Polymerase Chain Reaction (PCR)

1- Principle of the PCR

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Introduction

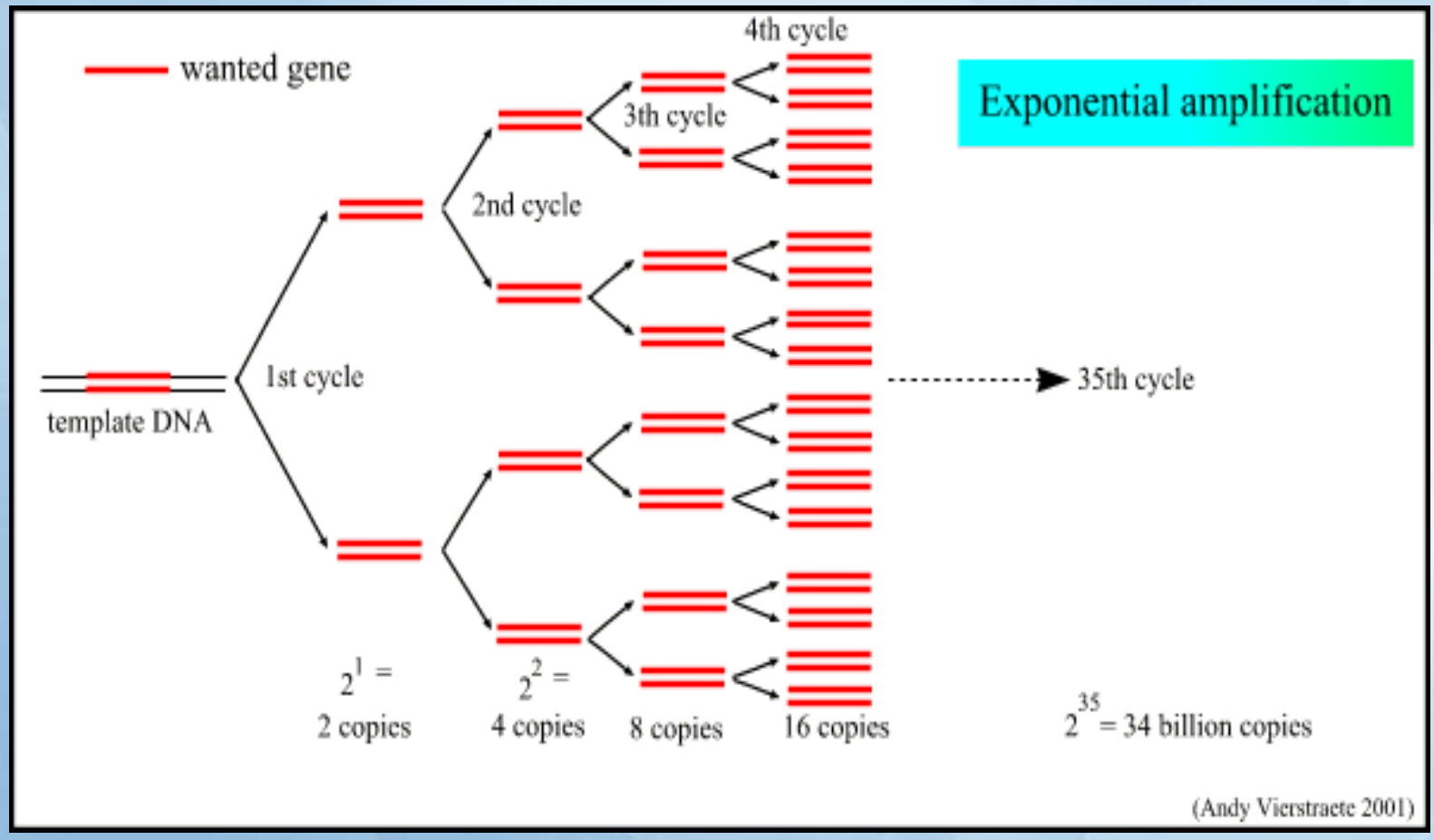
- The technique was invented by Dr. Kary Mullis, 1983,
- for which he received the Nobel Prize in Chemistry in 1993.
- *in vitro* method (test tube system) for DNA replication.

