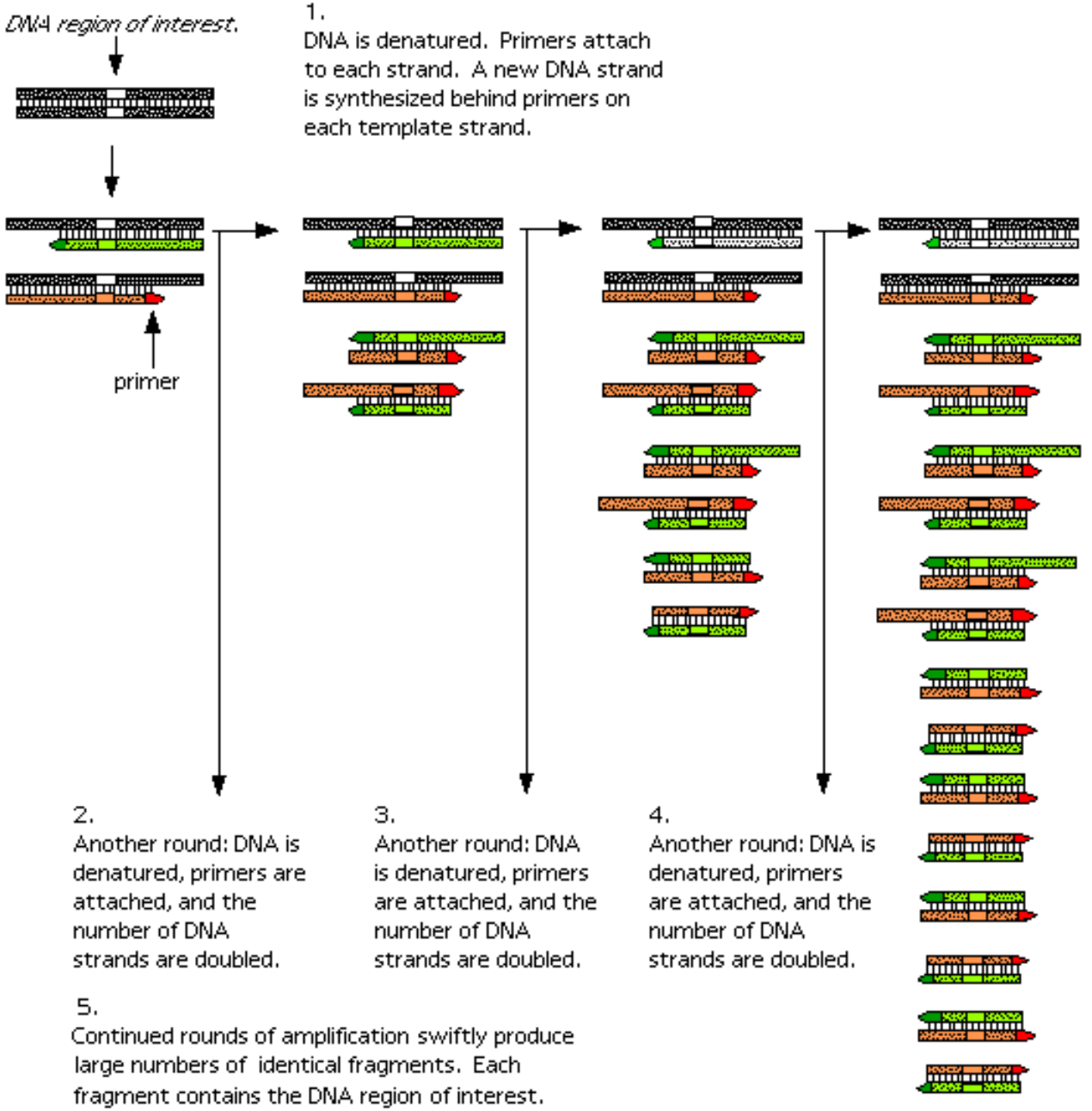
Step 3 Extension



2 minute at 72°C

DNTP’s

POLYMERASE CHAIN REACTION



1.
DNA is denatured. Primers attach to each strand. A new DNA strand is synthesized behind primers on each template strand.

