An Introduction to Polymerase Chain Reaction (PCR)

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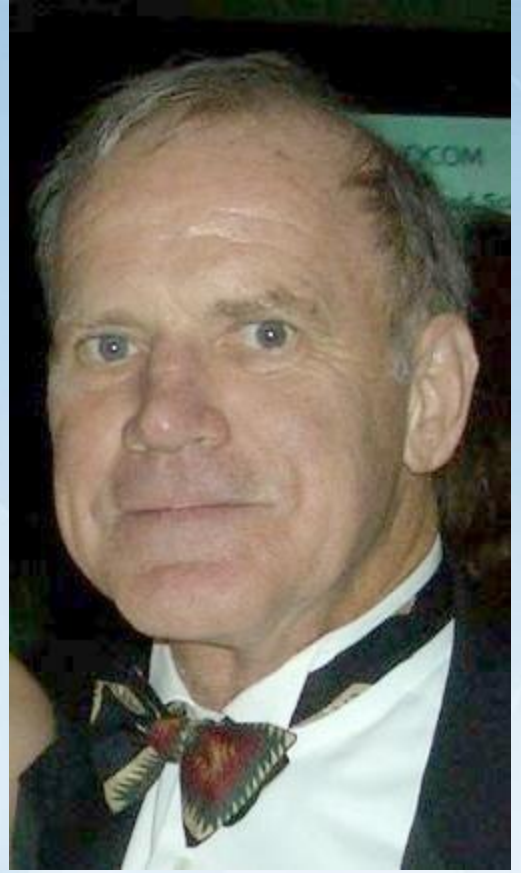
Introduction

- The technique was invented by Dr. Kary Mullis, 1986
- for which he received the Nobel Prize in Chemistry in 1993.



PCR Achieves Fame and Fortune

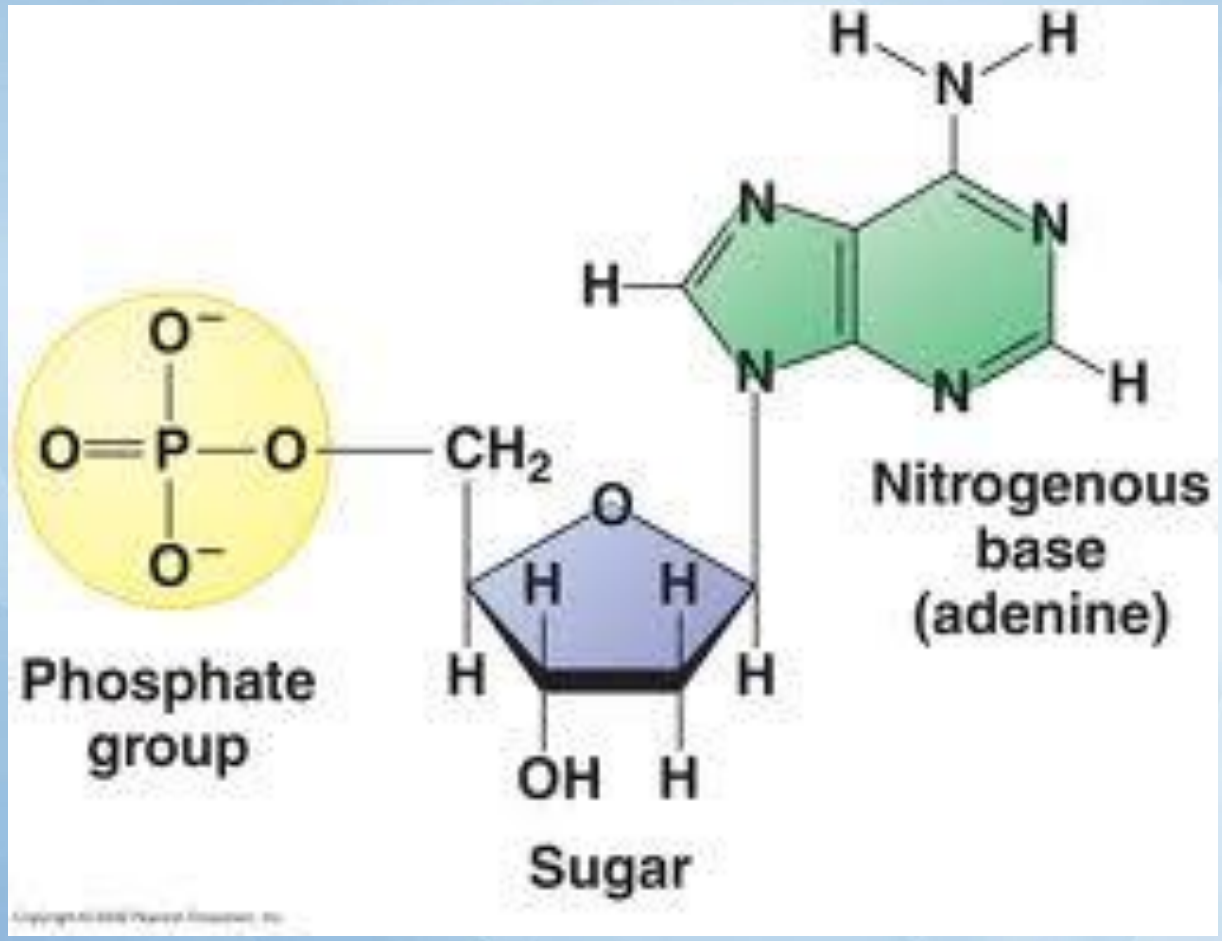
--becomes standard in molecular biology tool box--

