Polymerase Chain Reaction (PCR)

Professor

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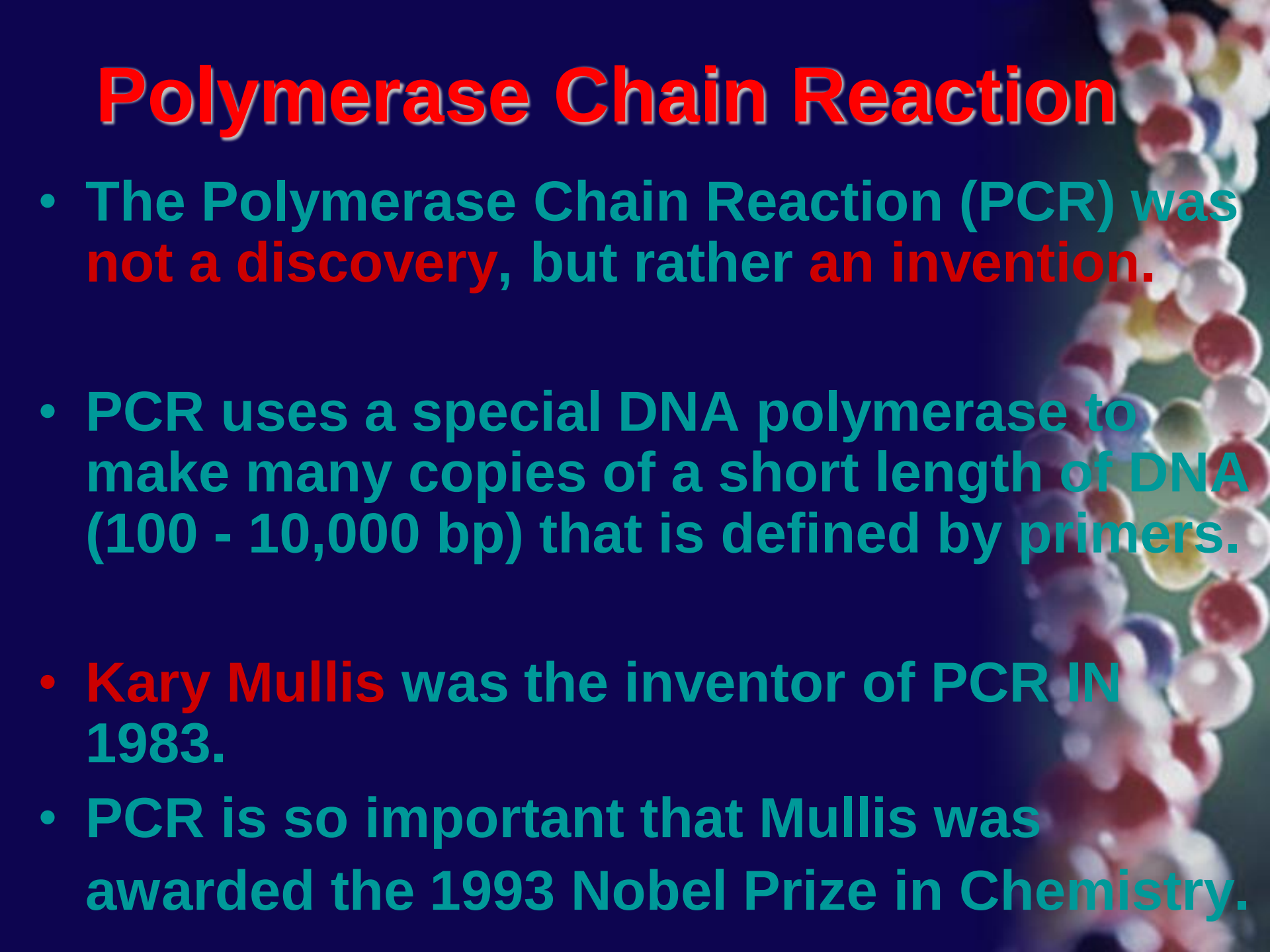
Polymerase Chain Reaction

- The Polymerase Chain Reaction (PCR) was not a discovery, but rather an invention.
- PCR uses a special DNA polymerase to make many copies of a short length of DNA (100 - 10,000 bp) that is defined by primers.
- Kary Mullis was the inventor of PCR IN 1983.
- PCR is so important that Mullis was awarded the 1993 Nobel Prize in Chemistry.