Introduction

- PCR will allow a short stretch (specific sequence) of DNA (usually fewer than 3000 base pairs) to be amplified to more than million fold.
- Millions of copies of a segment of DNA can be made within a few hours.

Introduction

- **DNA amplification by PCR (overview)**



What do we need for PCR?

- Target DNA (Template).
- Two primers: (forward and reverse)
- Nucleotides: (the 4 dNTP'S: A, T, C, G)
- Heat-stable DNA polymerase:
  (like Taq DNA Polymerase)
- Buffer and Cofactor $MgCl_2$ (Mg^{++}, K^{+}).
- Thermal cycler.

PCR Procedure

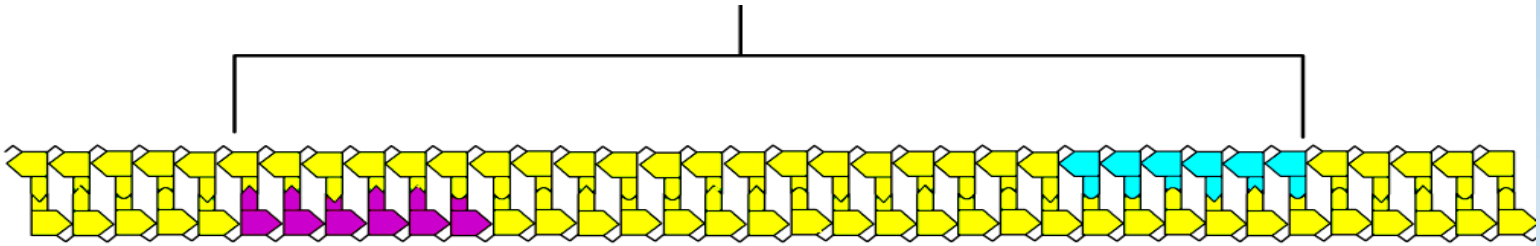
- All the required components are inserted into an Eppendorf tube and placed in a thermal cycler that varies the temperature.
- Each cycle of PCR contains 3 steps which are usually repeated for 30 – 40 cycles.

PCR cycle (round)

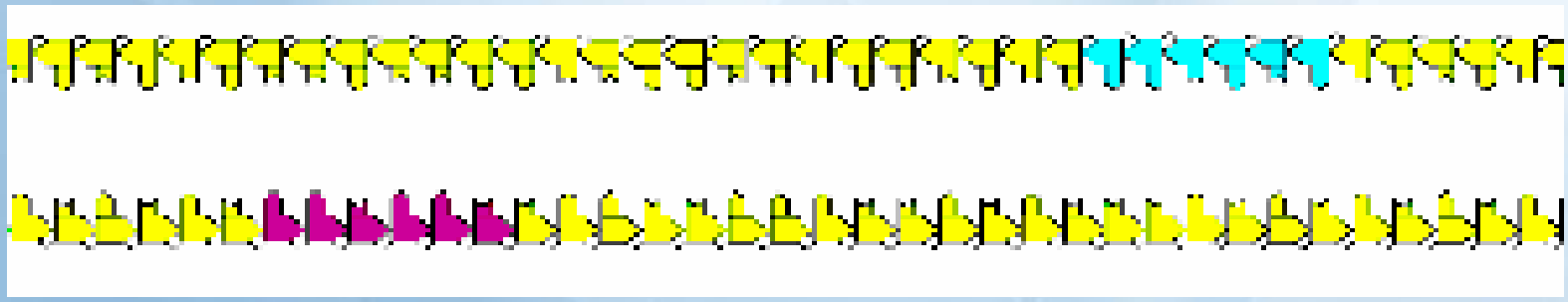
1. Denaturation at 93-95°C (~1 min):

- The two strands separate due to breakage of the hydrogen bonds holding them together.