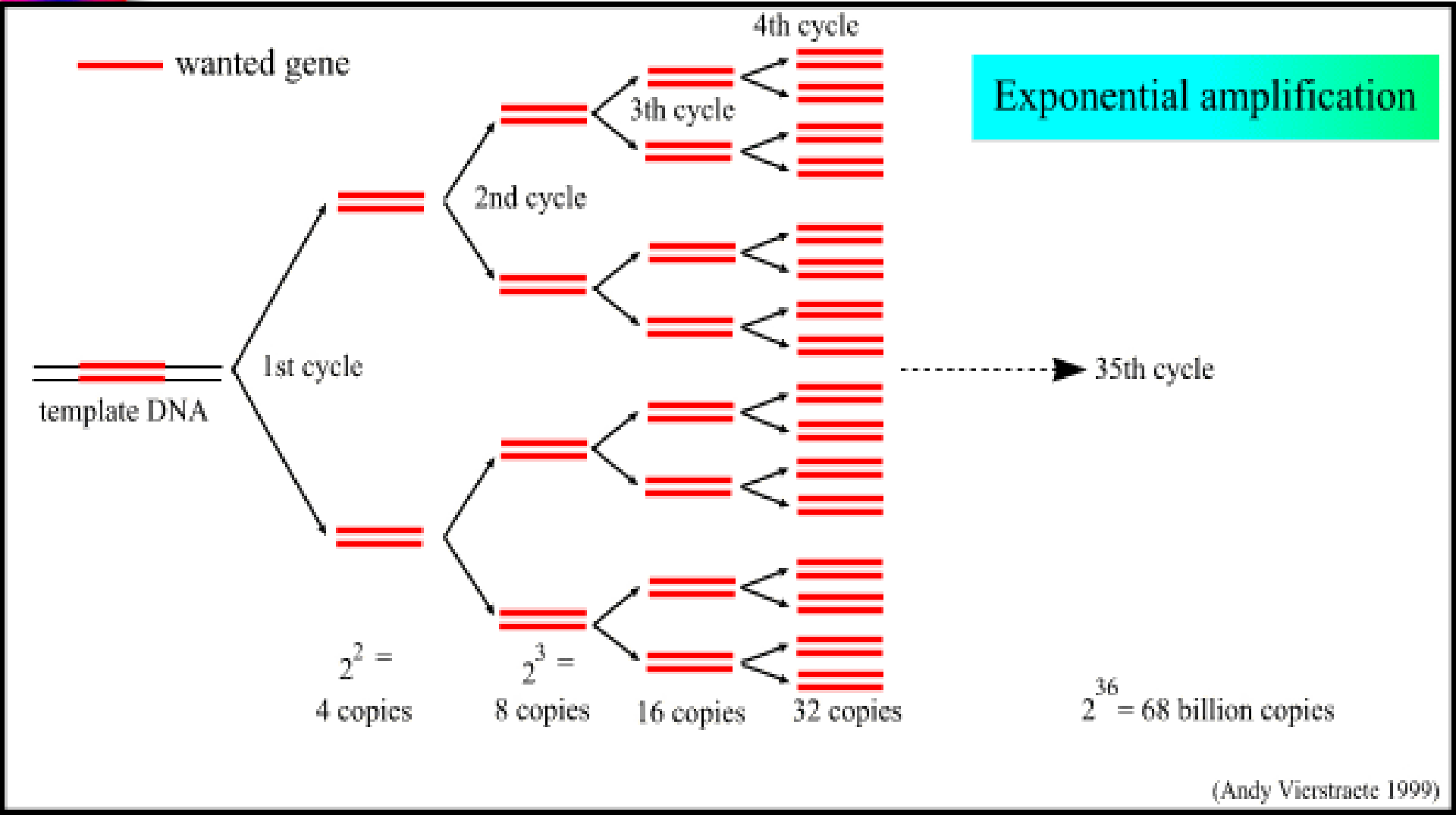
2.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

3.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

4.
Another round: DNA is denatured, primers are attached, and the number of DNA strands are doubled.

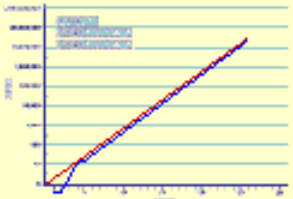
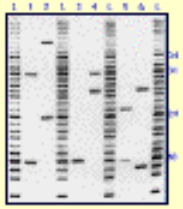
5.
Continued rounds of amplification swiftly produce large numbers of identical fragments. Each fragment contains the DNA region of interest.