Essential components of PCR mixture

- Buffer (containing Mg^{++})
- Template DNA
- Two Primers that flank the fragment of DNA to be amplified
- dNTPs
- *Taq* DNA Polymerase (or another thermally stable DNA polymerase)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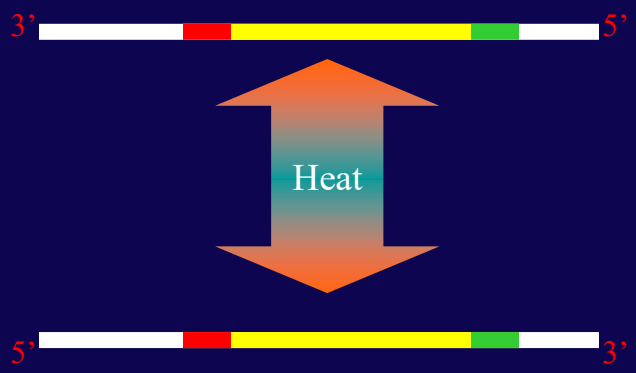
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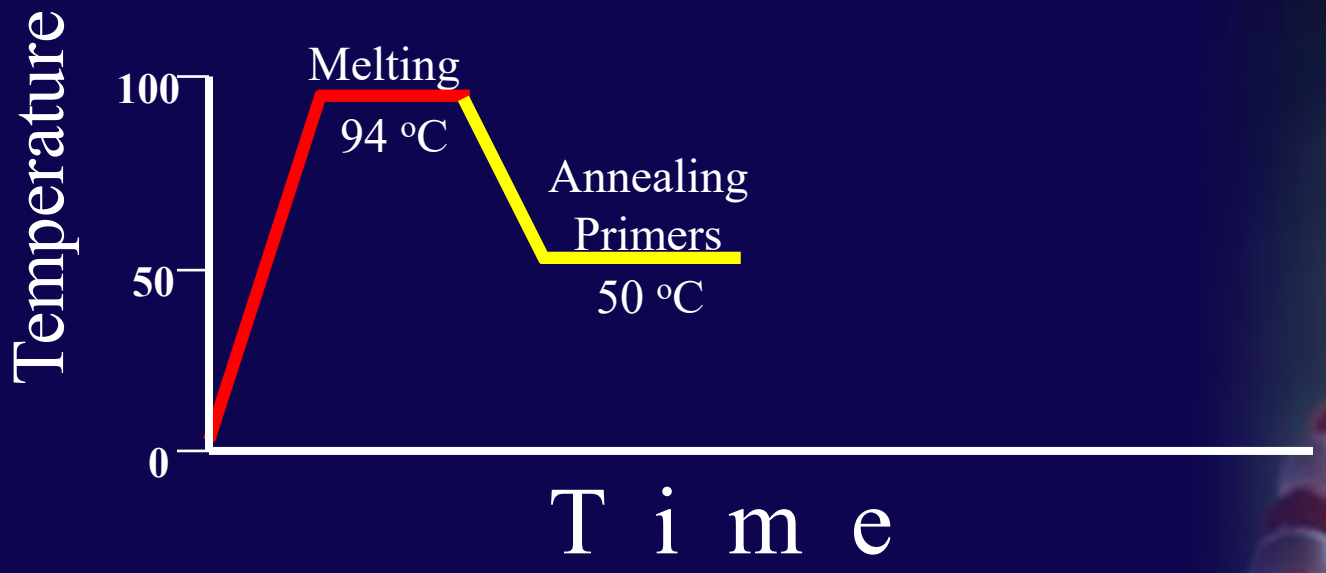
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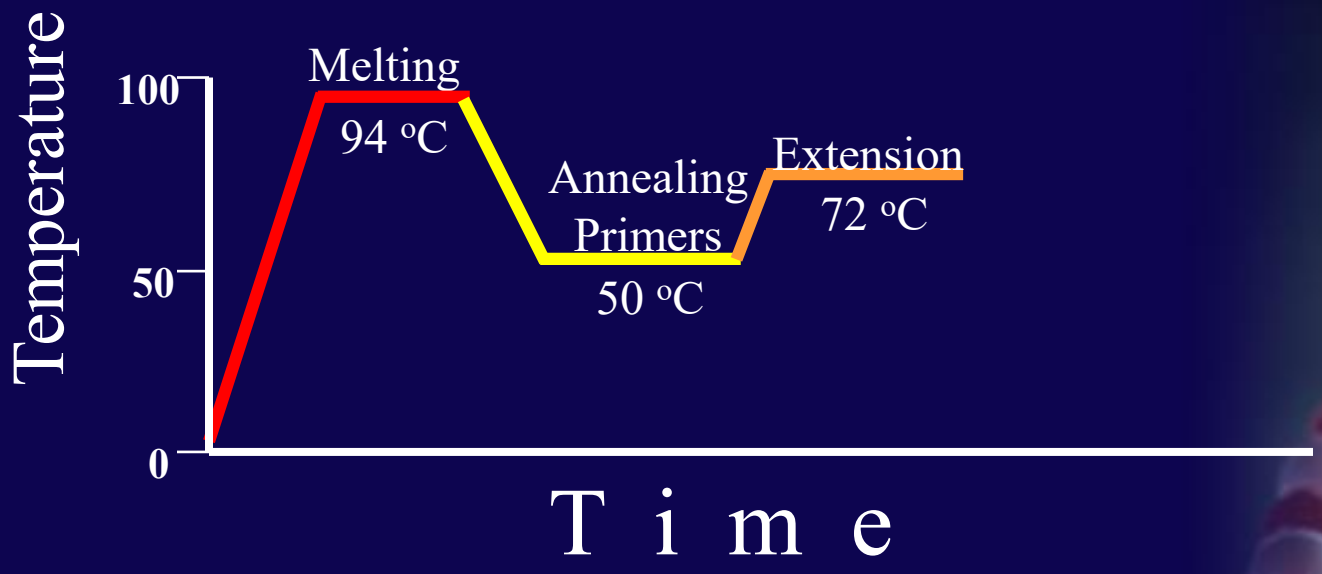