Target DNA Sequence



Denaturation (95°C)
~ 1 min.

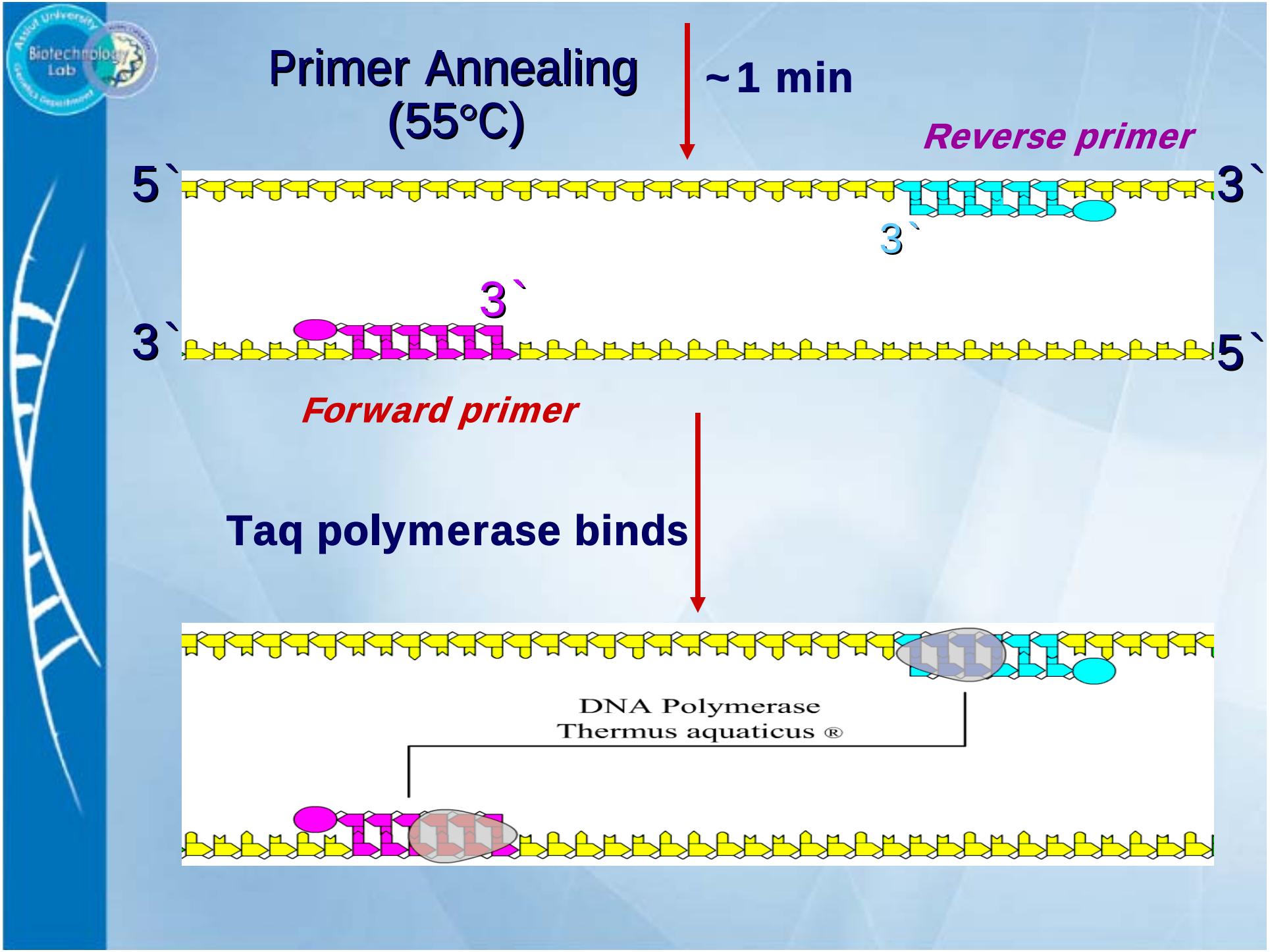


2. Primer annealing at 55°C (1 min):

- The two primers bind to their complementary sequences on the single strands of DNA.
- The PCR reaction will amplify the DNA section between the forward and reverse primers.

