Lecture 13:

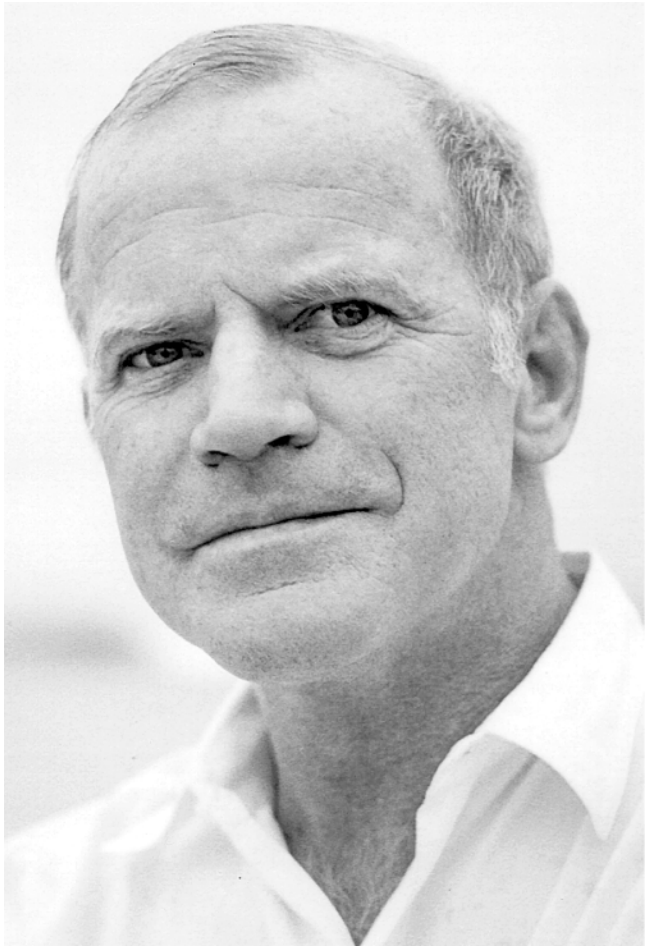
Finding the allele 3:
Polymerase Chain Reaction

Course 410

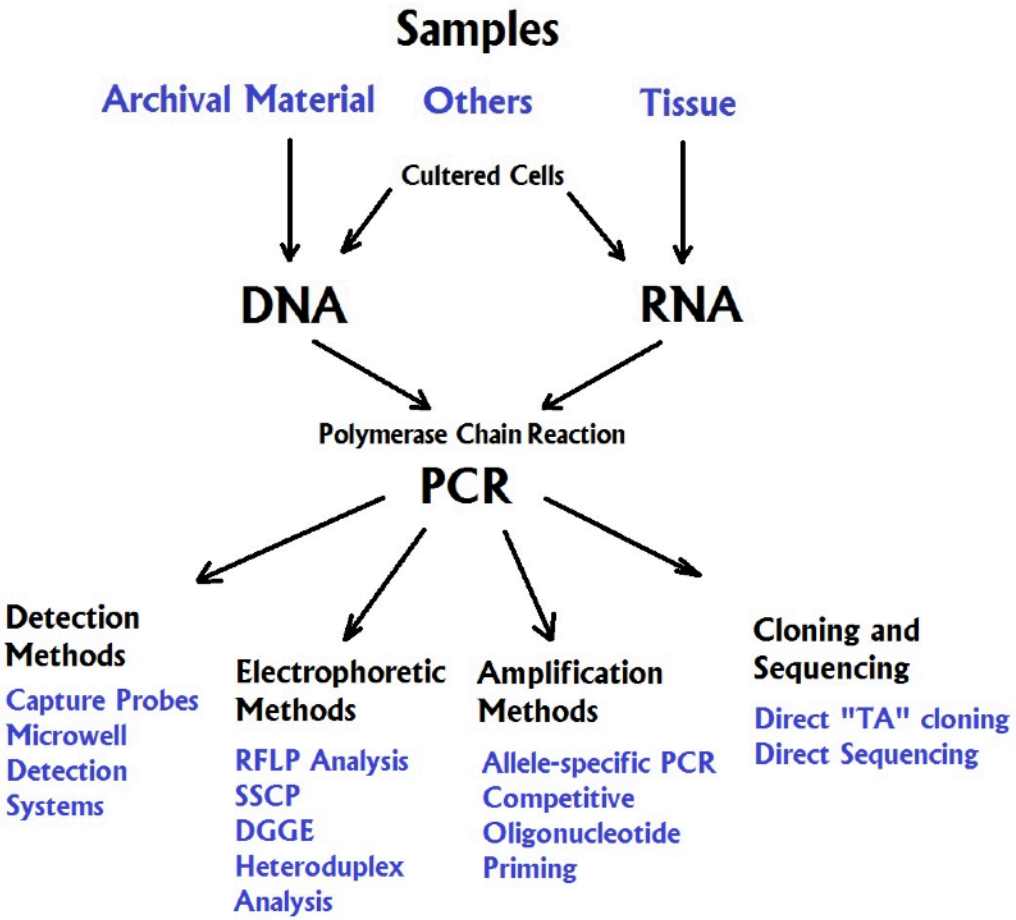
Molecular Evolution

• Polymerase Chain Reaction (**PCR**) allows the amplification (copying) of small amounts of DNA millions of copies.