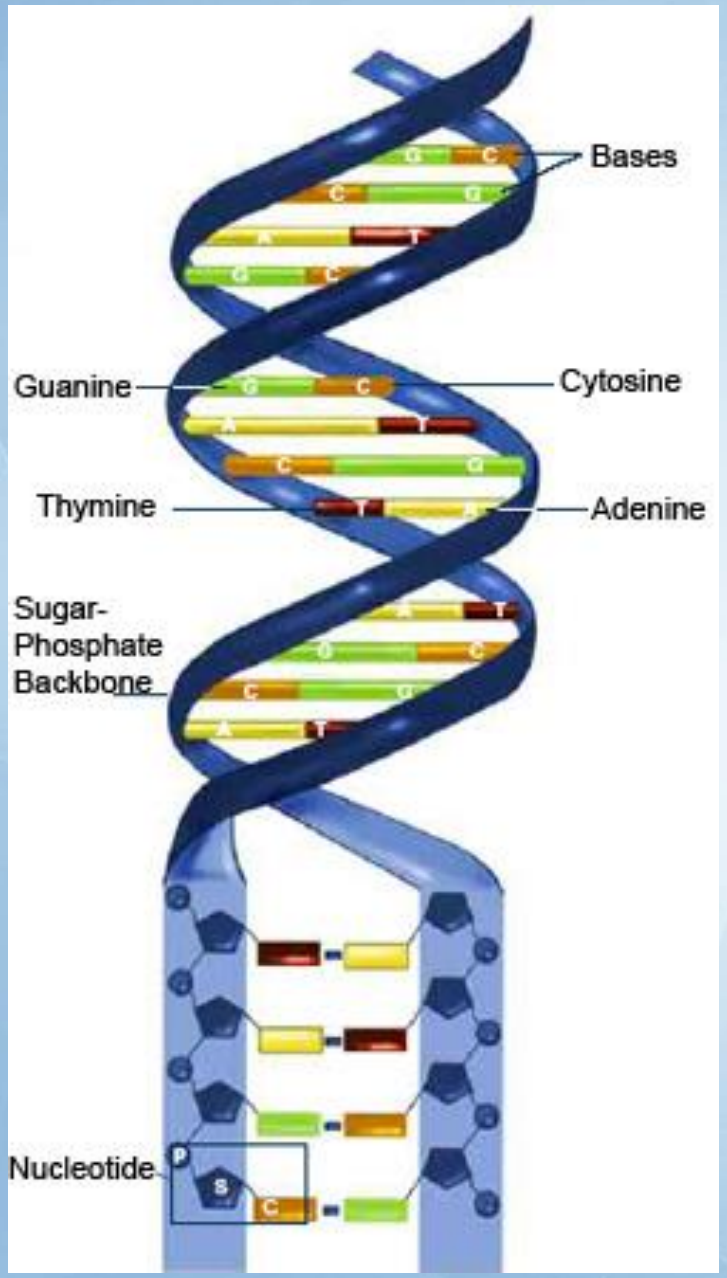
The Molecule of the Year

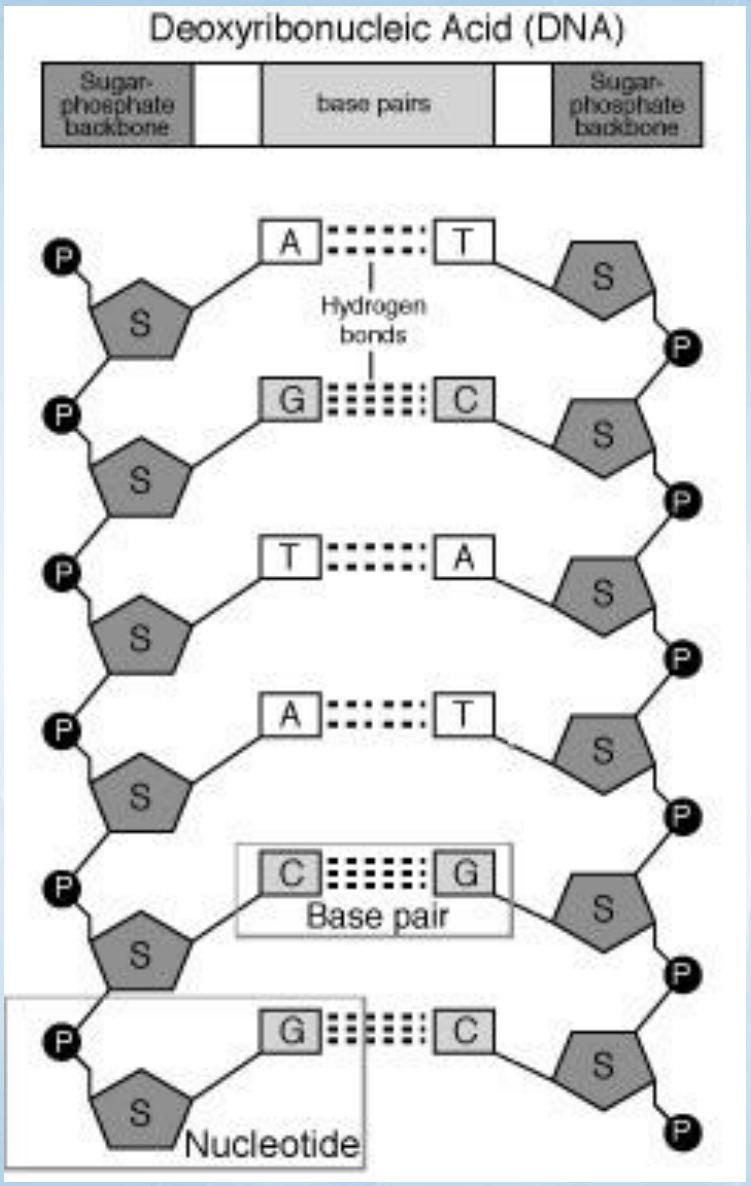
Ruth Levy Guyer and Daniel E. Koshland, Jr.

Science has selected the polymerase chain reaction as the major scientific development of 1989 and has chosen for its first "Molecule of the Year" the DNA polymerase molecule that drives the reaction. The list from which the polymerase chain reaction (PCR) was chosen included an impressive array of accomplishments in many areas of science and technology; additional kudos are therefore conferred below to 17 of the other big "stories" that made 1989 an exciting year for scientists and for followers and beneficiaries of science. Although the PCR procedure was introduced several years ago, use of the technique truly burgeoned in 1989; in much the same way, the full potentials of many of the interesting "runner-up" scientific achievements of this year are likely to be realized sometime in the years to come.

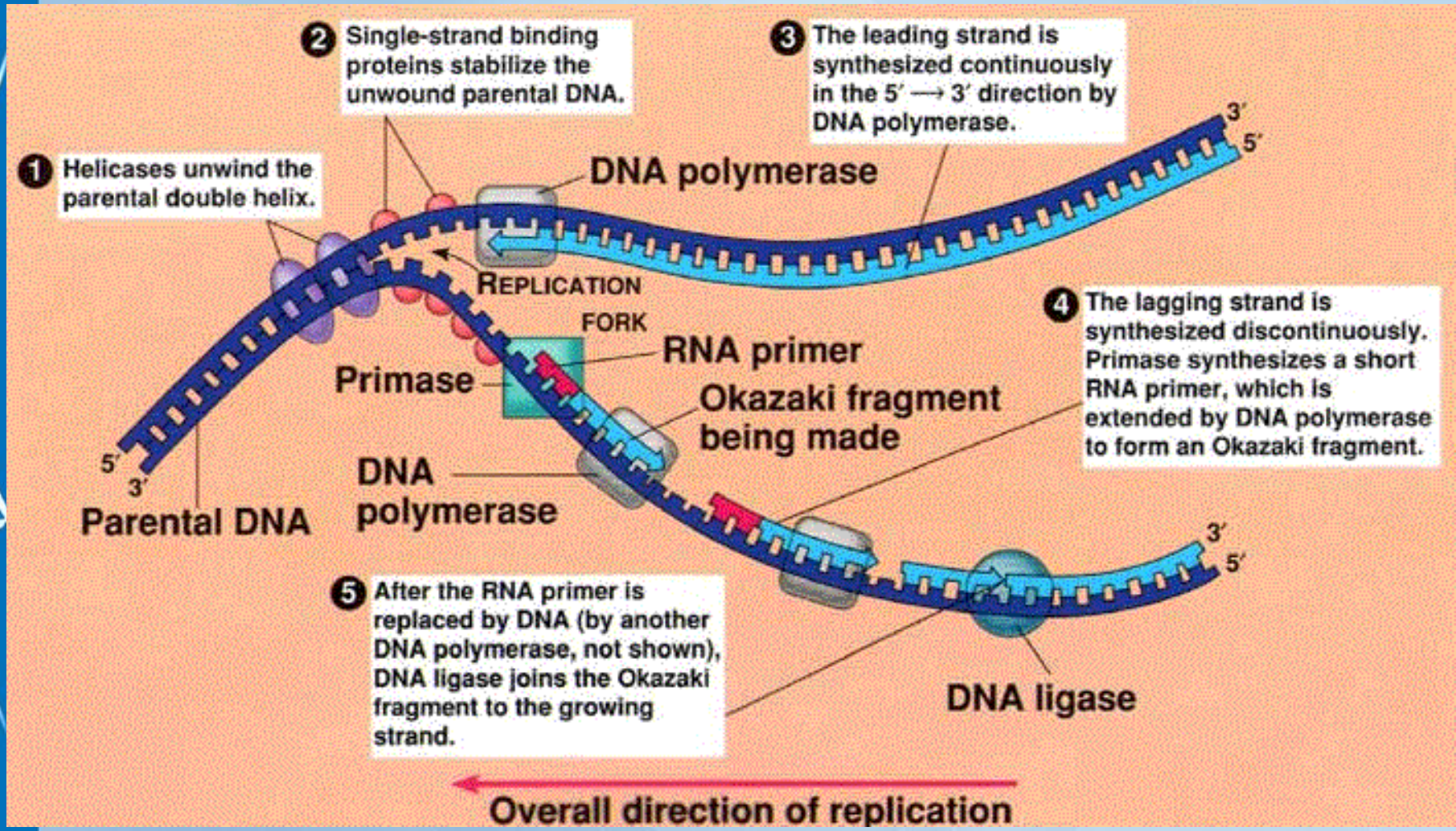
DNA Structure




Bases
Guanine
Cytosine
Thymine
Adenine
Sugar-
Phosphate
Backbone
Nucleotide


Deoxyribonucleic Acid (DNA)
Sugar-phosphate backbone
base pairs
Sugar-phosphate backbone
Hydrogen bonds
Base pair
Nucleotide

DNA Replication



Polymerase Chain Reaction (PCR)

- PCR is a technique which is used to amplify the number of copies of a **specific region of DNA**, (usually fewer than 3000 base pairs) in order to produce enough DNA to be adequately tested.
- **Millions** of copies of a segment of DNA can be made within a few hours
- As a result, it now becomes possible to analyze and characterize the DNA.