→ Forward primer anneals to lower strand

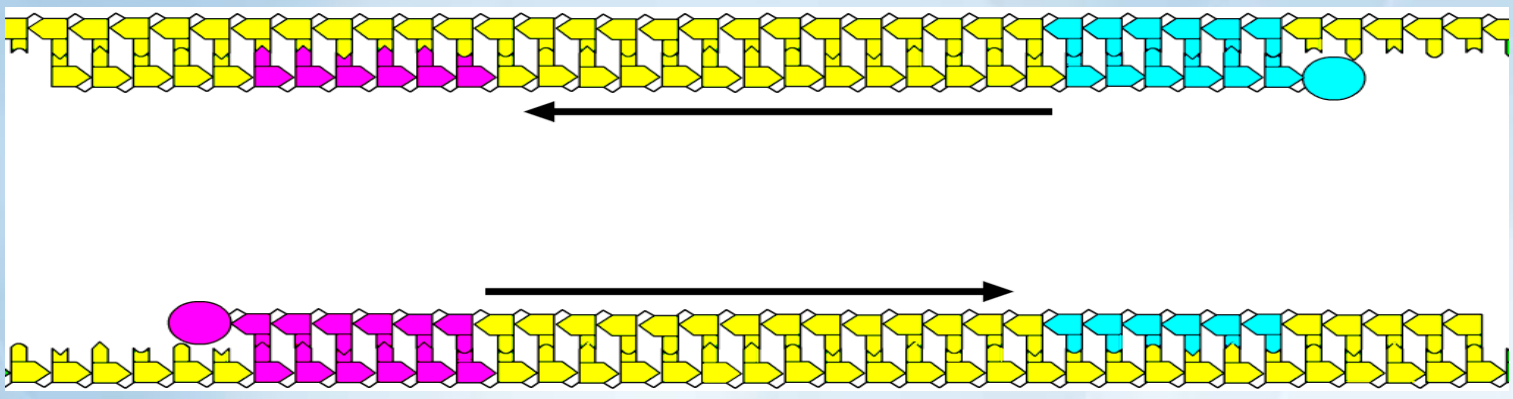
← Reverse primer anneals to upper strand


Primer Annealing
(55°C)
~1 min
Reverse primer
5`
3`
3`
3`
3`
5`
Forward primer
Taq polymerase binds
DNA Polymerase
Thermus aquaticus ®

3. Primer Extension at 72°C (1kb/min):

- Taq DNA Polymerase extends the DNA chain by adding nucleotides to the 3' ends of the primers.
- DNA polymerase catalyzes the extension of the strand in the 5'→3' direction, starting at the primers, attaching the appropriate nucleotide (A-T, C-G).