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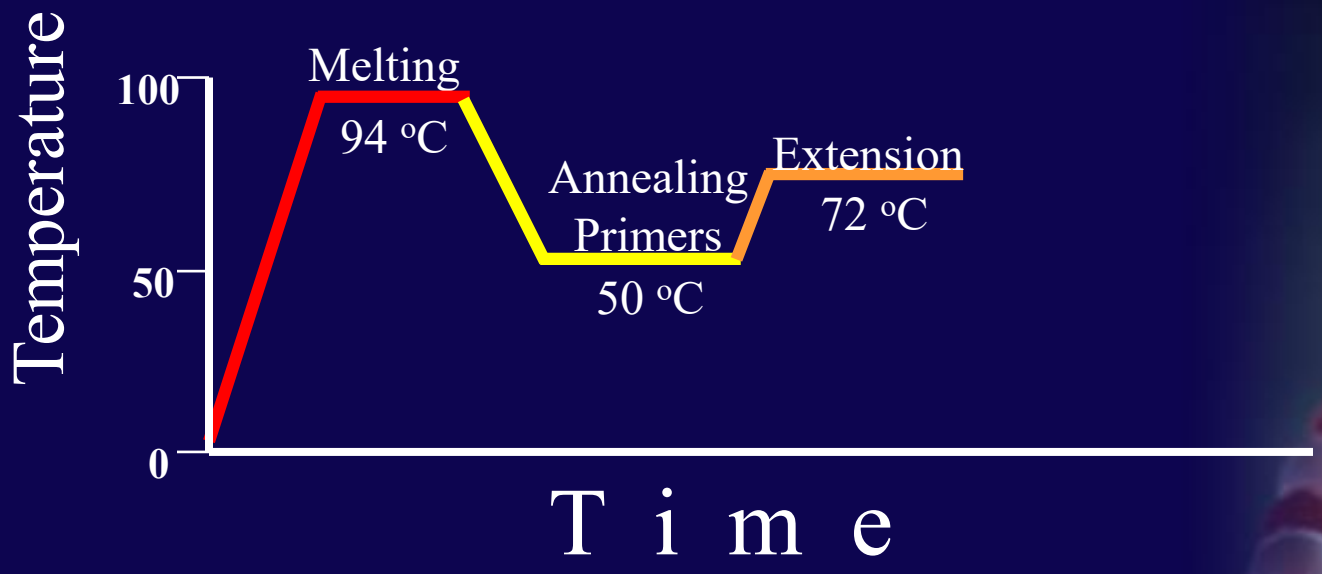
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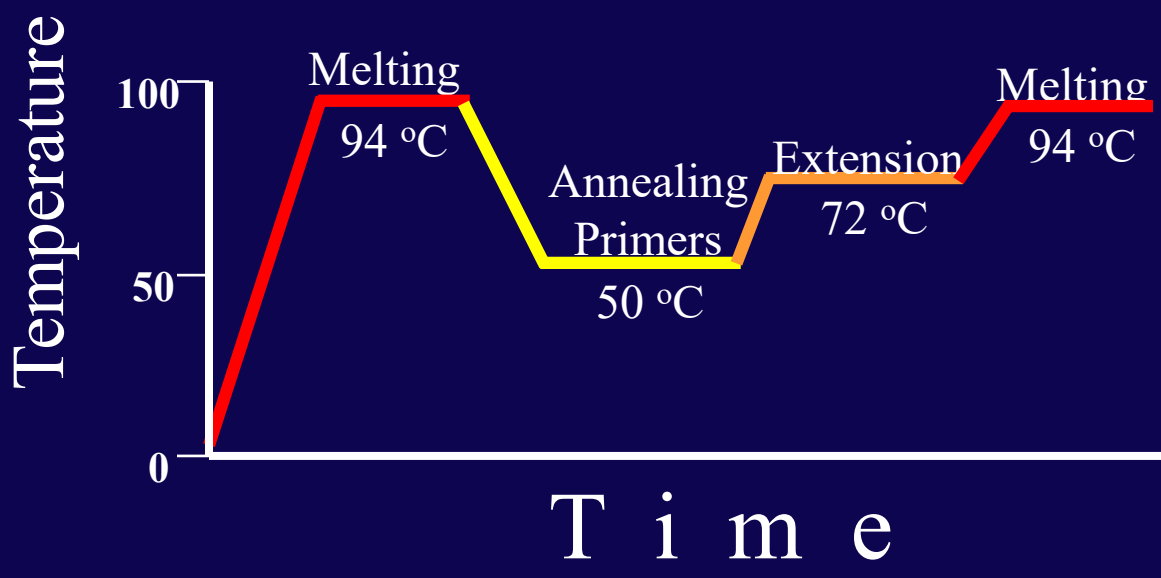
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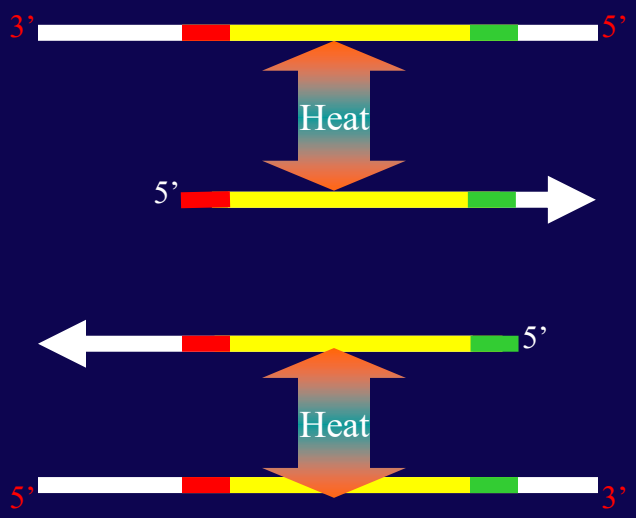
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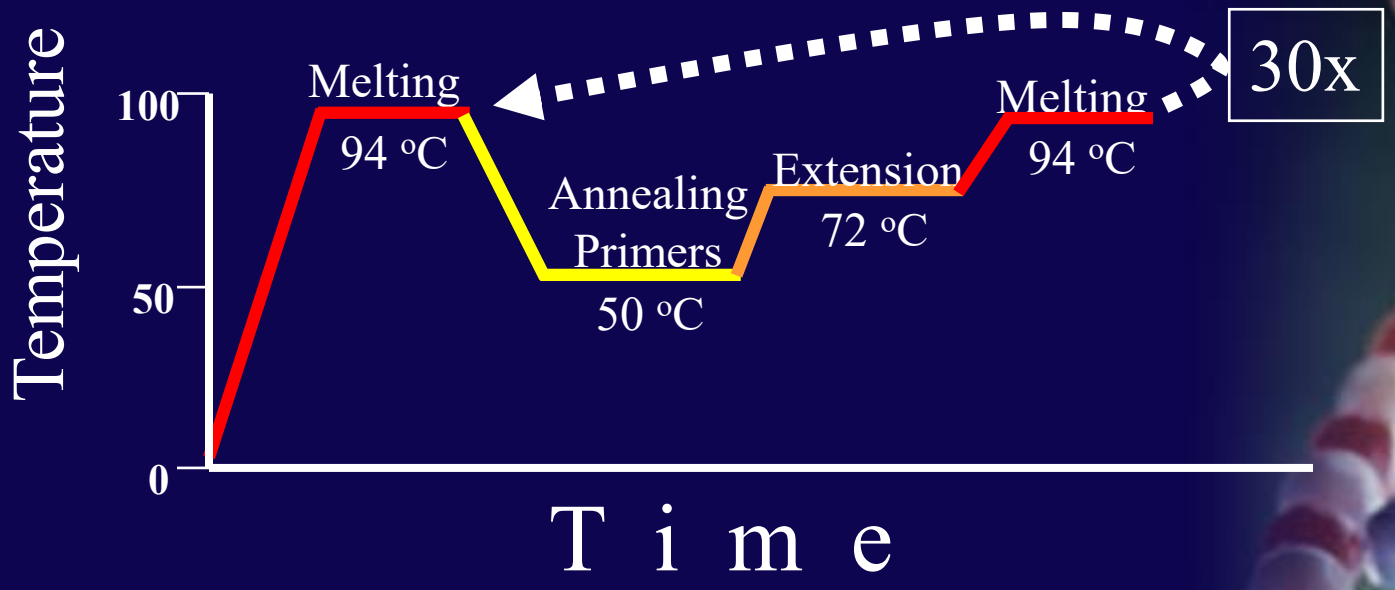
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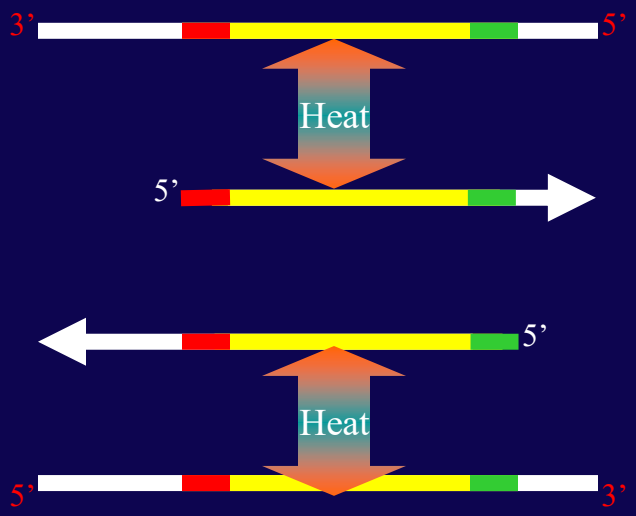