The exponential amplification of the gene in PCR.

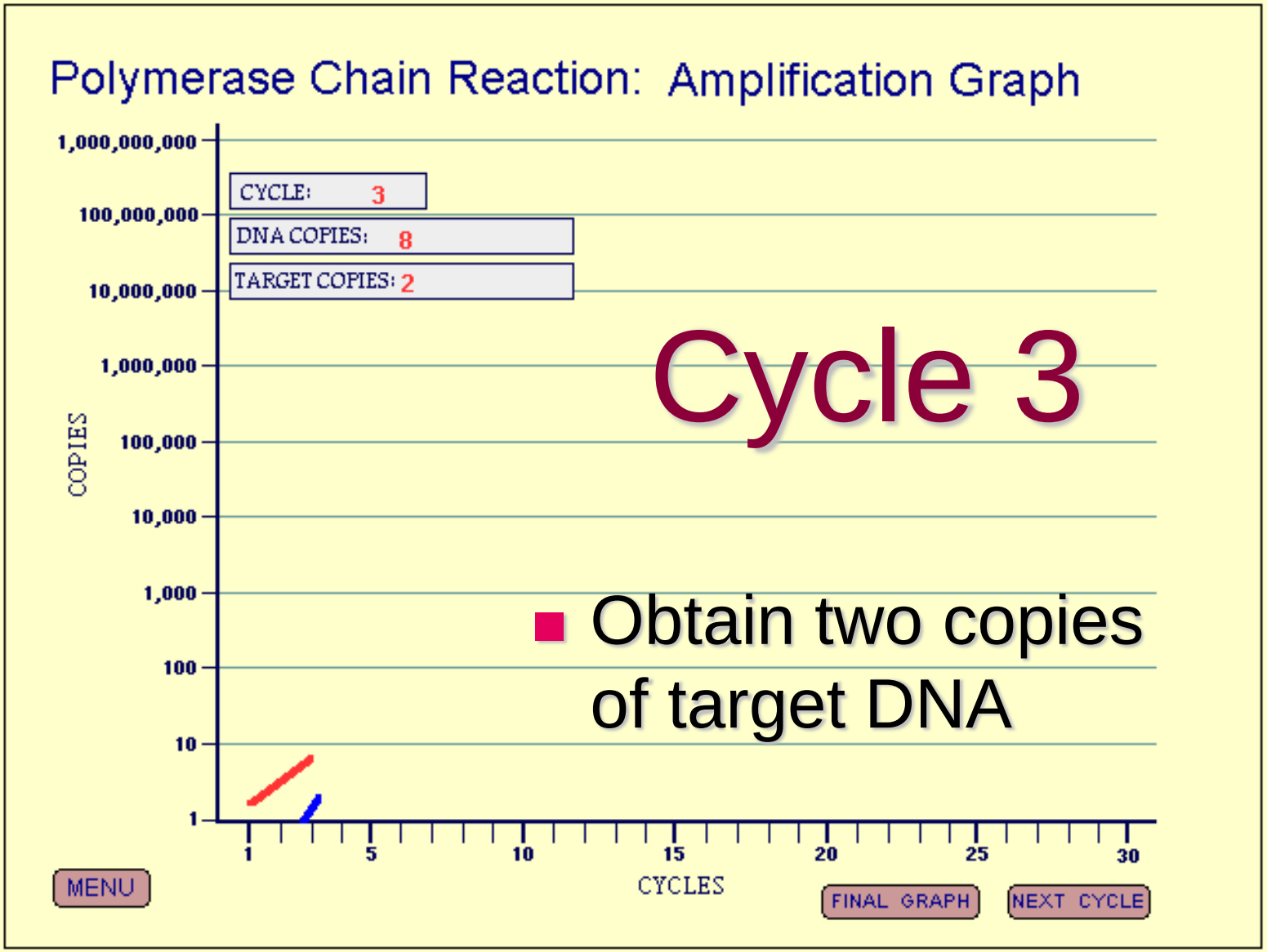


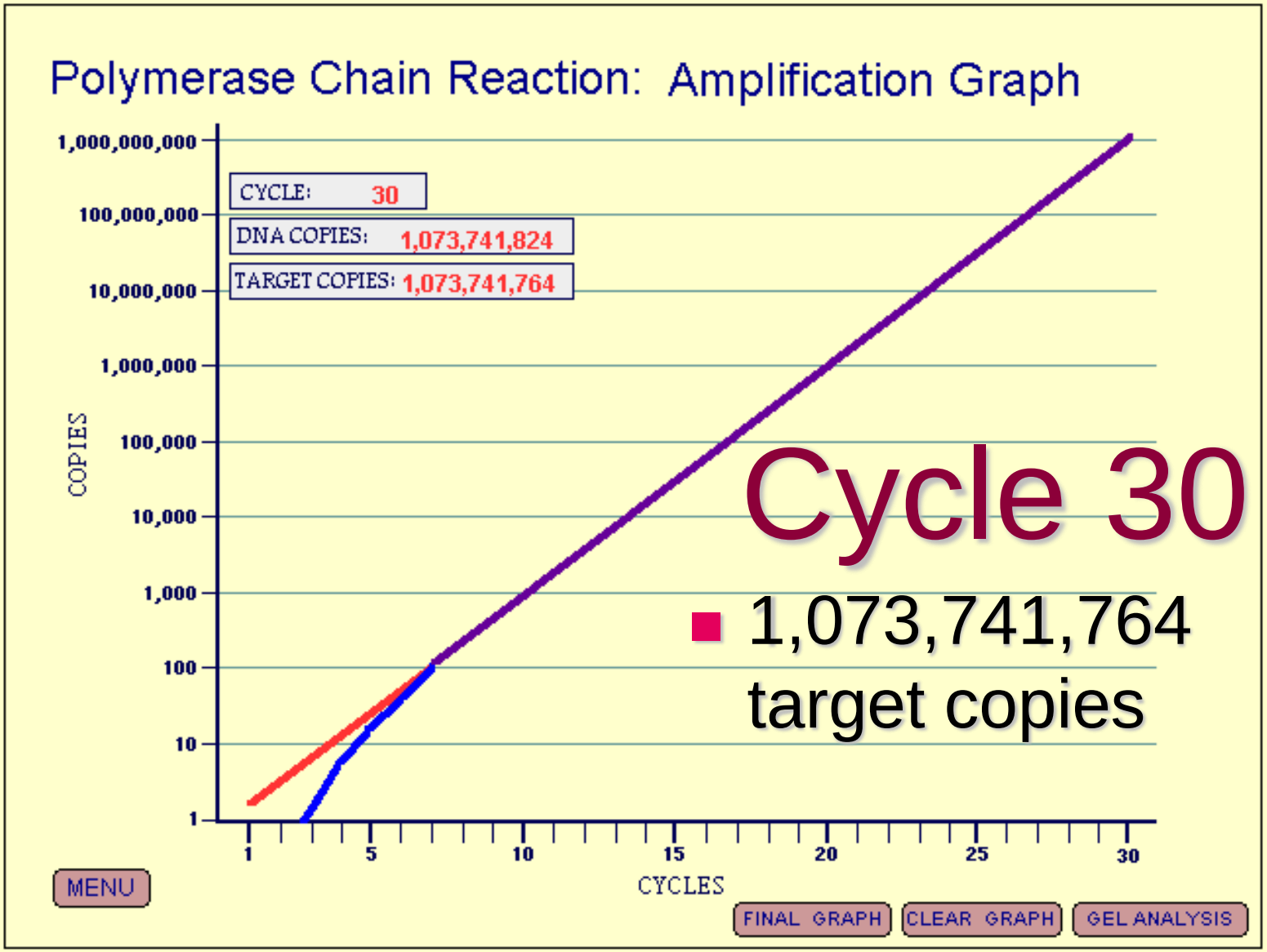
Polymerase Chain Reaction: Menu

QUIT

- Introduction
- Amplification 
- Amplification Graph 
- Gel Analysis 
- Human DNA Polymorphisms