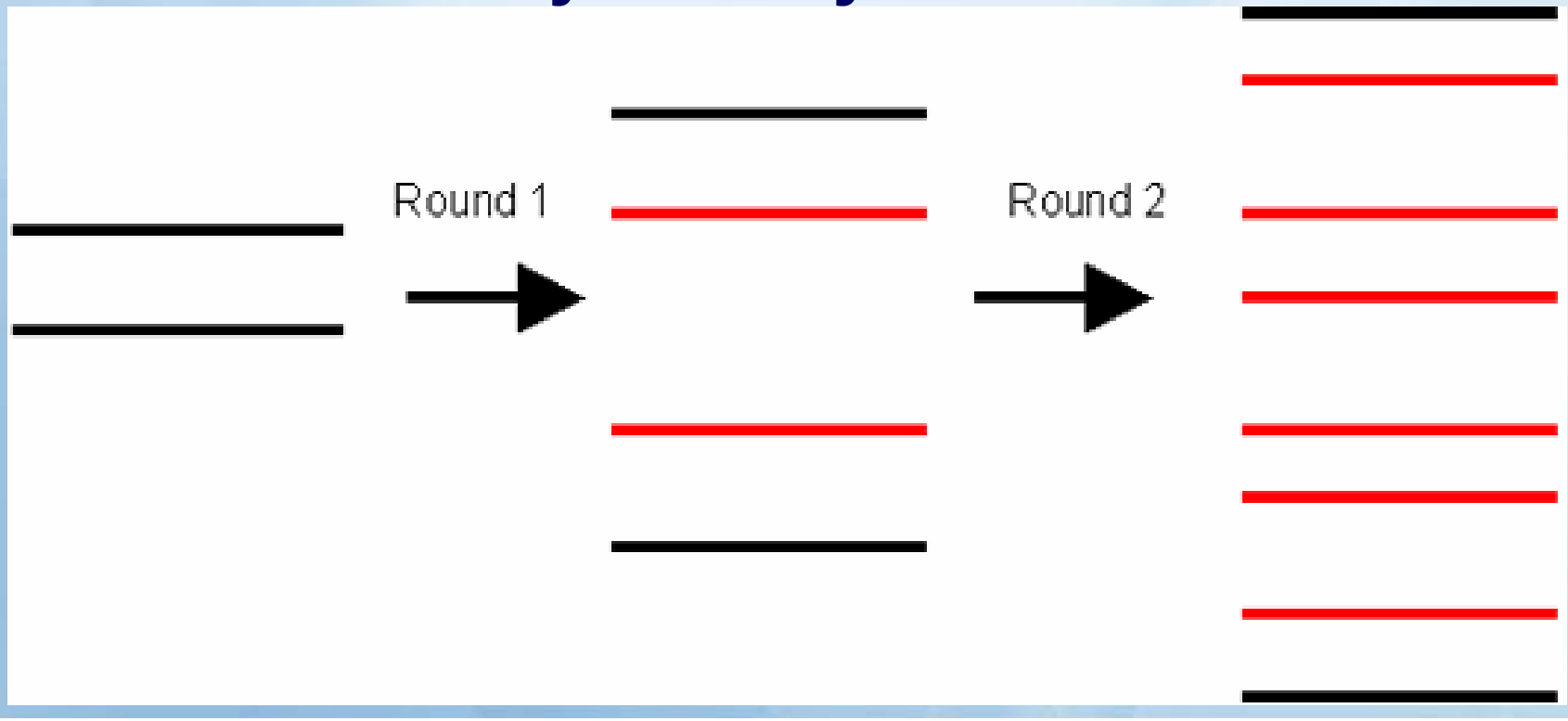
Primer Extension At 72°C (1 min/kilobase)