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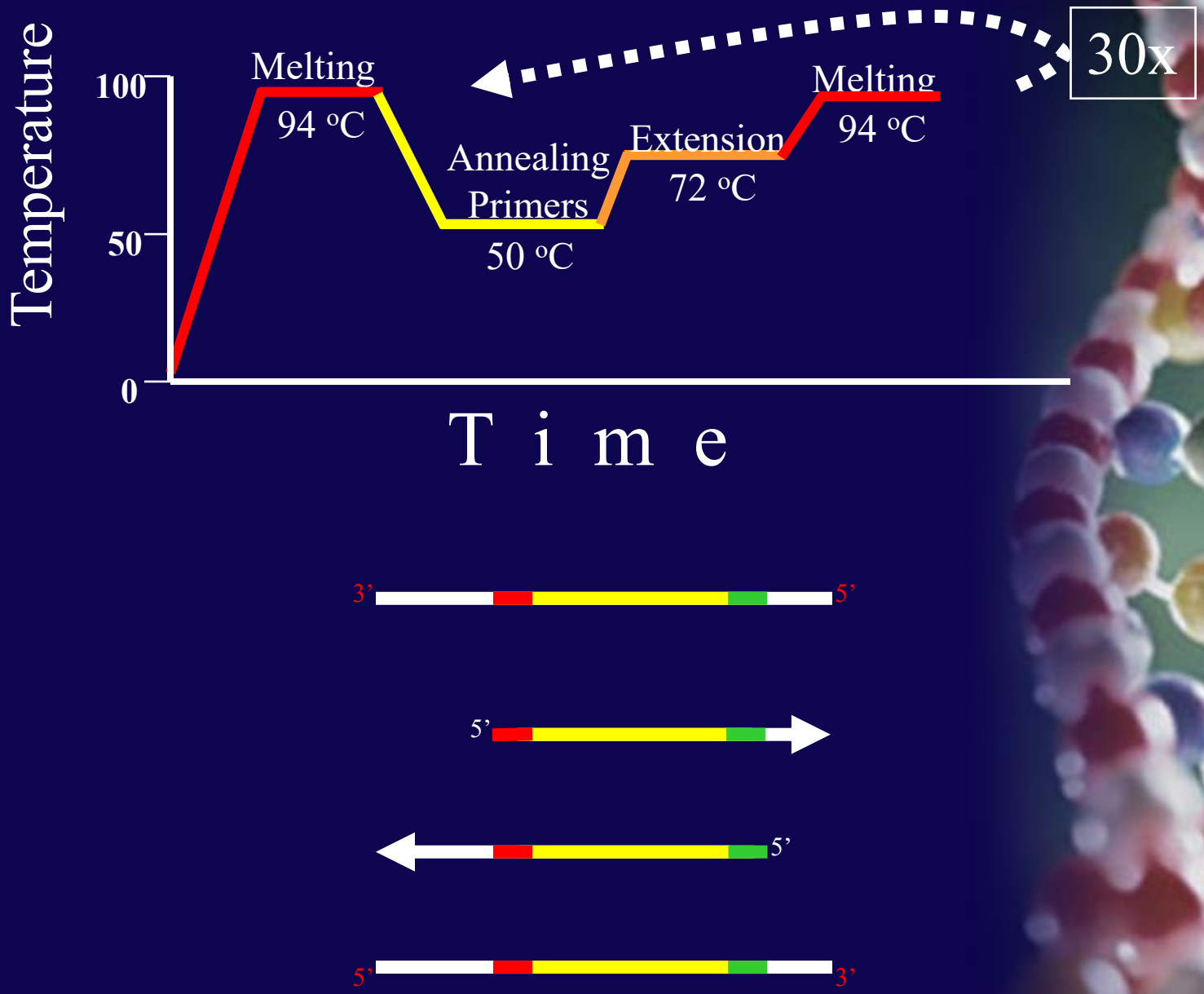
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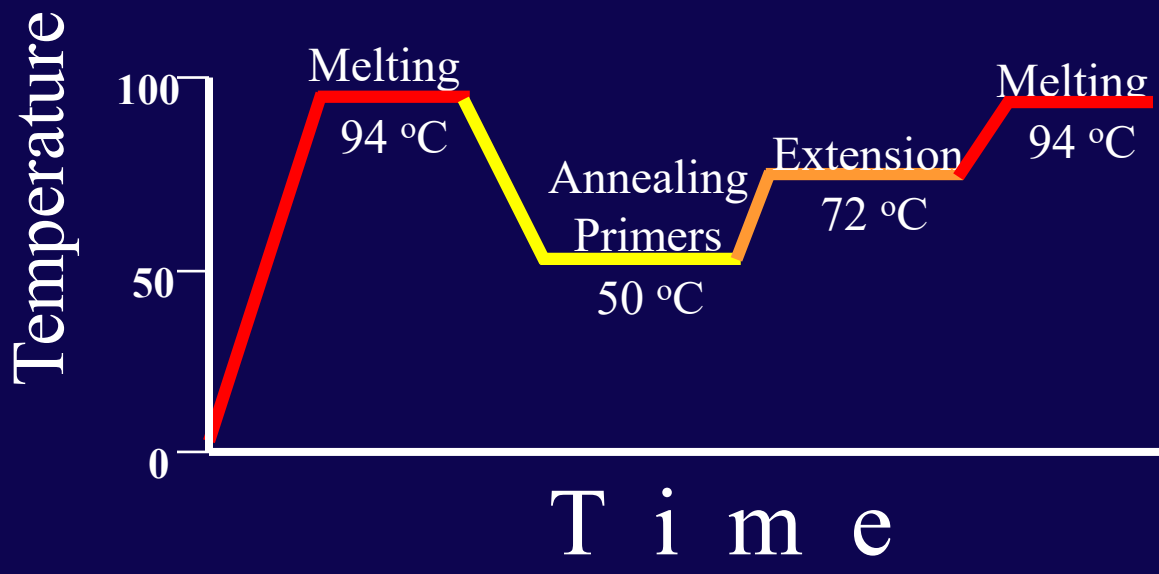
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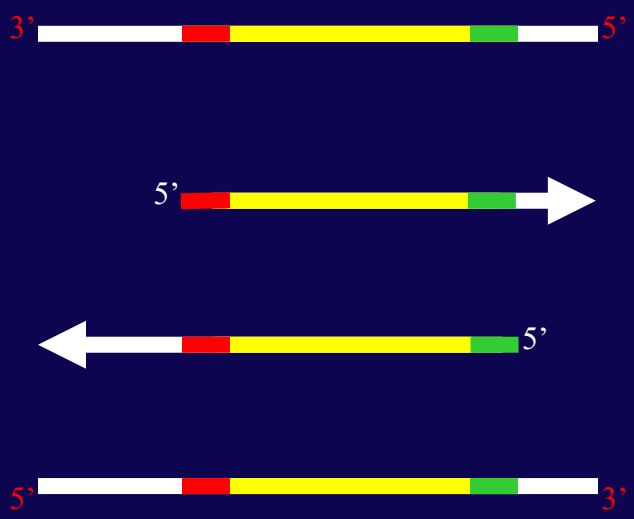
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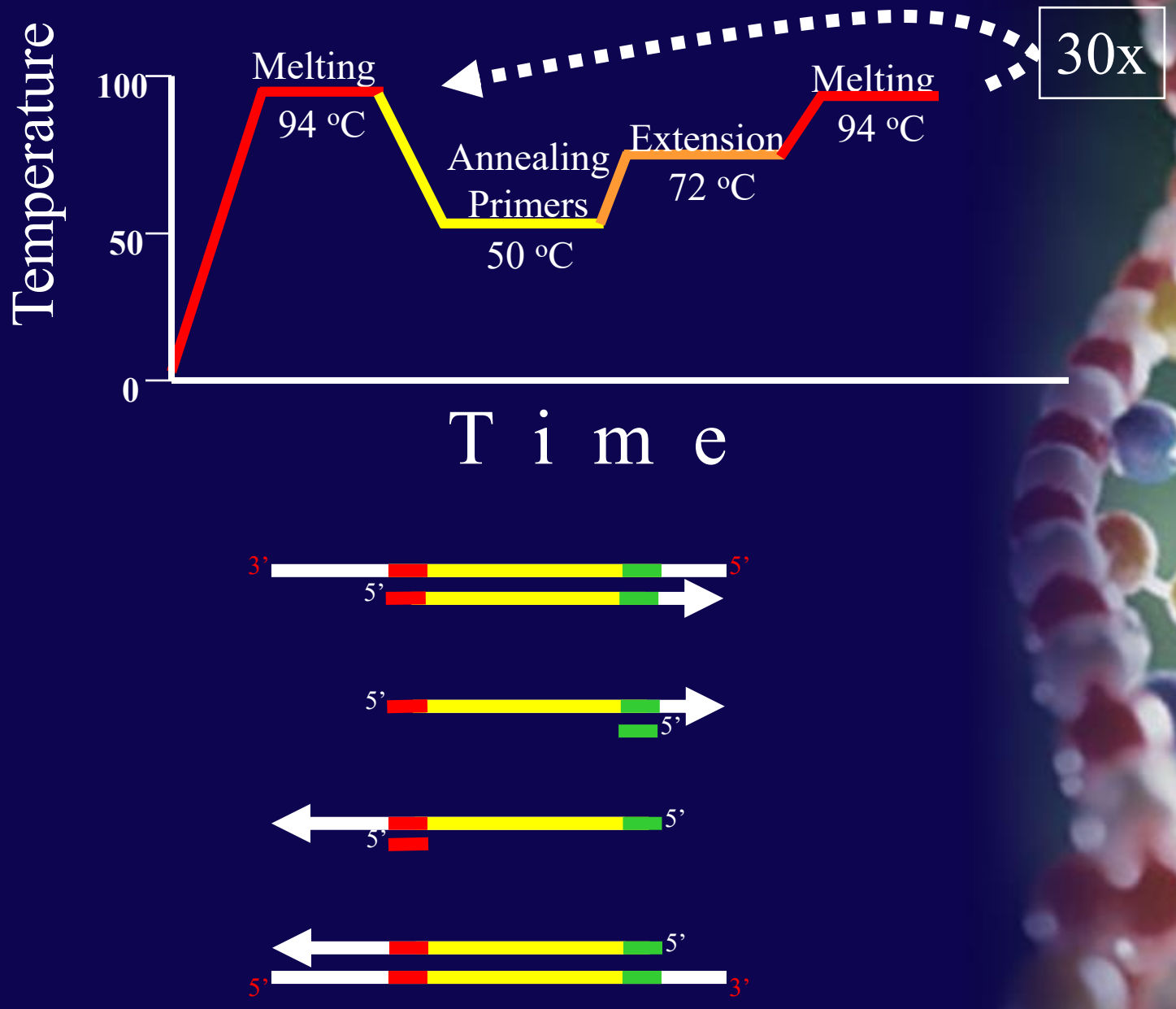
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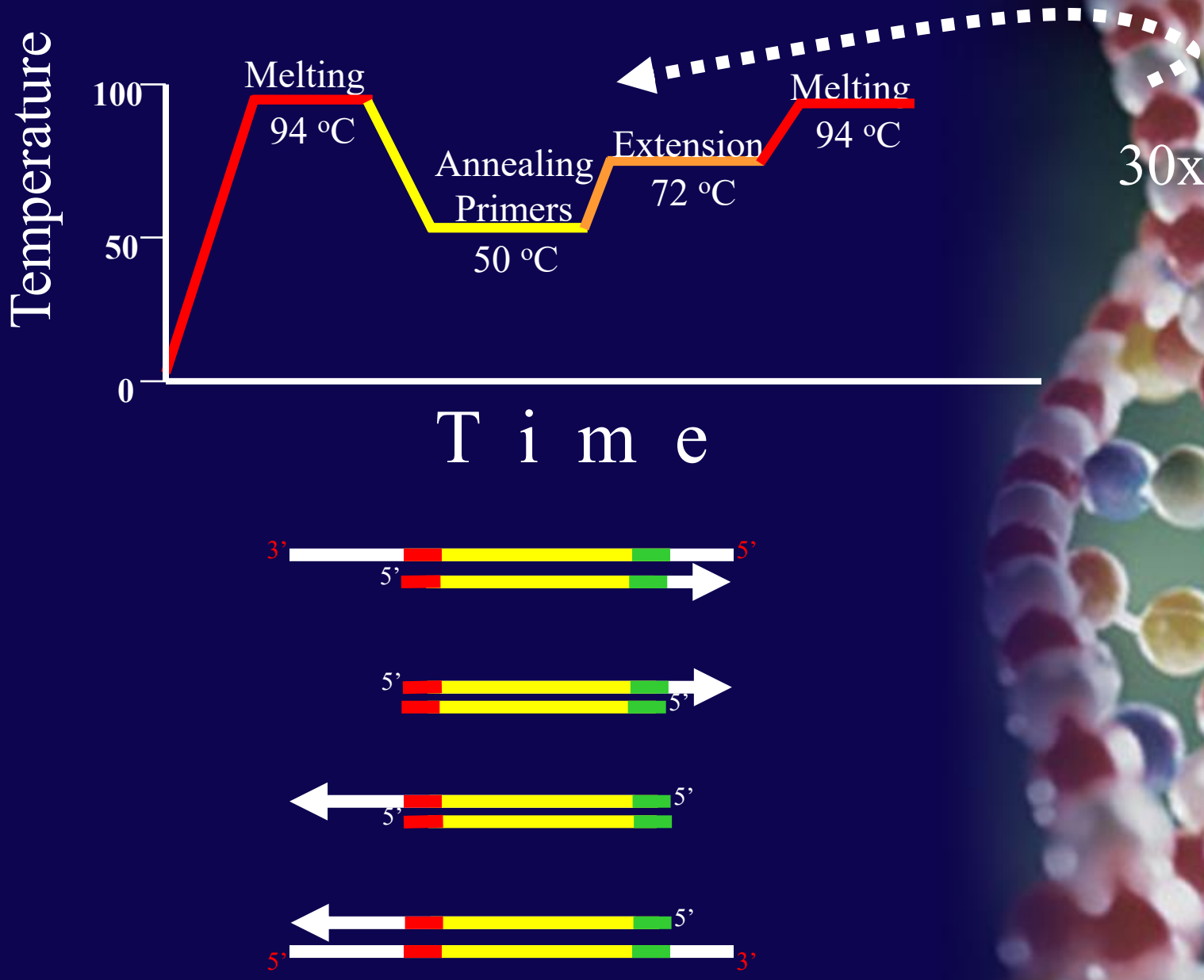