Before PCR

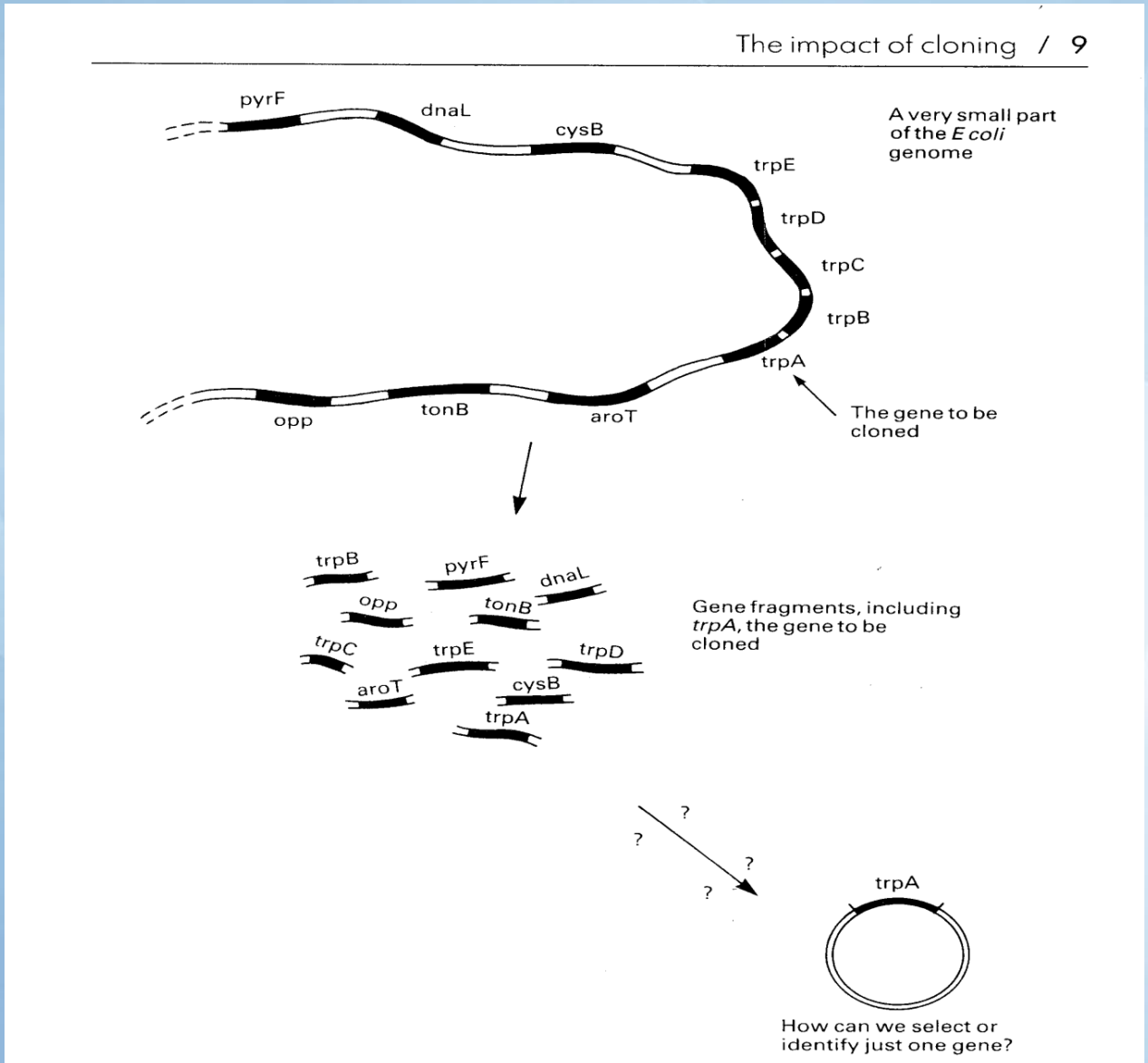
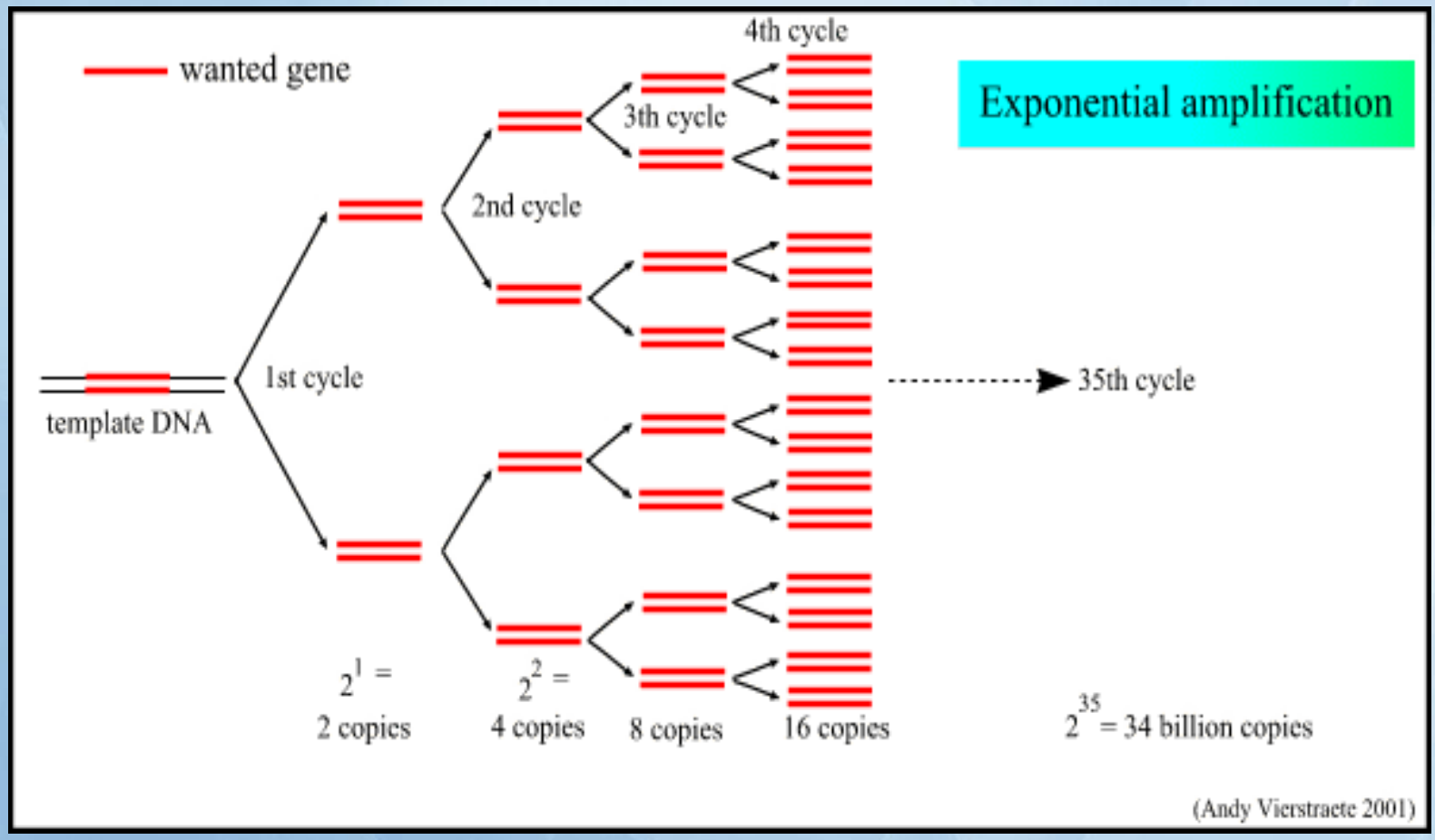


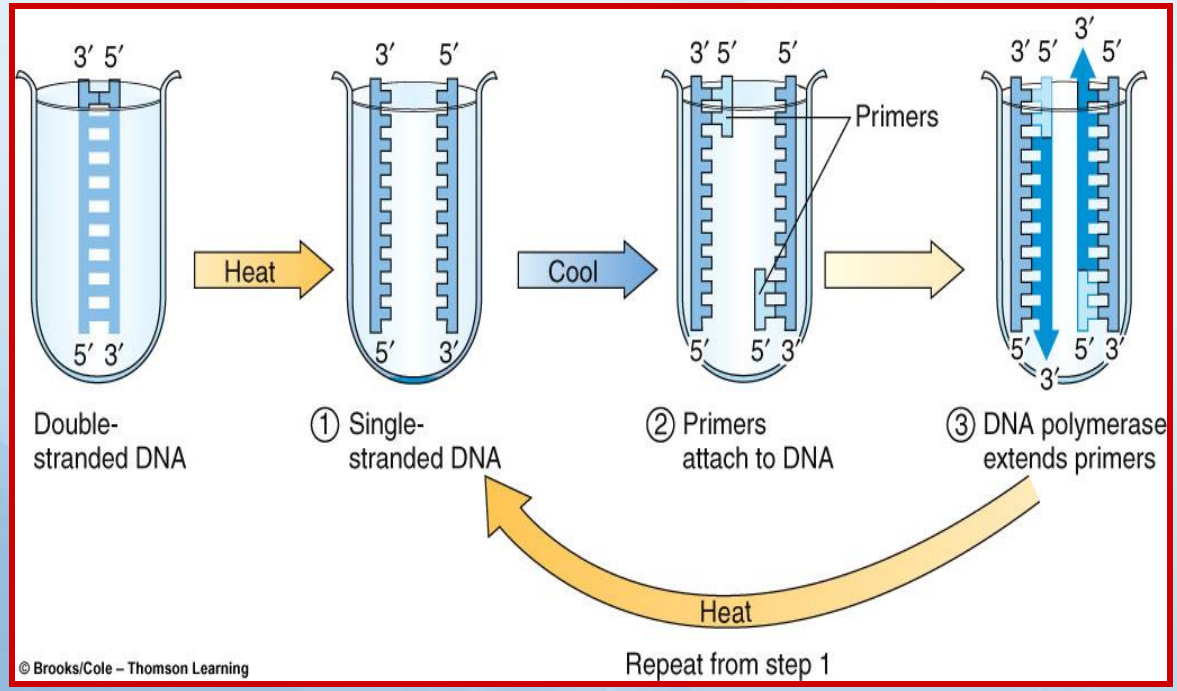
Figure 1.3 The problem of selection.