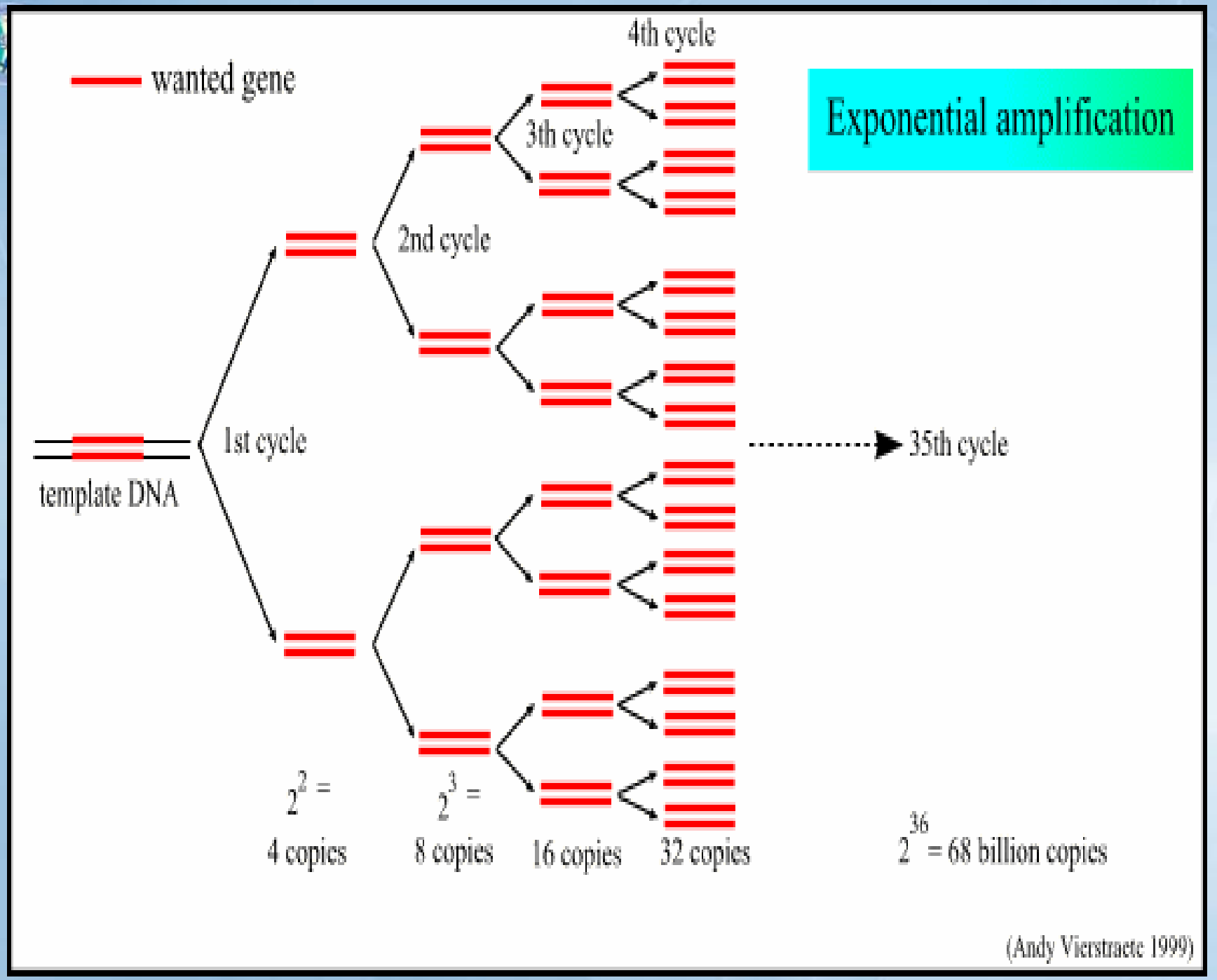


- **The first round of synthesis occur on each of the DNA templates.**

- **The mixture is heated again at 90-95°C to denature the molecules and separate the strands and the cycle is repeated.**

- **Each new strand then acts as a template for the next cycle of synthesis.**




wanted gene
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
35th cycle
Exponential amplification
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies
2^{36} = 68 billion copies
(Andy Vierstraete 1999)