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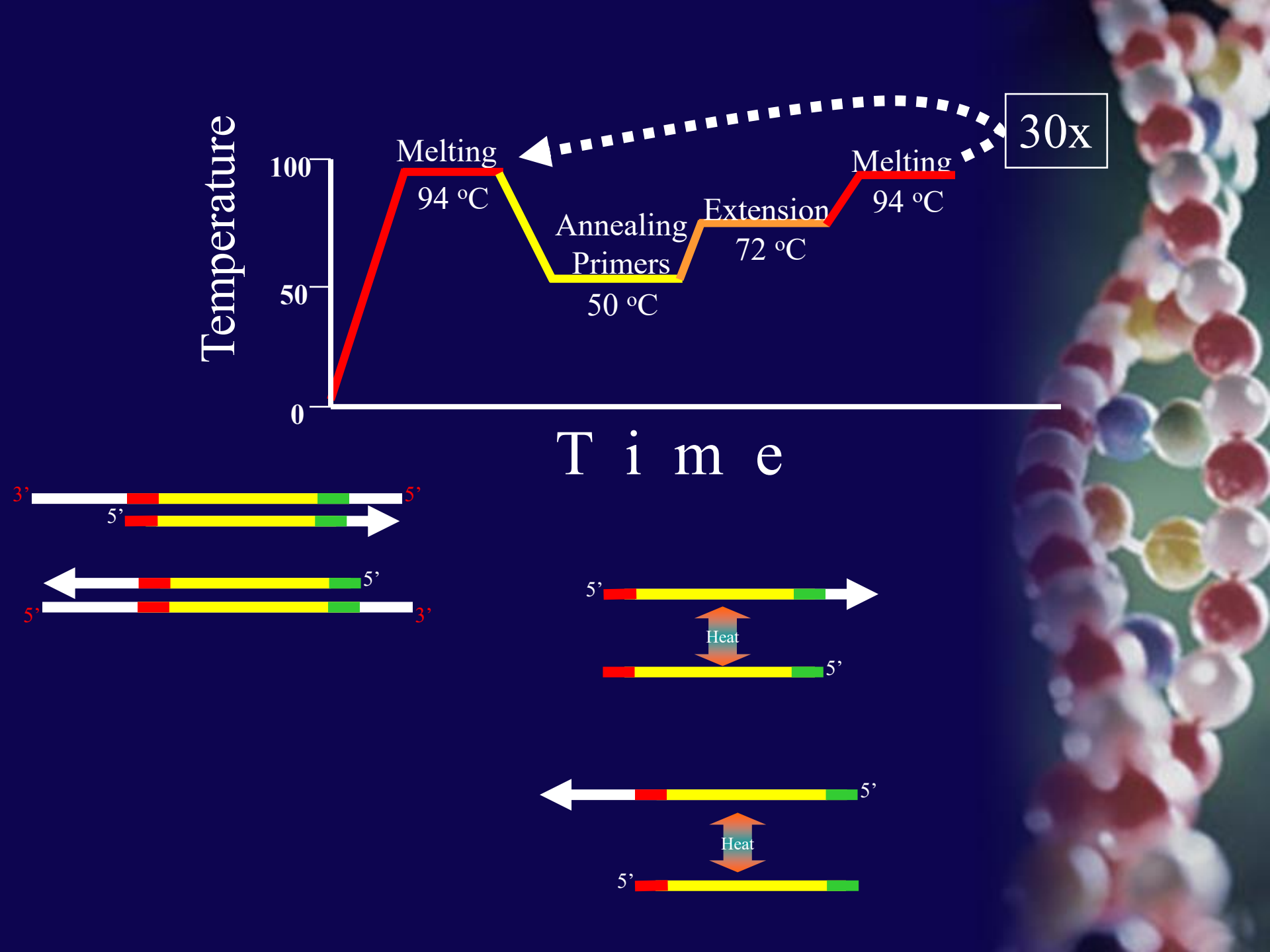
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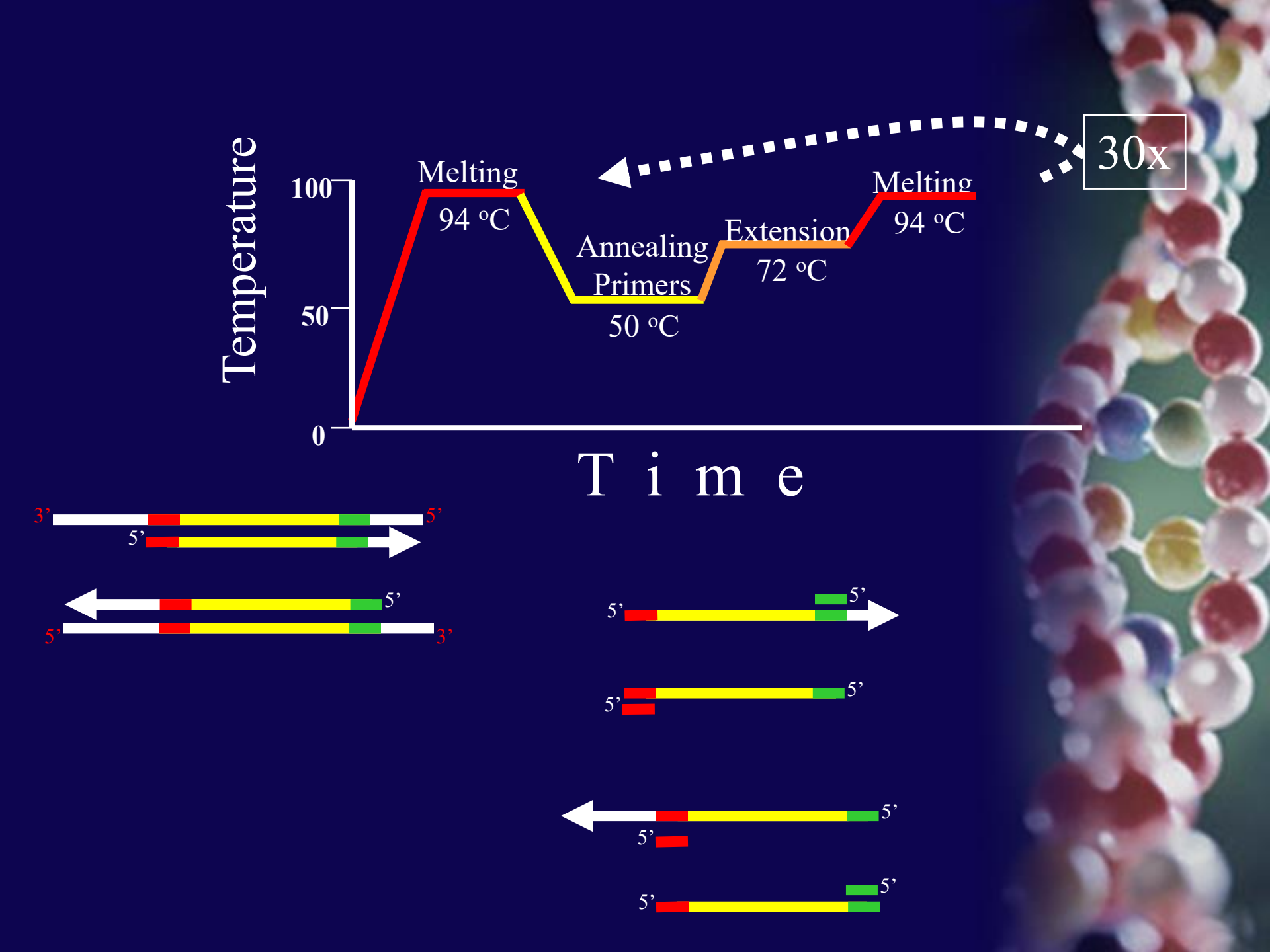
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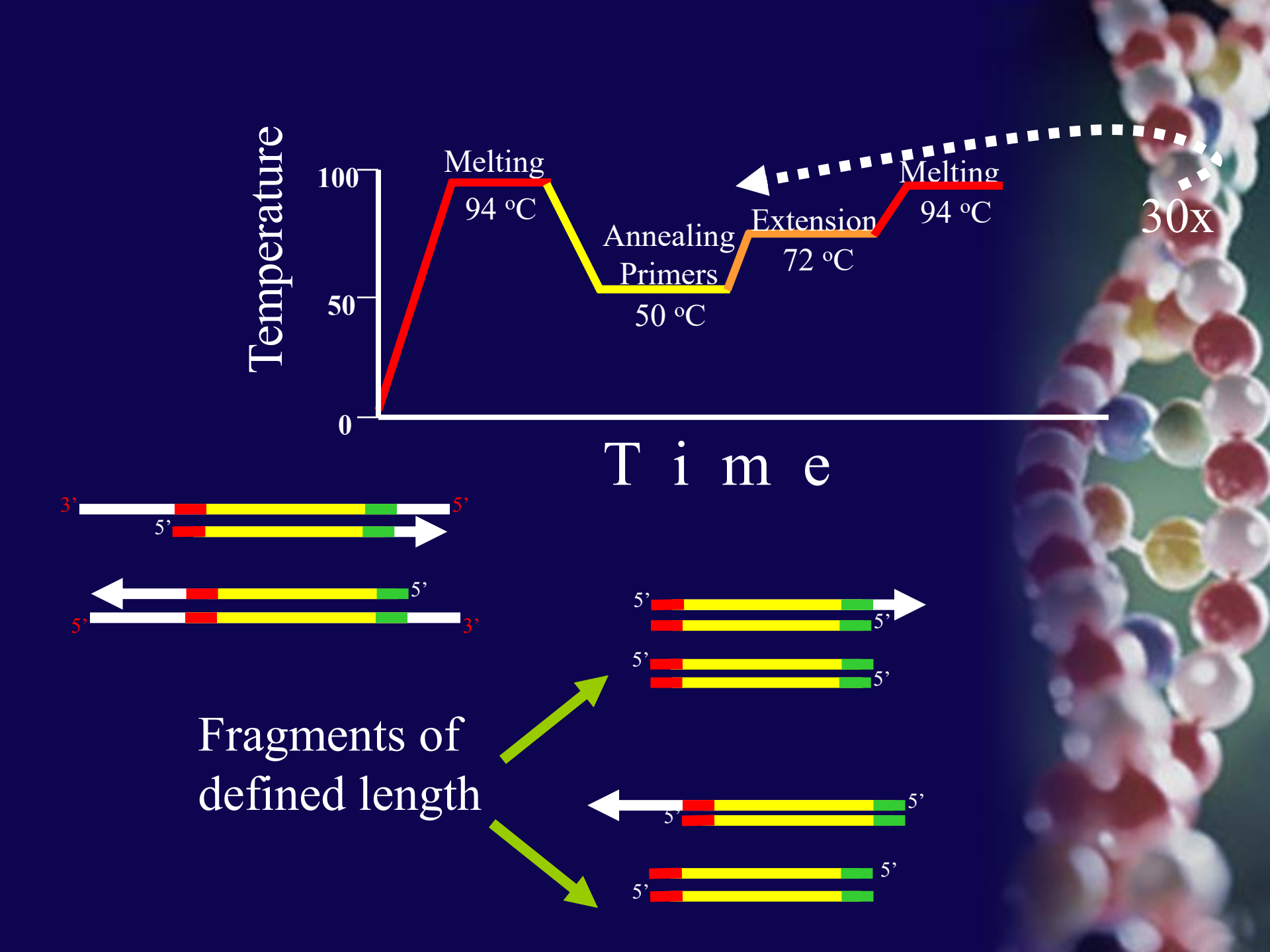
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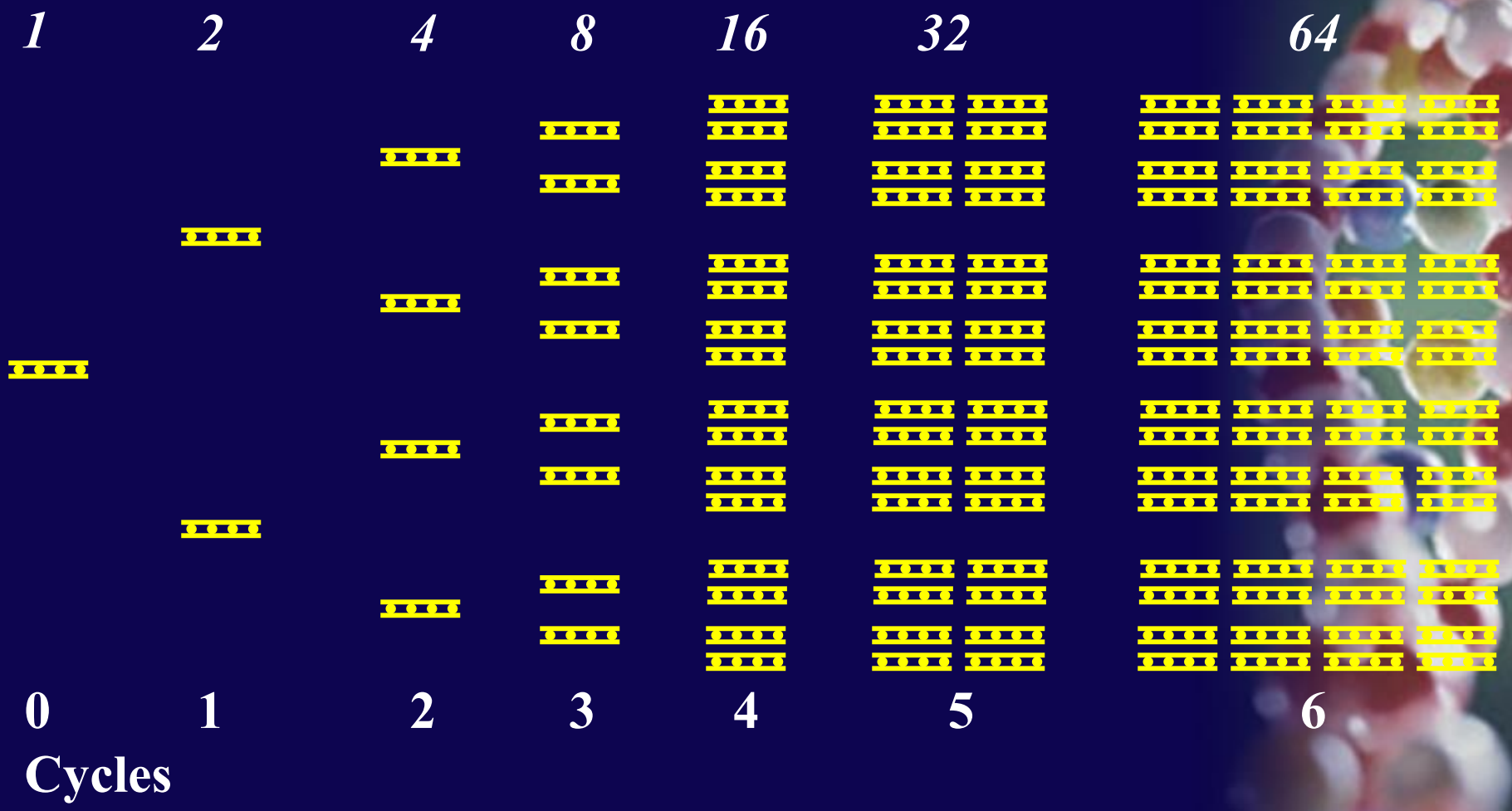
DNA Between The Primers Doubles With Each Thermal Cycle