DNA amplification by PCR (overview)



PCR Cycle

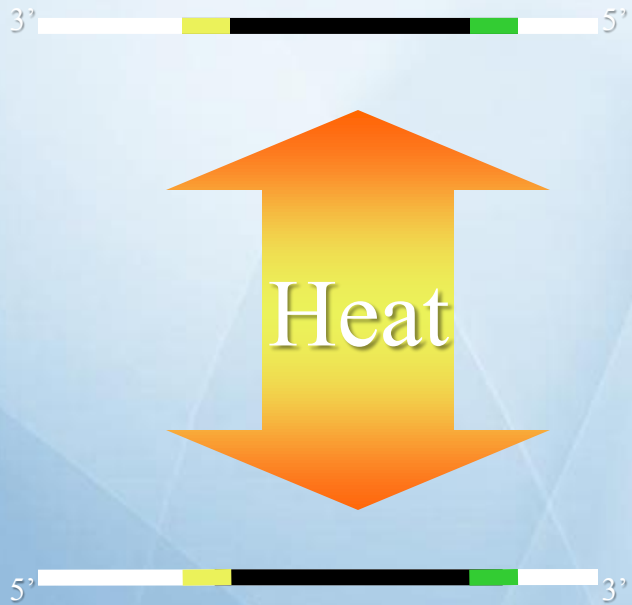
- **Each cycle (Round) of PCR contains 3 steps:**
  - **1- Denaturation**
  - **2- Primer annealing**
  - **3- Primer extension**
- **The cycle usually repeated for 25 – 40 times.**