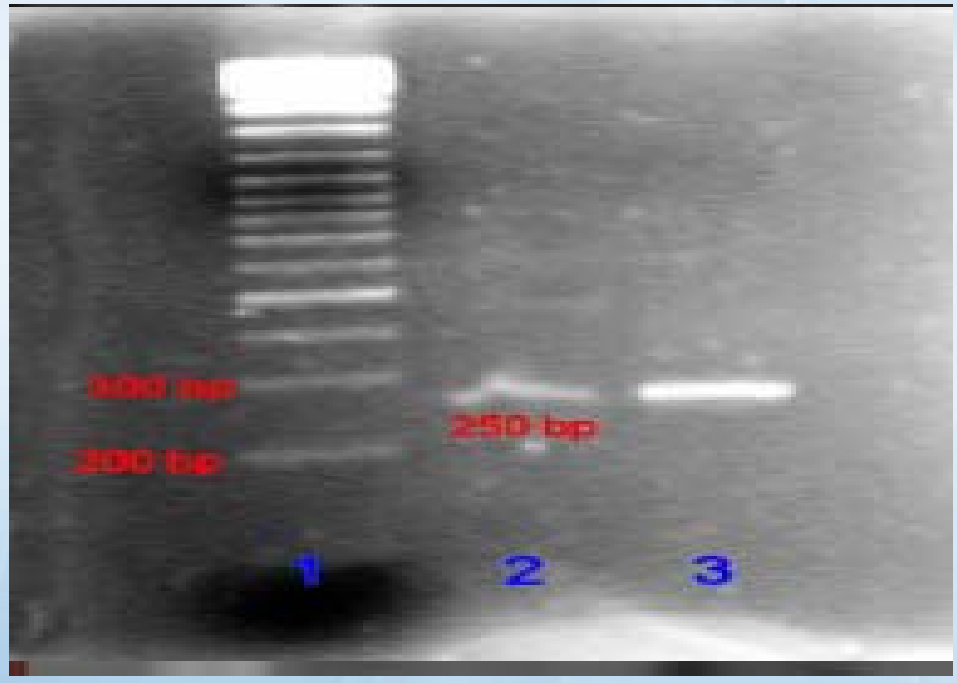
- **A thermal cycler automatically changes the temperature at the correct time for each of the stages.**

- **The DNA of interest is amplified by a power of 2 for each PCR cycle:**
- **5 cycles = 2^5 (or 64) DNA copies.**
- **35 cycles = 2^{35} (or 68 billion) DNA copies.**

- **PCR animation and Video**

- **The amplified product can be detected using gel electrophoresis to view the band containing DNA fragments.**

- **Animation.**



Primer features:

- short (10-24 bases), single stranded DNA molecules.

*as the primer increases in size, the chances of matching the target size increase.

- forward and reverse primers, should have similar Tm (melting temperature).

- Primers are sequence specific.