Theoretical Yield Of PCR

Theoretical yield = 2^n x y

Where y = the starting number of copies and

n = the number of thermal cycles

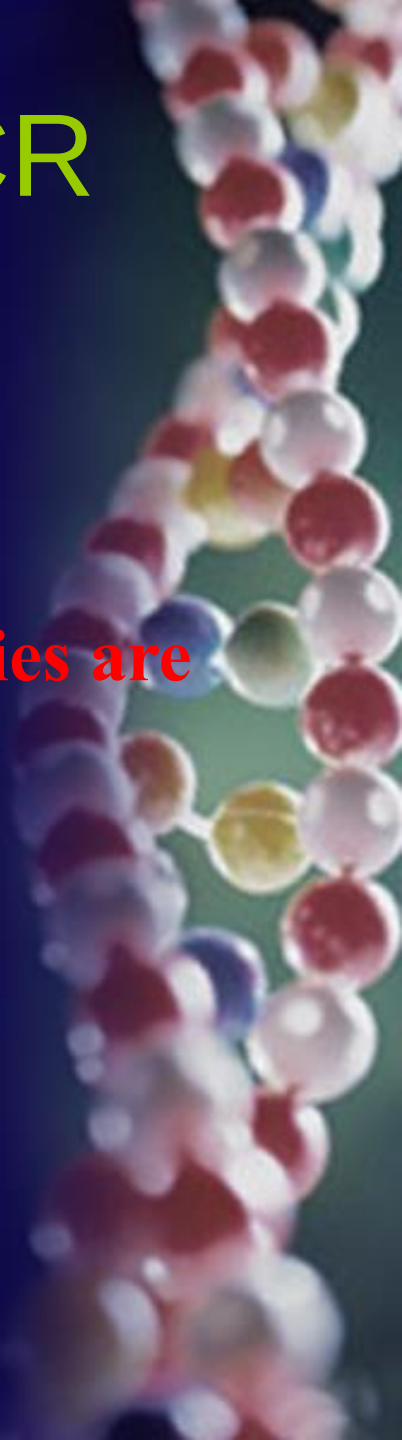
If you start with 100 copies, how many copies are made in 30 cycles?

2^n x y

= 2^{30} x 100

= 1,073,741,824 x 100

= 107,374,182,400



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How do we identify and detect a specific sequence in a genome?

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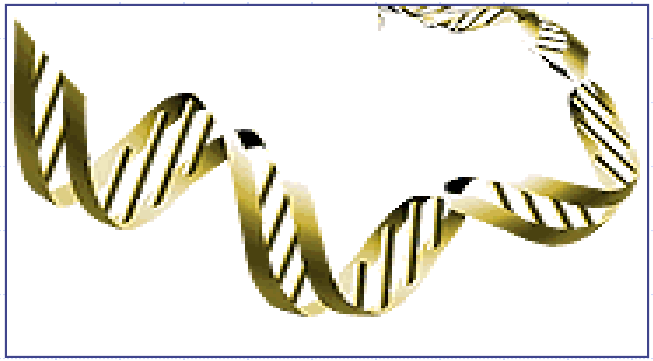

- TWO BIG ISSUES:
  - There are a LOT of other sequences in a genome that we're not interested in detecting. (SPECIFICITY)
  - The amount of DNA in samples we're interested in is VERY small. (AMPLIFICATION)

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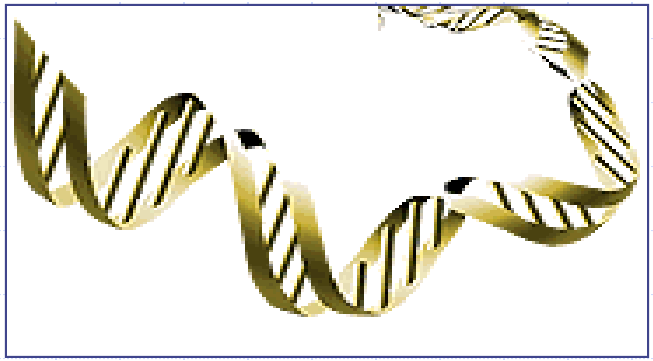

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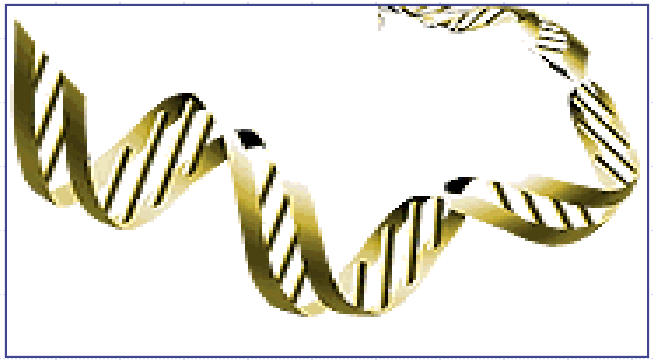

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Just How Big Is 3.4 Billion?

- Human genome is 3.4 B bp
- If the bases were written in standard 10-point type, on a tape measure...
- ...The tape would stretch for 5,366 MILES!

- Identifying a 500bp sequence in a genome would be like finding a section of this tape measure only 4 feet long...

How many molecules do we need to be able to see them?

- To be visible on an agarose gel, need around 10 ng DNA
- For a 500-bp product band, weighing 660 g/mol.bp, therefore need 10e-9 / (500*660) = 3.03e-14 moles
- Avogadro's number = 6.02e23
- Therefore need 1.8e10 copies!

- In other words, to "see" a single "gene", the DNA in a sample of 100 cells would have to be multiplied 180 million times!!!!!

The Problem...

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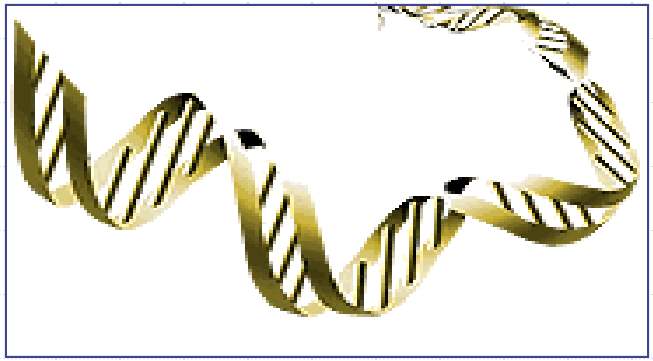

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Genetic engineering and its application in medicine:

-To produce large amounts of biologically useful proteins. These include:

A-recombinant human proteins used as drugs or hormones like the following:

Proteins	**Their importance**
Chorionic gonadotropin	Treatment of inferility
Insulin	Treatment of Diabetes mellitus
Interleukins	Treatment of Cancer
Interferons (β,γ)	Treatment of Viral infection, cancer
Growth hormones	Treatment of Growth retardation

B-Recombinant enzymes with industrial uses:

Proteins	**Industrial use**
Rennin	Cheese makingff Cheese making
Protease	Detergents
Lipase	Cheese making
Catalase	Antioxidants in food

2- To create organisms (plant, animal, and microorganisms) with altered characteristics, for example, plants are resistance to disease.

3- In the diagnosis of most medically important bacteria e.g., tubercle bacilli (*Mycobacterium tuberculosis)* is diagnosed with the technique of polymerase chain reaction which is highly sensitive and specific .

4-For understanding the molecular basis of a number of disease e.g., familial hypercholesterolemia, sickle cell disease, thalassemias, cystic fibrosis.

5- For production of proteins for vaccination e.g., vaccine of hepatitis B surface antigen (HBsAg),and for diagnostic test (e.g., AIDS test)

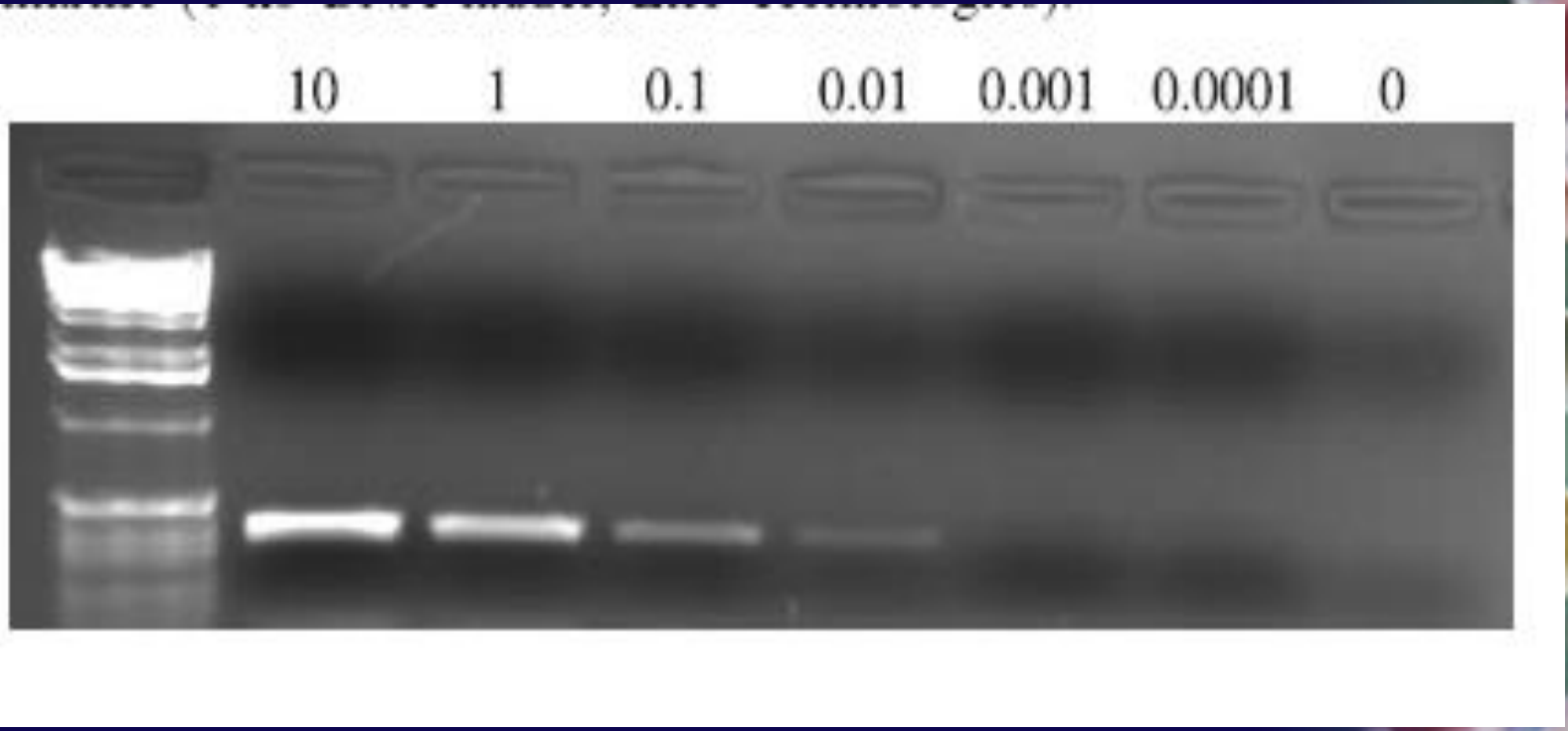
6- In the gene therapy of sickle cell disease, thalassemias and other diseases

PCR History

In what has been called by some the greatest achievement of modern molecular biology, **Kary B. Mullis** developed the **polymerase chain reaction** (PCR) in 1983. PCR allows the rapid synthesis of designated fragments of **DNA**. Using the technique, over one billion copies can be synthesized in a matter of hours.