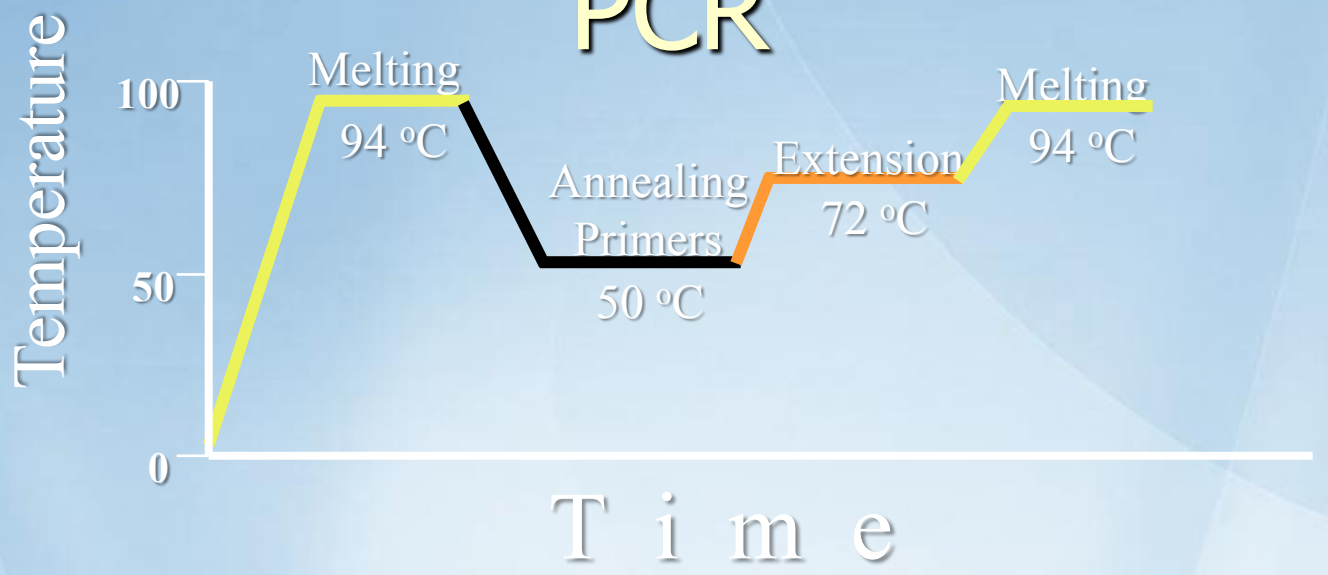
Movie

PCR

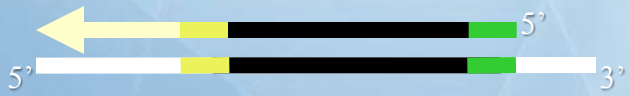
PCR

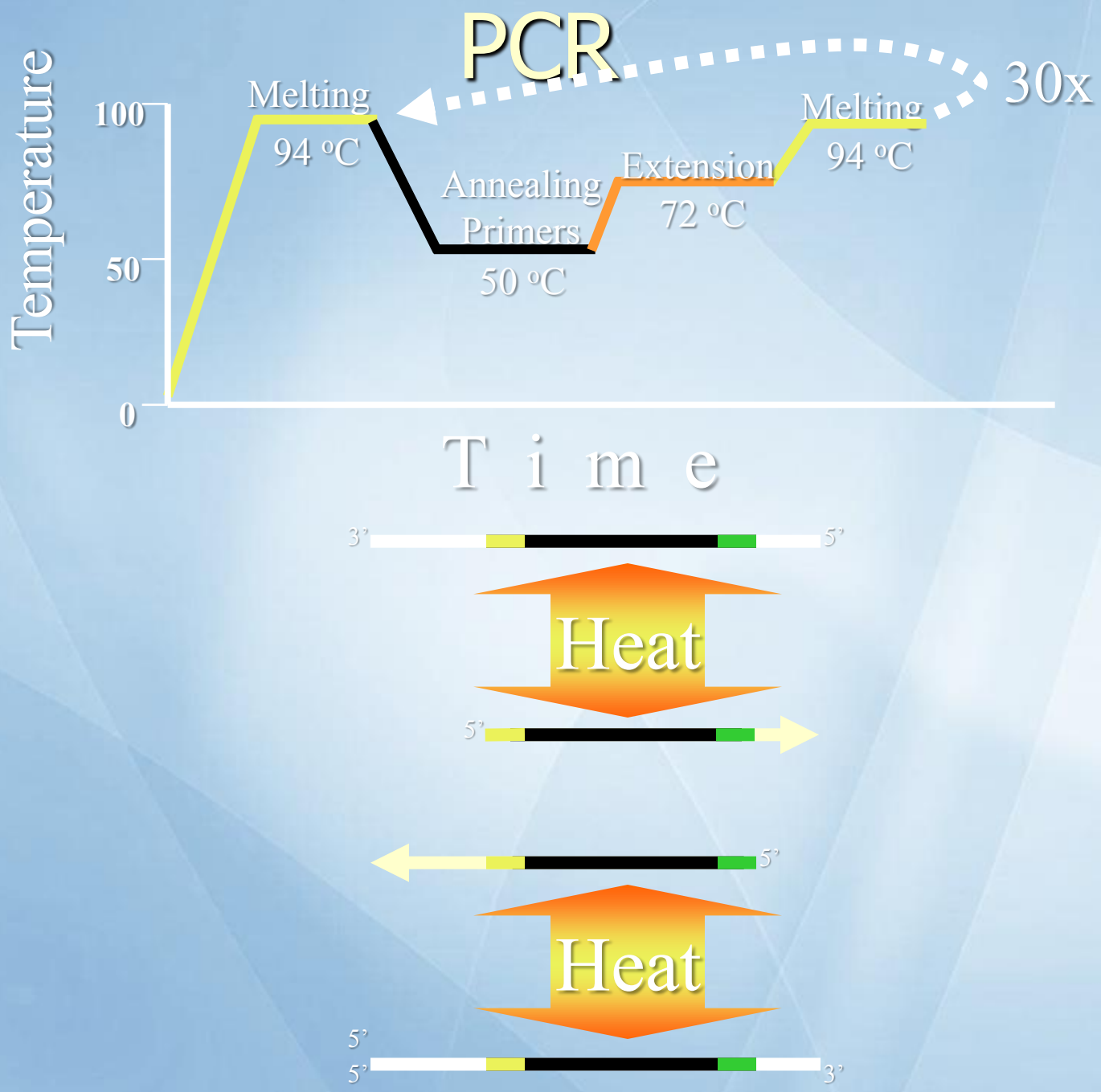


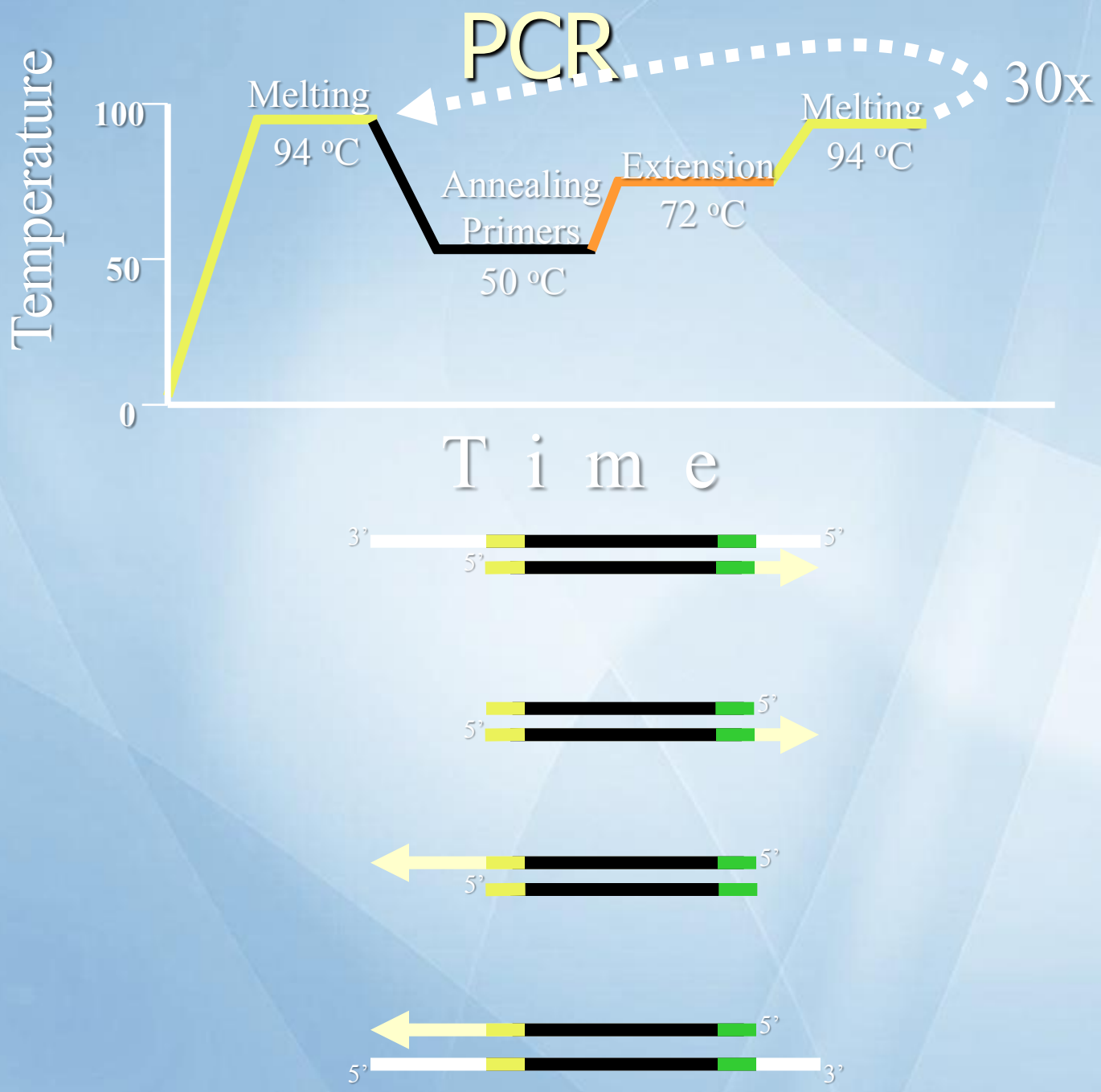
PCR


Temperature
100
50
0
Melting
94 °C
Annealing
Primers
50 °C
Extension
72 °C
Melting
94 °C
T i m e


3'
5'
5'


5'
5'
3'


PCR
30x
Temperature
100
50
0
Melting
94 °C
Annealing
Primers
50 °C
Extension
72 °C
Melting
94 °C
T i m e
3'
5'
Heat
5'
5'
Heat
5'
5'
3'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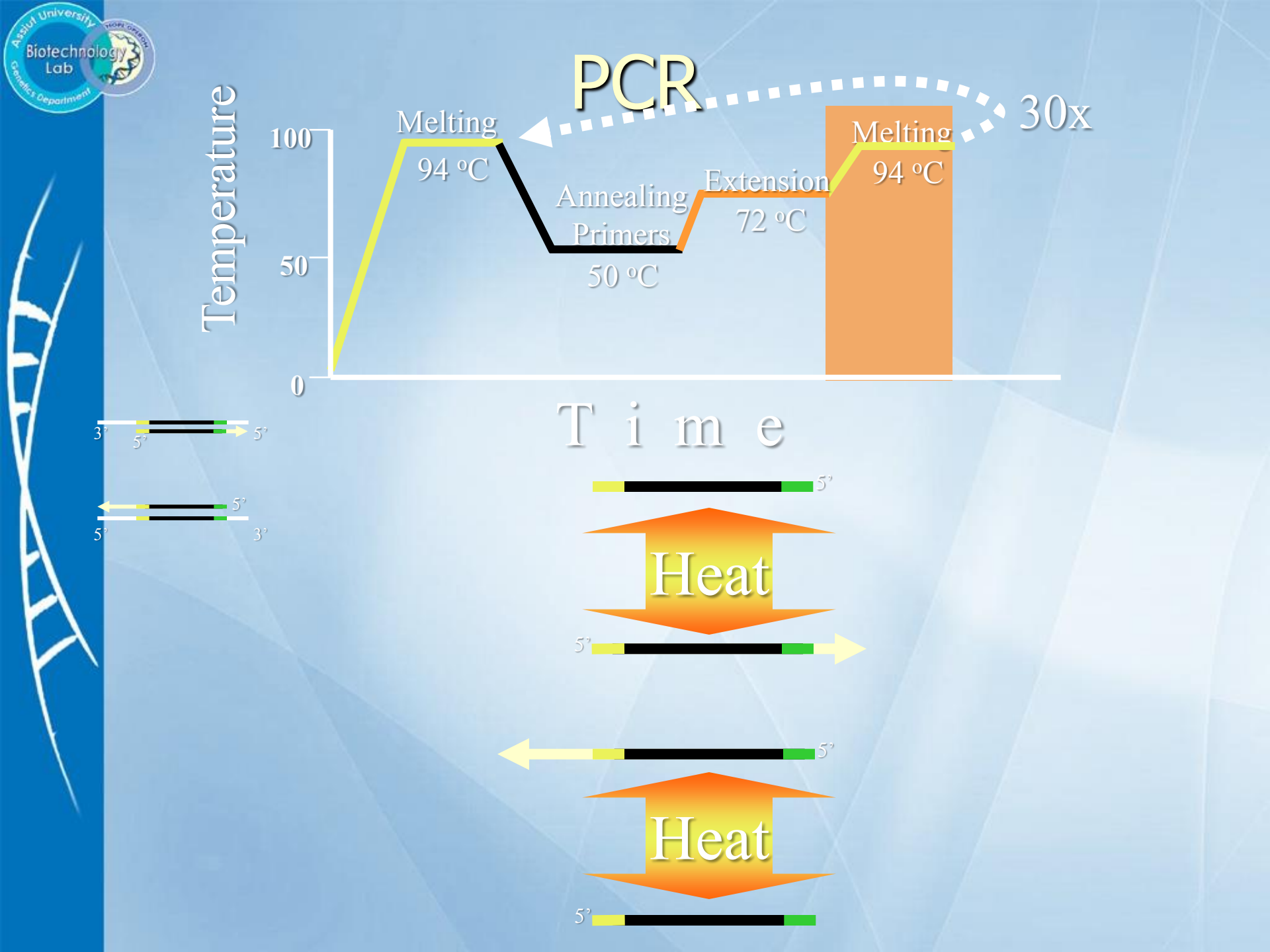
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3'
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Heat
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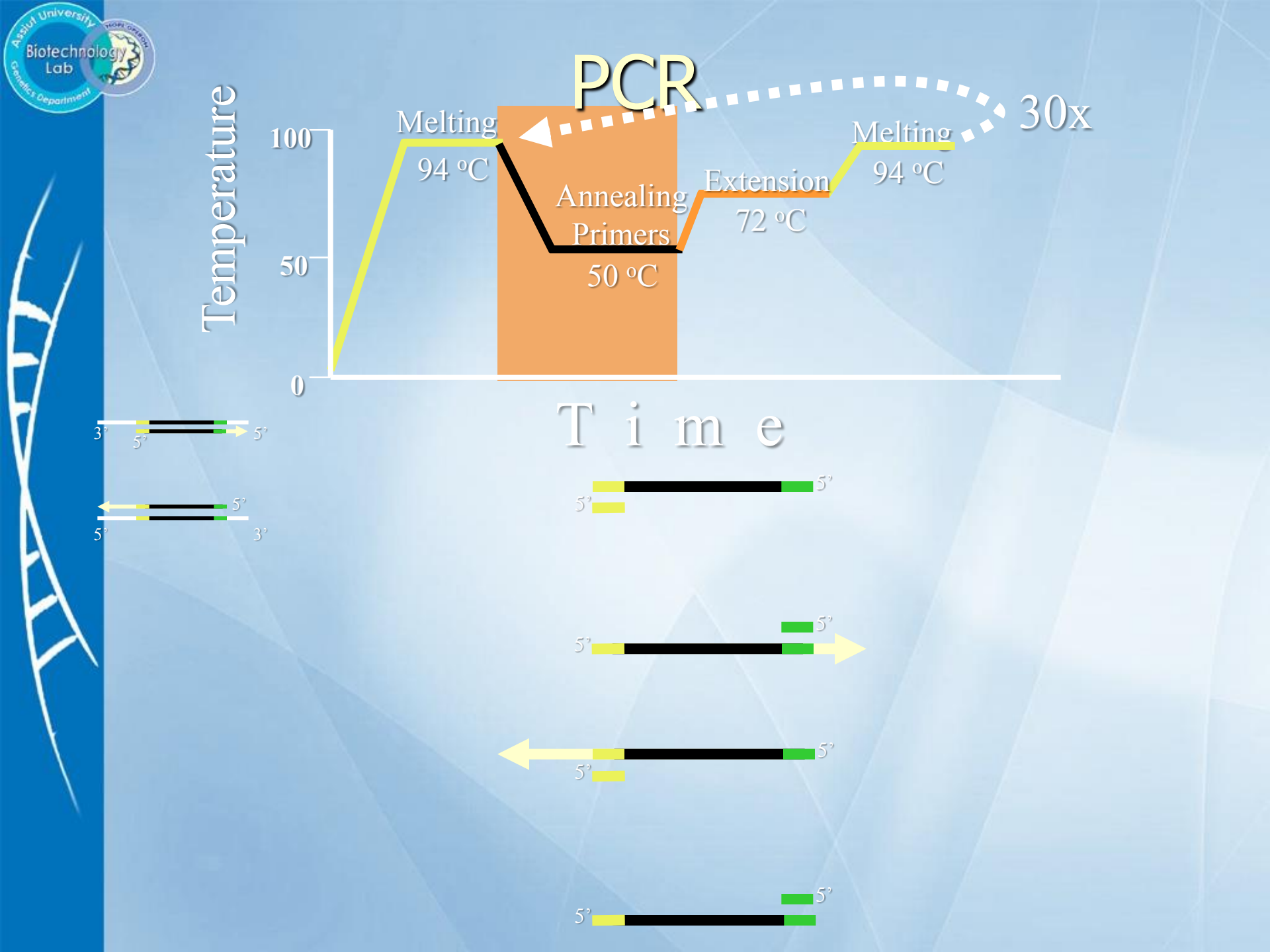
Heat
5'