Copyright 1994, DNA Learning Center, Cold Spring Harbor Laboratory

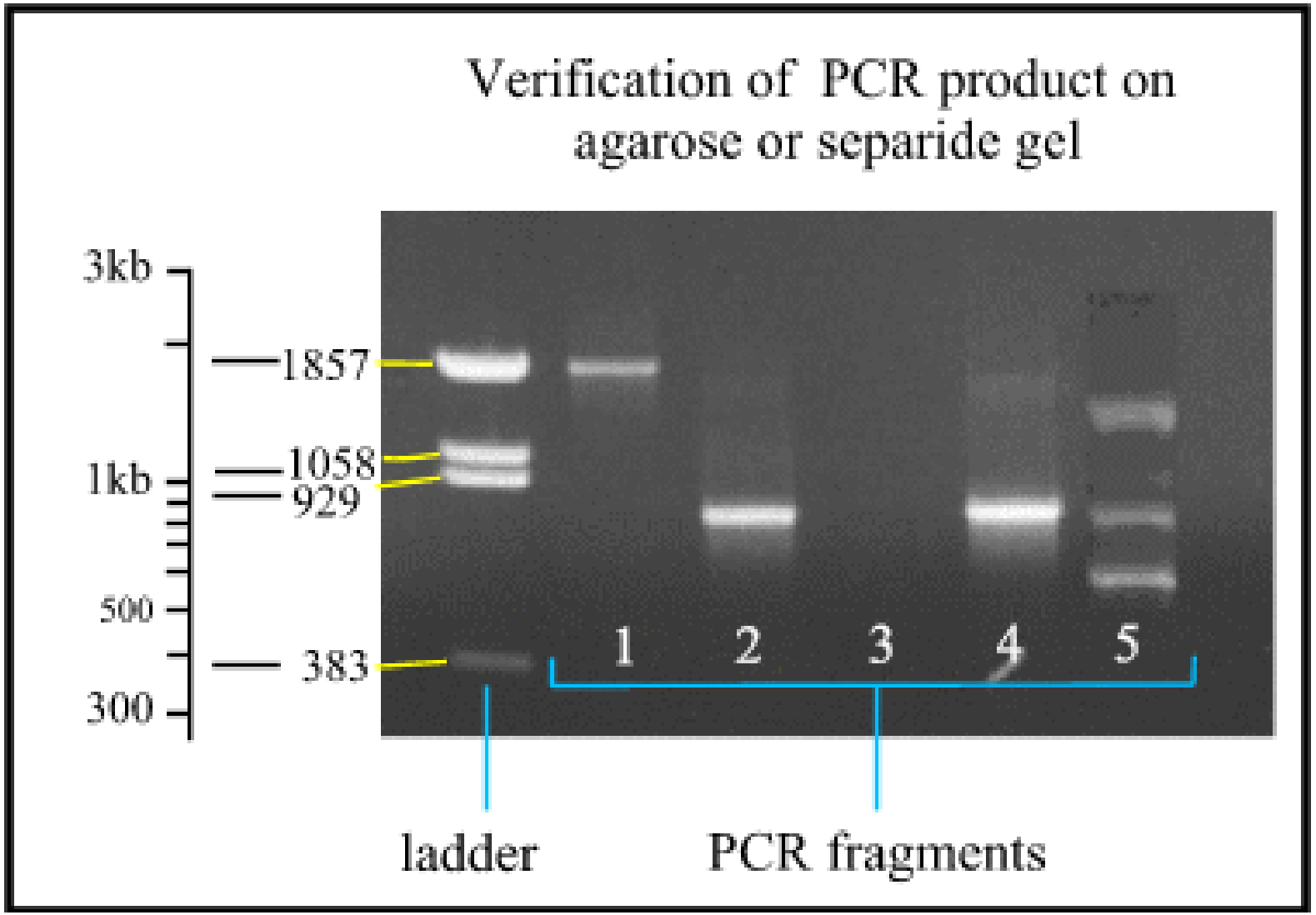

Polymerase Chain Reaction: Amplification Graph
1,000,000,000
100,000,000
10,000,000
1,000,000
100,000
10,000
1,000
100
10
1
COPIES
CYCLE: 3
DNA COPIES: 8
TARGET COPIES: 2
Cycle 3
Obtain two copies of target DNA
1
5
10
15
20
25
30
CYCLES
MENU
FINAL GRAPH
NEXT CYCLE



Cycle 30

- 1,073,741,764 target copies

Is there a gene copied during PCR and is it the right size ?



Applications of PCR

- quick, reliable method for detecting all manner of mutations associated with genetic disease - from insertions, to deletions, to point mutations.
  - Duchene muscular dystrophy
- Detect unwanted Genetic material
  - Bacterial or viral infection
    - HIV infection
- Amplify degraded DNA samples
  - Egyptian mummy
  - Termite in amber