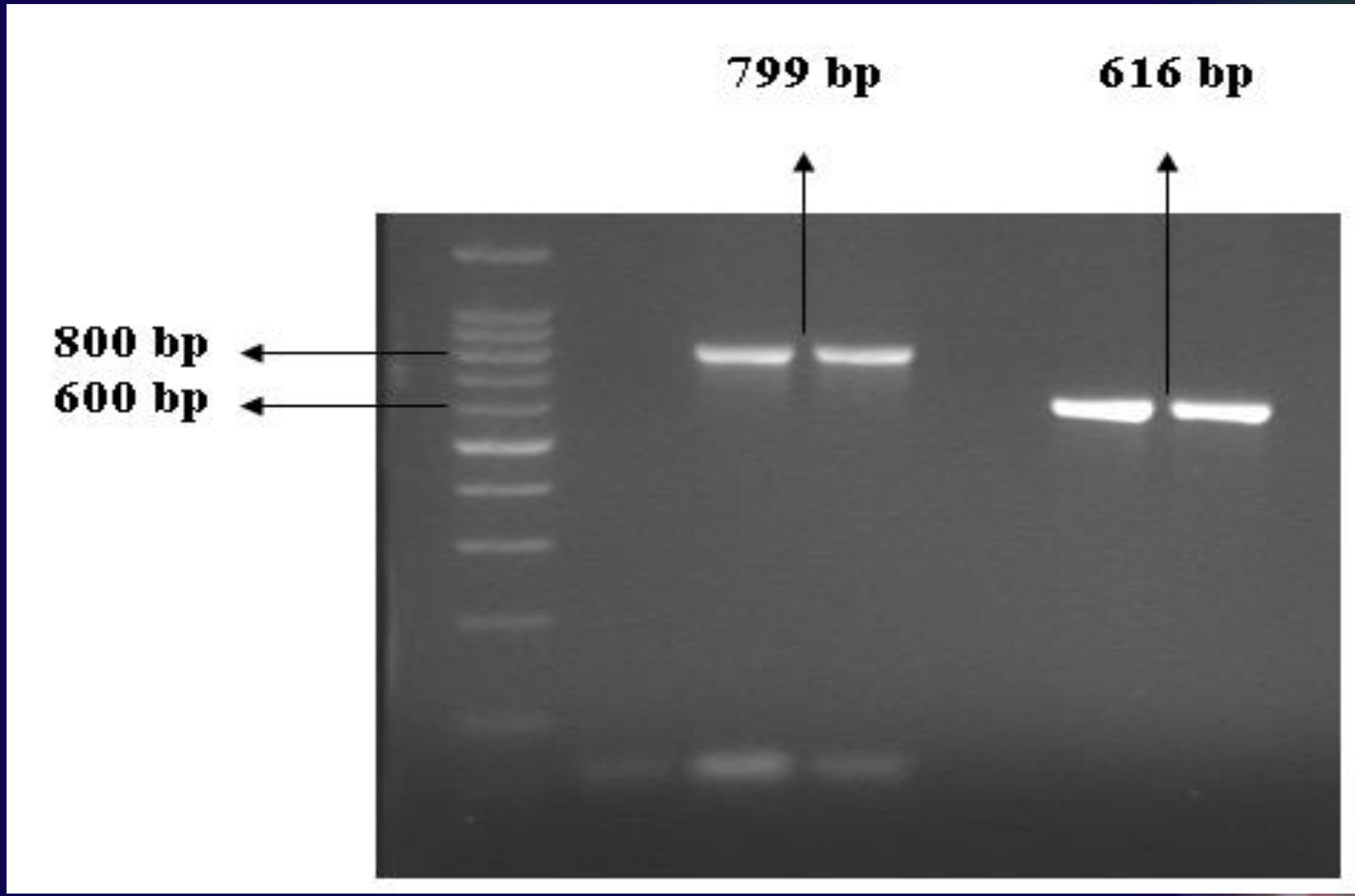
PCR is valuable to scientists by assisting **gene mapping**, the study of gene functions, cell identification, and to forensic scientists in criminal identification. Cetus Corporation, Mullis' employer at the time of his discovery, was the first to commercialize the PCR process. In 1991, Cetus sold the PCR patent to Hoffman-La Roche for a price of $300 million. It is currently an indispensable tool for molecular biologists and the development of genetic engineering.

Isolation and PCR amplification of a speciesspecific oxidoreductase-coding gene region in *Listeria grayi*



Determination of the sensitivity of *Listeria grayi* specific PCR. *Listeria grayi* ATCC 25400 DNA was serially diluted and amplified with primers lgr20-246F and lgr20-246R.

PCR products



Requirements for a PCR reaction

- Template (DNA, cDNA, whole cells)
- dNTPs (dATP, dCTP, dGTP, dTTP)
- Thermostable polymerase
- Primers: oligonucleotides that are complimentary to your gene of interest, and prime DNA synthesis (5'-3') towards each other

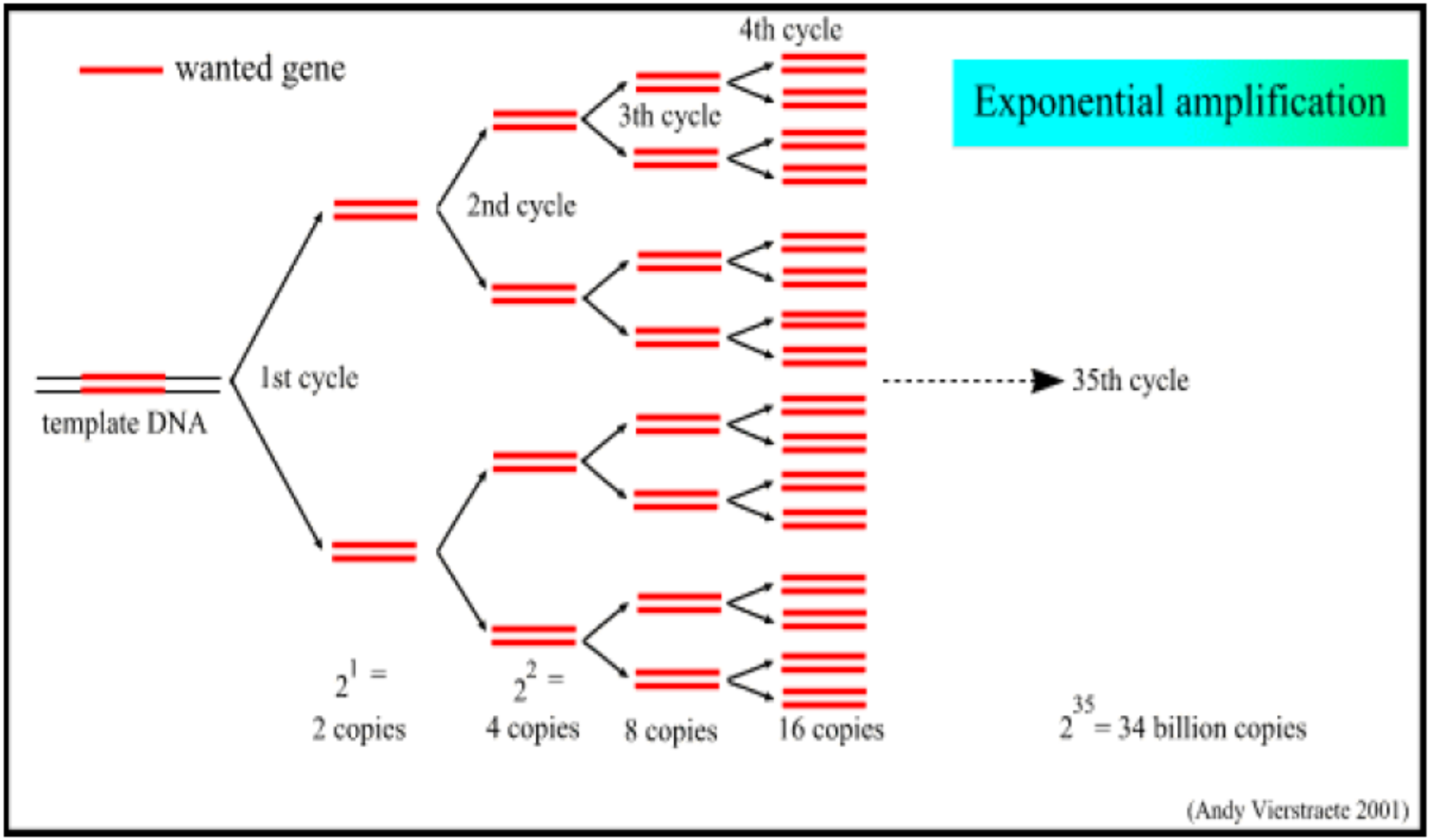
The Polymerase Chain Reaction (PCR)

- An alternative method was developed in the 1980's (Kary Mullis) that allowed the selective amplification of any piece of DNA in a heterogeneous population-in less than 4 hrs!