PCR
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Temperature
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Melting
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Primers
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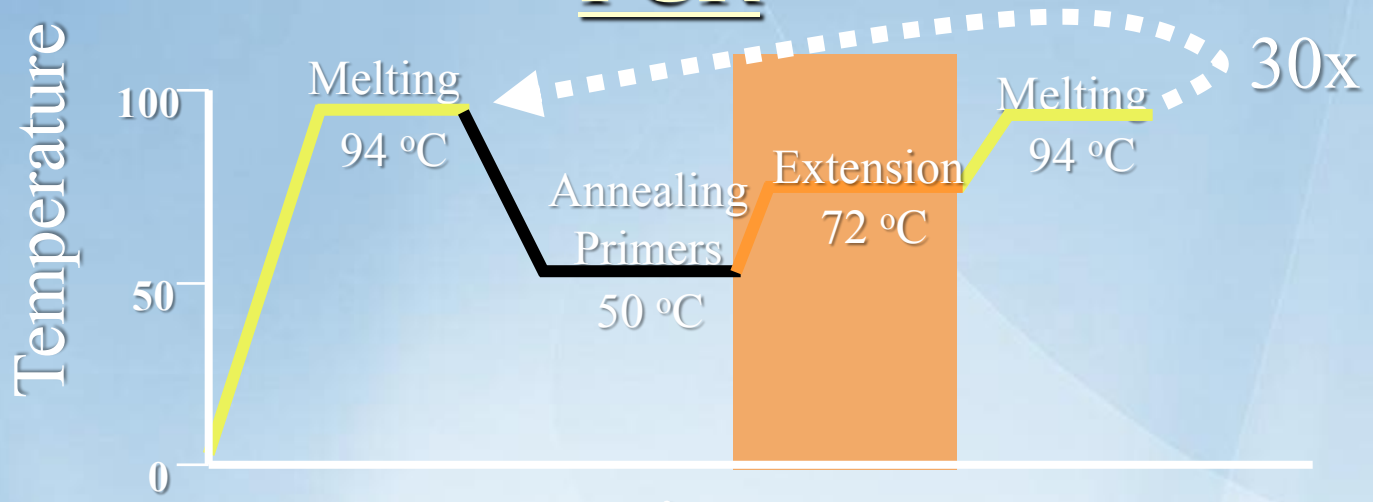
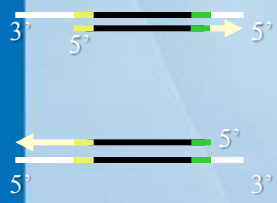
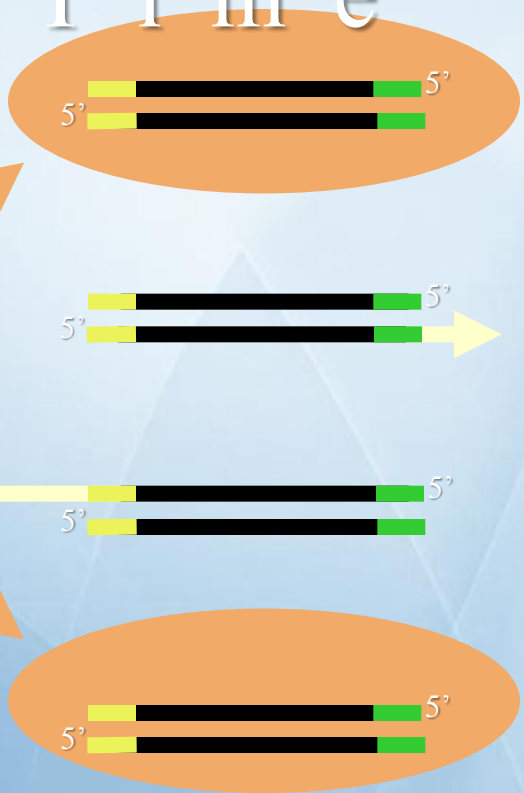
PCR