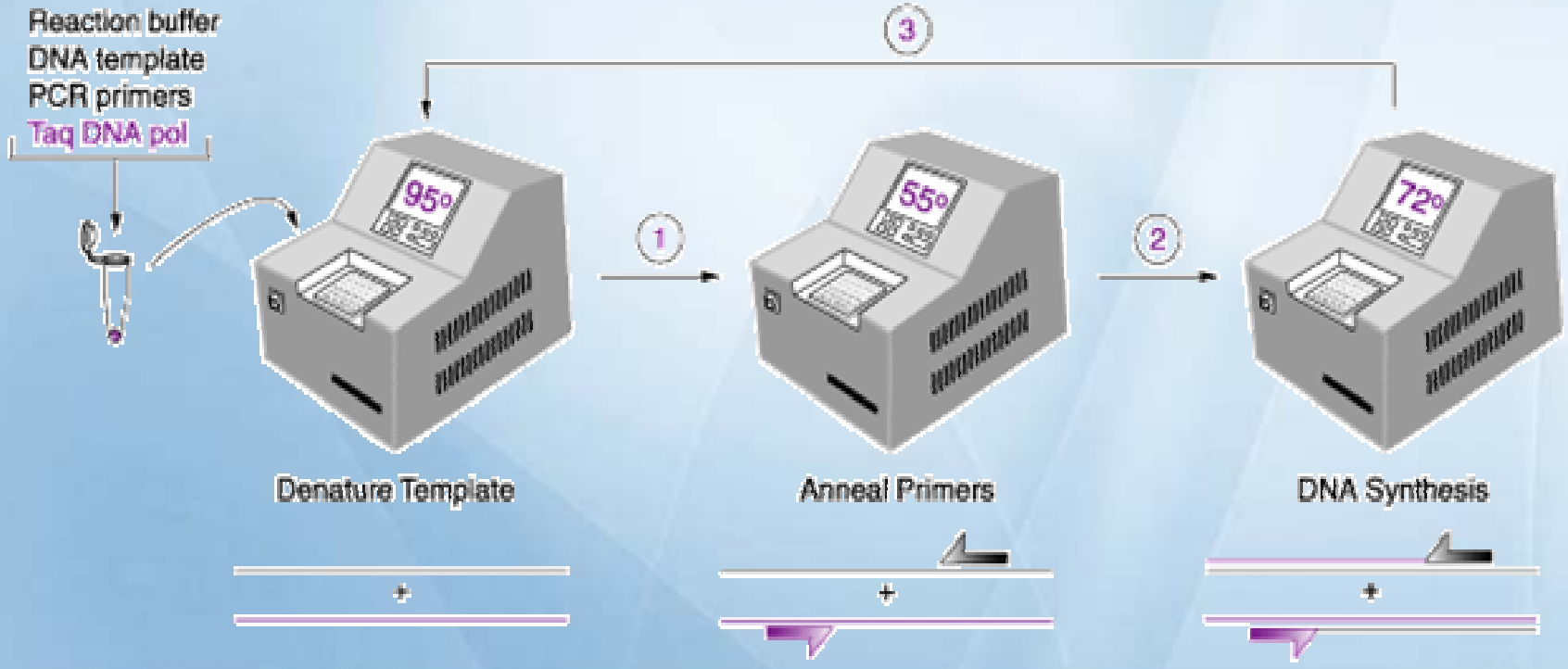
- The G+C content of the primers should be 40 - 60%, and having G or C at 3' end.
- Avoid complementarities between oligo-primers.
- They are manufactured commercially

Taq DNA Polymerase features :

- A heat-stable DNA polymerase must be used in the reaction.
- Heat stable Taq DNA polymerase was purified from the hot springs (hydrothermal vents) bacterium *Thermus aquaticus* in 1976.
- Taq polymerase has optimal enzymatic activity at 72°C .
- Its enzymatic halflife (at 95°C) is 40 min
- Taq Polymerase extends the DNA chain by adding ~150 nucleotides per second.

Thermal Cycler:

- It heats and cools the reaction tubes within it to the precise temperature required for each step of the reaction.
- It is available in different specificity and models.



PCR features and advantages

- This technique is flexible and powerful and has many different applications.
- Speed and ease of use in which each cycle takes 3-5 minutes.
- It will even work on degraded DNA or fixed DNA .
- One can start with a single sperm cell or stand of hair .

Disadvantages of PCR

- Need information about Target DNA sequence.
- There is an upper limit to the size of DNA synthesized by PCR .


Thank you
Prof. Dr. Hamdy El-Aref

References

- **Micklos, David, Greg Freyer and David Crotty. DNA Science a First Course. New York:Cold Spring Habor Laboratory Press, 2003.**
- **Purves, Sadava, Orians, Heller. "Life." 6th ed. Sinauer Associates, 2001.**
- **Demidov.V, Broude. N(2004). DNA Amplification: Current Technologies and Applications**
- **Websites:**
- http://faculty.plattsburgh.edu/donald.slish/PCRmov.html (Animation)
- http://www.accessexcellence.org/RC/AB/IE/PCR_Xeroxing_DNA.html
- http://www.people.virginia.edu/~rjh9u/pcranim.html (PCR Animation)
- http://www.escience.ws/b572/L3/L3.htm (PCR Animation)
- http://homepages.strath.ac.uk/~dfs99109/BB211/RecombDNAtechlect4.html
- http://en.wikipedia.org/wiki/Polymerase_chain_reaction
- http://www.escience.ws/b572/L3/L3.htm
- http://allserv.rug.ac.be/~avierstr/principles/pcrani.html