- The only requirement is that you know or can infer the sequence of your gene of interest.

- Furthermore, this technology has been co-opted to produce extraordinary advancements in a variety of fields of study.

Overview of PCR



Overview of PCR

- Each cycle of PCR results in an exponential increase in DNA.
- 35-cycles will produce 34,359,738,370 copies!
- For a small gene (less than 1kb), this can be carried out in a little over an hour!

Types of template DNA for PCR

- **Purified DNA or cDNA**: PCR operates most efficiently when purified DNA is used as a template.
- **Whole cells**: Although less efficient, PCR can be very effective.
- **Environmental samples**.

Uses of PCR

- Most applications involving DNA require relatively large quantities.

- Because PCR can generate a tremendous amplification of DNA, it facilitates many DNA manipulations.

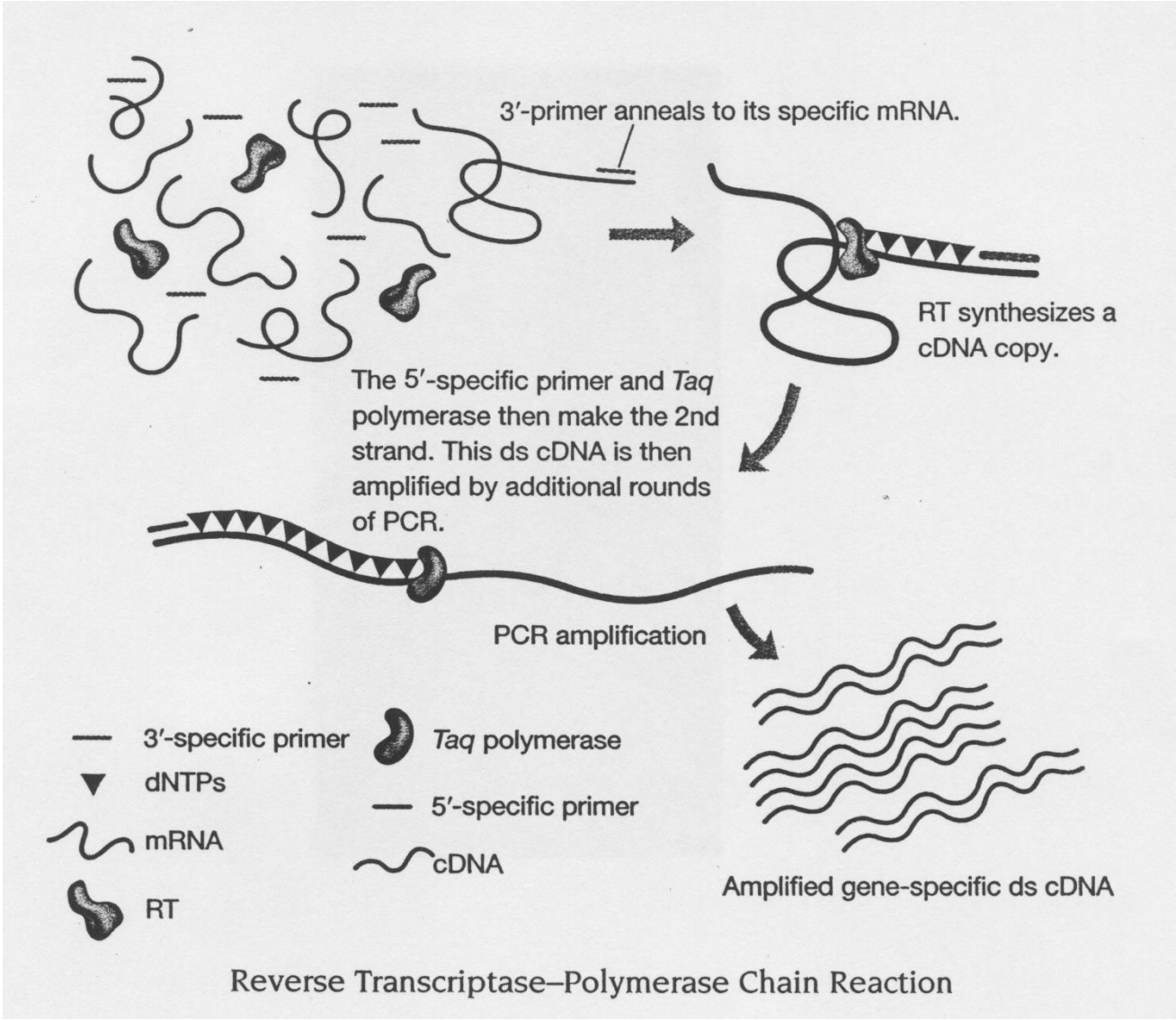
Uses of PCR

- **Cloning**: amplification of DNA for cloning genes, bypassing the need for restriction enzymes and electrophoresis.

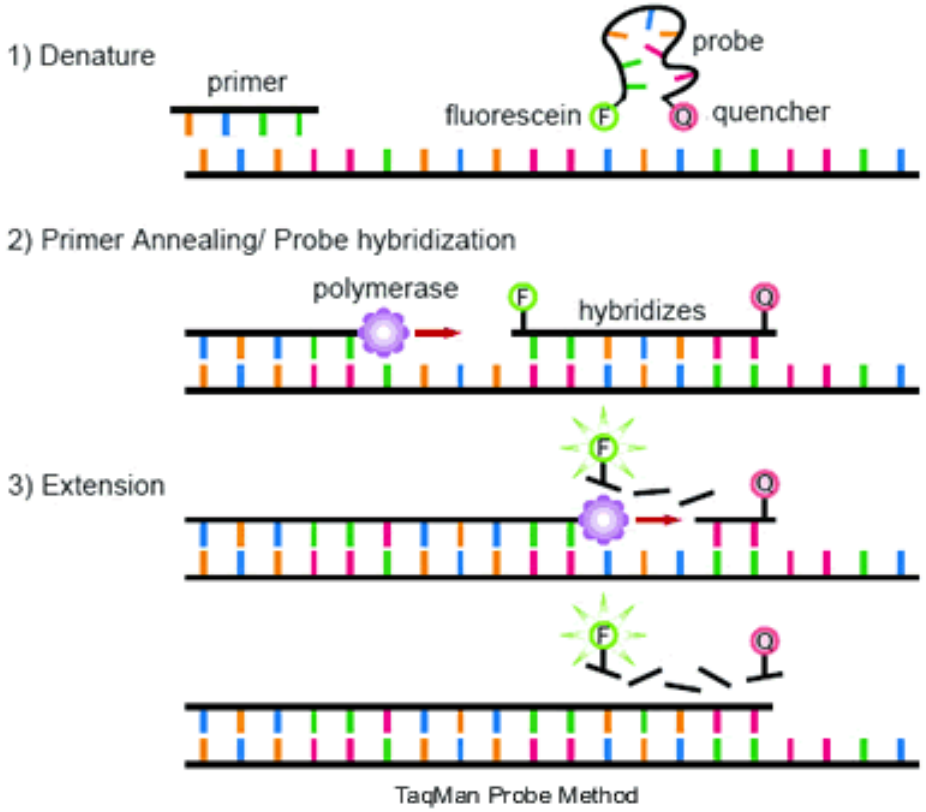
- **Examination of gene expression**: cDNA prepared from mRNA can be used as a template, thus allowing measurement of gene expression.

- **Diagnostic:** PCR allows specific amplification of a gene of interest from a very dilute sample. However, if the gene is not present in the sample, it cannot be amplified. Therefore, PCR can be an excellent diagnostic.

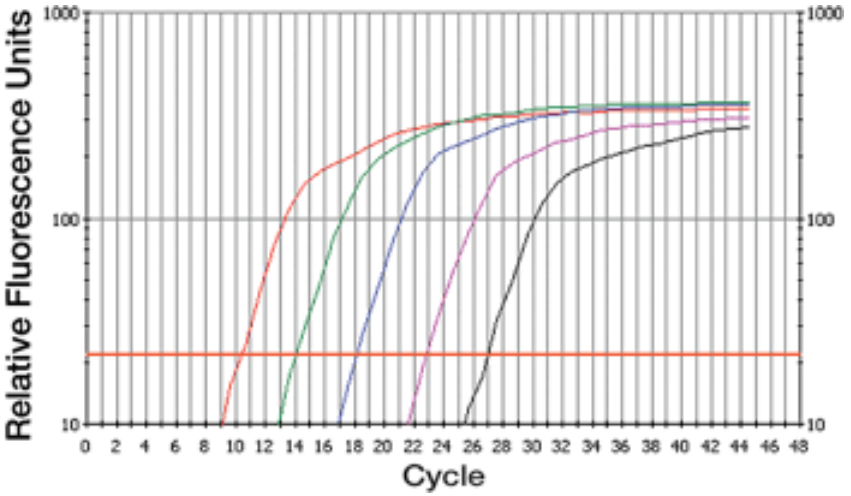
Examination of gene expression: RT-PCR



Real Time PCR



TaqMan Probe Method



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Specificity and contamination

- **Purity of samples:** the extraordinary power of PCR means that contaminants can also be amplified.
- **Primer choice:** if primers anneal to the wrong genomic region, this region will be amplified.
- **Temperature:** Specific base pairing of primers to template DNA only occurs in a narrow range of temperature and salt concentration.

Technical Considerations

- Polymerase fidelity: the thermostable Taq polymerase is commonly used for PCR. However, it incorporates the incorrect base at a rate of 0.0002-0.001 errors/bp.

- The so called “proof-reading” polymerases (e.g. PFU polymerase) result in a lower error rate (1.6×10^{-6} errors/base), but are often more difficult to use.

- The choice of polymerase depends on the required outcome (e.g. gene cloning vs analytical experiment).

Technical Considerations

- Length of amplified sequence. PCR works best for shorter sequences (<5Kb).

- Longer sequences can be amplified, but require the use of alternative protocols (e. g. mixed polymerases).