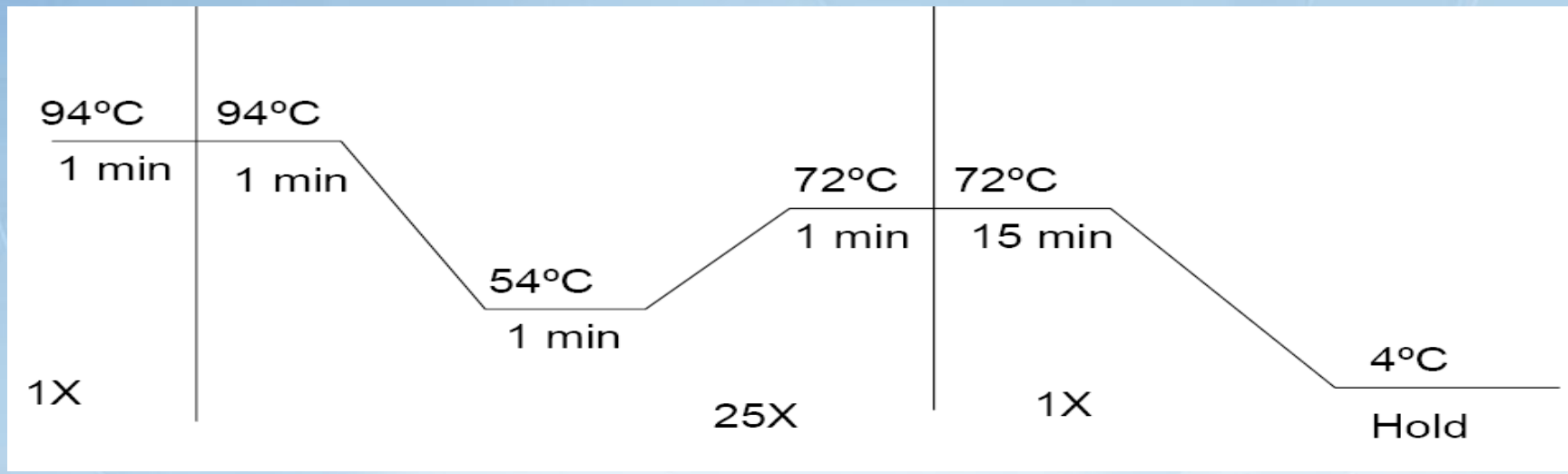


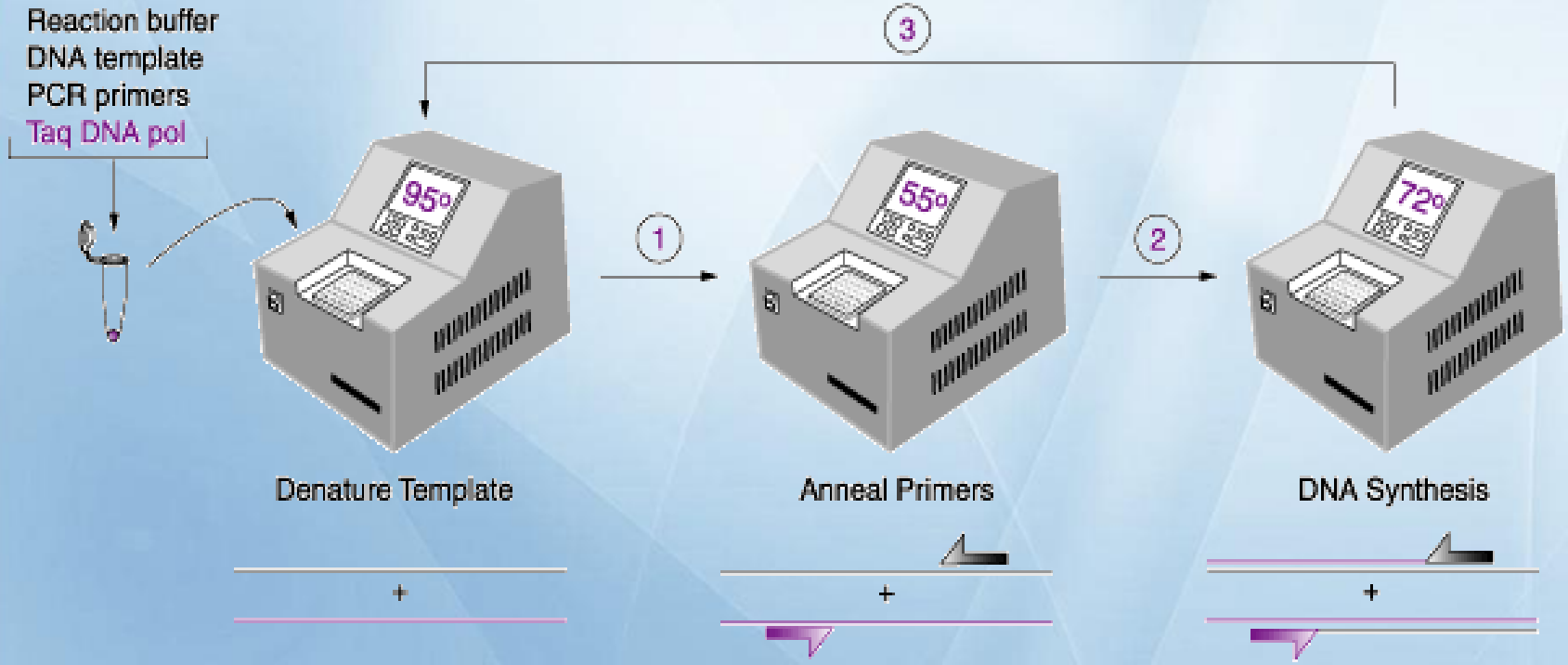
Fragments of defined length



Animation

Programming the Thermocycler





What do we need for PCR?

PCR tube

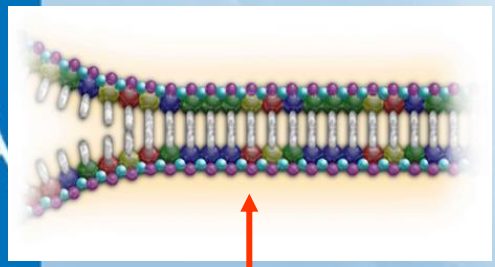
THERMOCYCLER

What do we need for PCR?

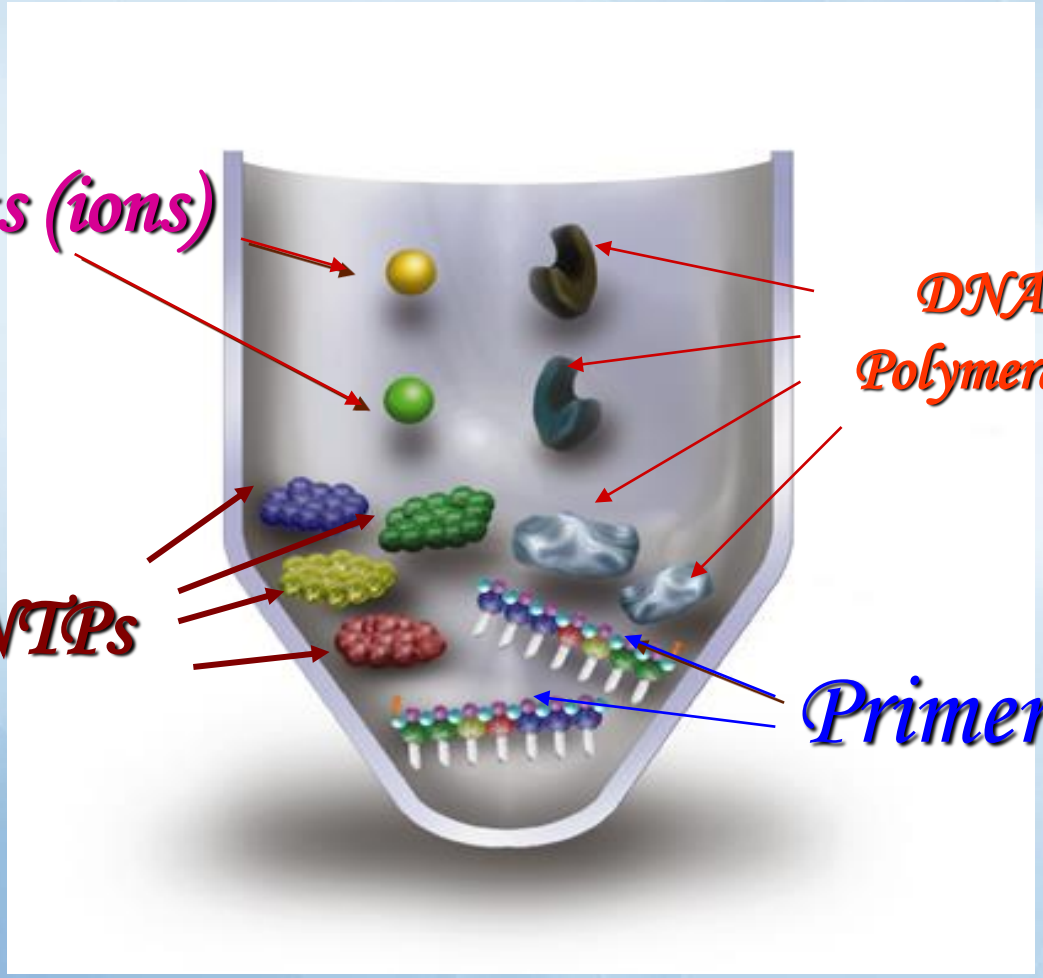
- Target DNA (Template).
- Two primers: (forward and reverse)
- Nucleotides: (the 4 dNTP'S: A, T, C, G)
- Heat-stable DNA polymerase:
  (like Taq DNA Polymerase)
- Buffer and Cofactor $MgCl_2$ (Mg^{++}, K^{+}).
- Thermal cycler.

PCR Procedure

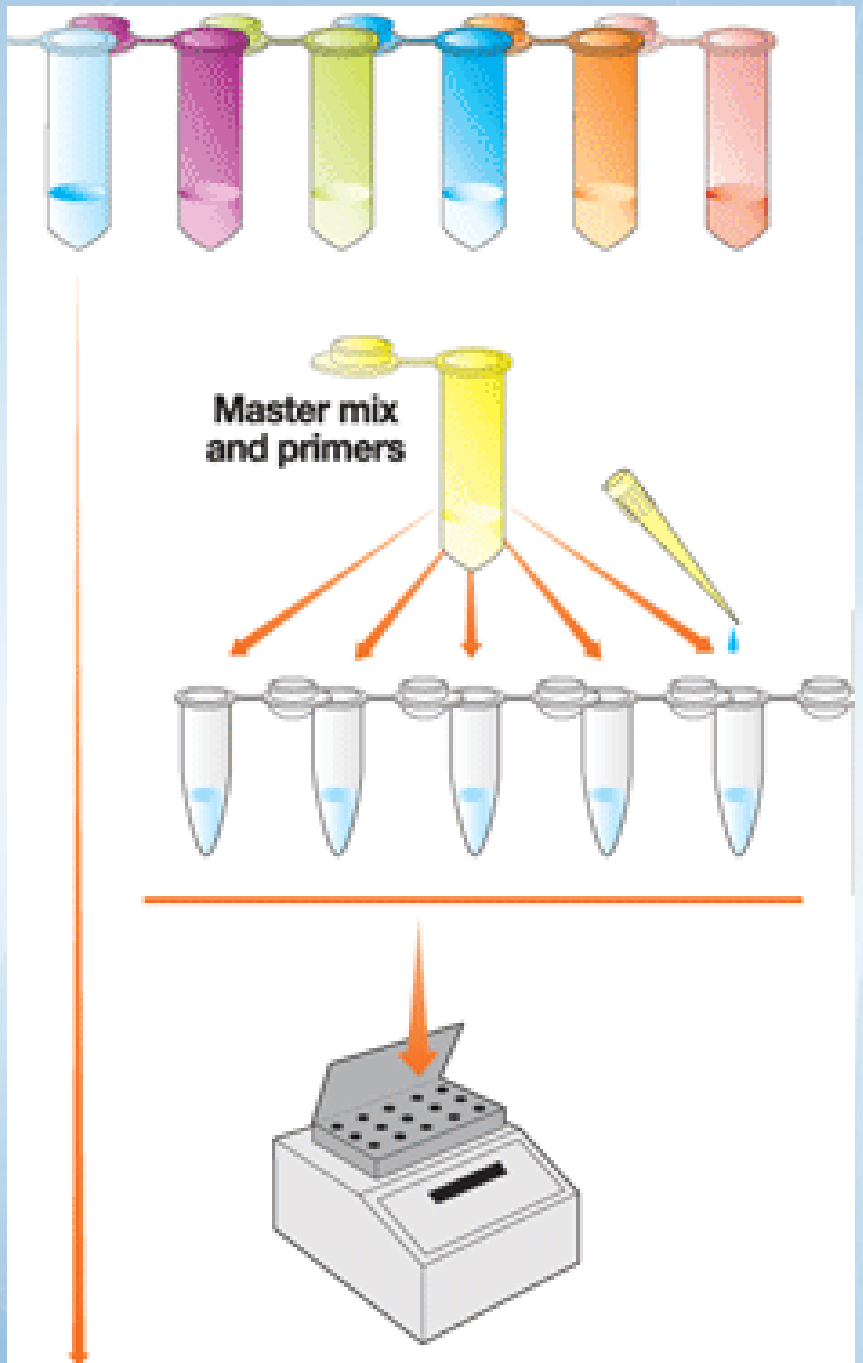
All the required components are inserted into an Eppendorf tube



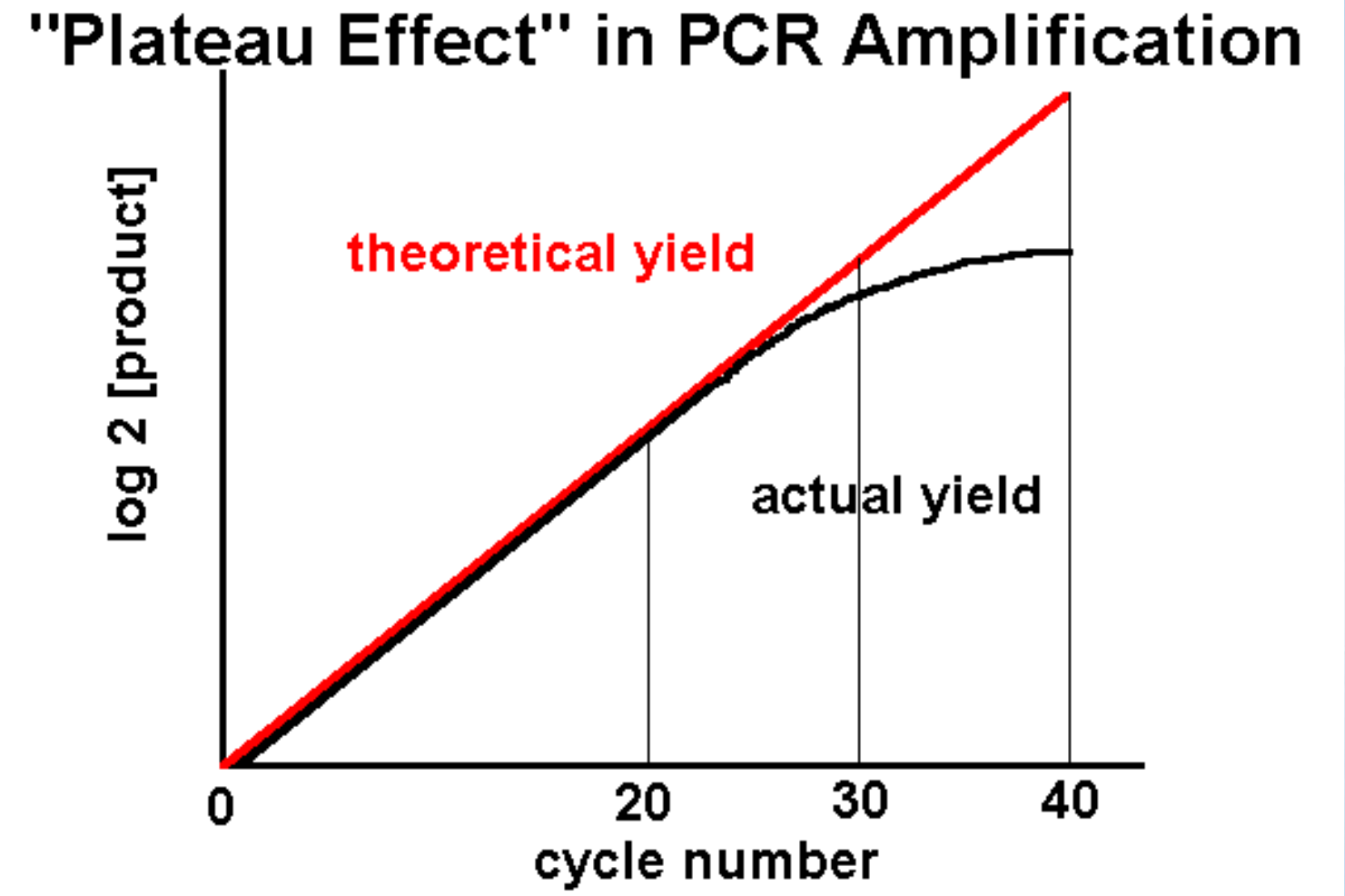
Template DNA



- DNA (Template).
- Forward primer
- Reverse primer
- dNTP's
- *Taq* DNA Polymerase
- Buffer
- H_2O



Cycle Number

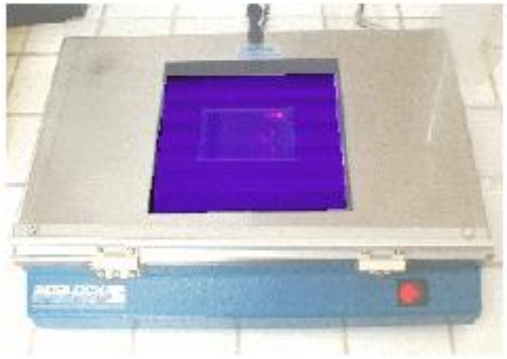


PROCEDURE .....

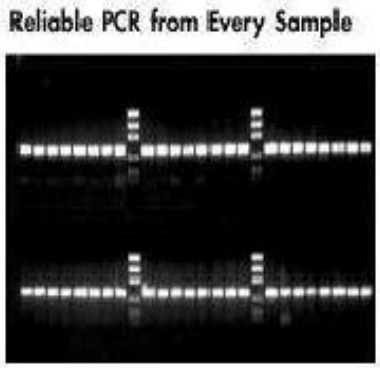
PCR

Agarose gel electrophoresis

3-4 hours



UV visualisation



The final product

Development….

- PCR work was first published (1985) using Klenow polymerase – unstable with heat

- First reports using DNA polymerase from *Thermus aquaticus* (1988)

- Developed automatic "thermocycler" programmable heat block

Taq DNA Polymerase features :

Polymerase	T ½, 95°C	Extension Rate (nt/sec)	Source
Taq pol	40 min	75	*T. aquaticus*
Amplitaq	80 min	>50	*T. aquaticus*
Vent	400 min	>80	*Thermococcus litoralis*
Deep Vent	1380 min	?	*Pyrococcus GB-D*
Pfu	>120 min	60	*Pyrococcus furiosus*
Tth	20 min	>33	*T. thermophilus*

Disadvantages of PCR

- Need information about Target DNA sequence.
- Highly susceptible to contamination or false amplification.
- Amplification may not be 100% specific.
- Analysis and product detection usually takes longer than the PCR reaction itself.
- There is an upper limit to the size of DNA synthesized by PCR.

Applications of PCR

- Classification of organisms
- Genotyping
- Mutagenesis
- Mutation detection
- Sequencing
- Detection of pathogens
- DNA fingerprinting
- Genetic engineering
- Research

Variations of the PCR

- Colony PCR
- Nested PCR
- Multiplex PCR
- AFLP PCR
- Hot Start PCR
- Inverse PCR
- Long PCR
- Long Accurate PCR
- Reverse Transcriptase PCR
- Allele specific PCR
- Real time PCR

Published papers with 'PCR'

- 1989 - 219
- 1990 - 496
- 1991 - 711
- 1992 - 906
- 1993 -1030
- 1994 - 857 (>4000)
- 1995 - 823
- 1996 - 796
- 1997 - 732
- 2006- 257,737
- 2007 - 286,486