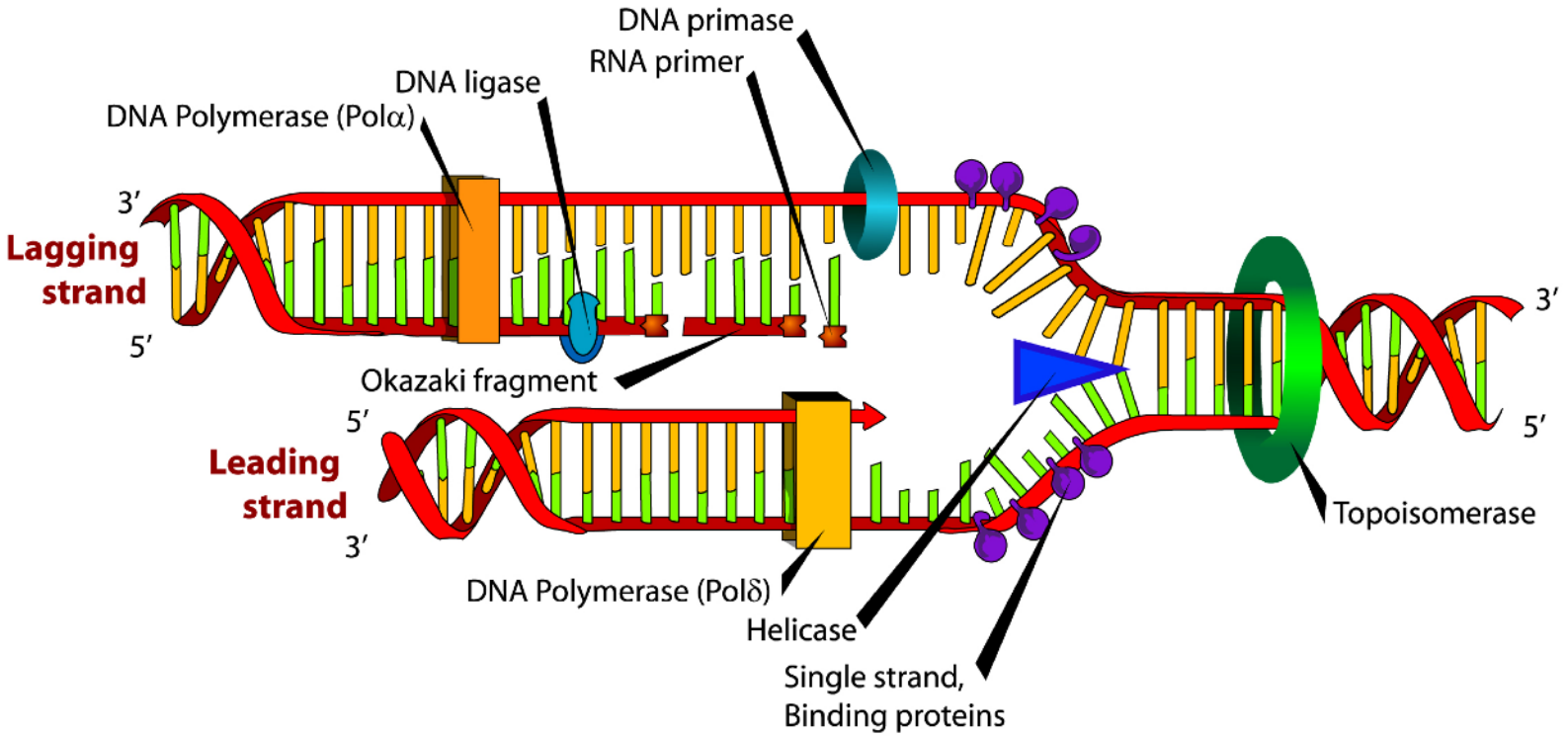
• The method was developed by Kary Mullis (1983) and he was awarded the Nobel Prize for his invention.



- It is considered in many cases the first step before any genetic analysis.
- Many methods and applications involve PCR.



- DNA replication in the cells involves making an identical copy of the genome (DNA).
- PCR uses the same procedure but to generate millions of copies of a small section of the genome in a tube!



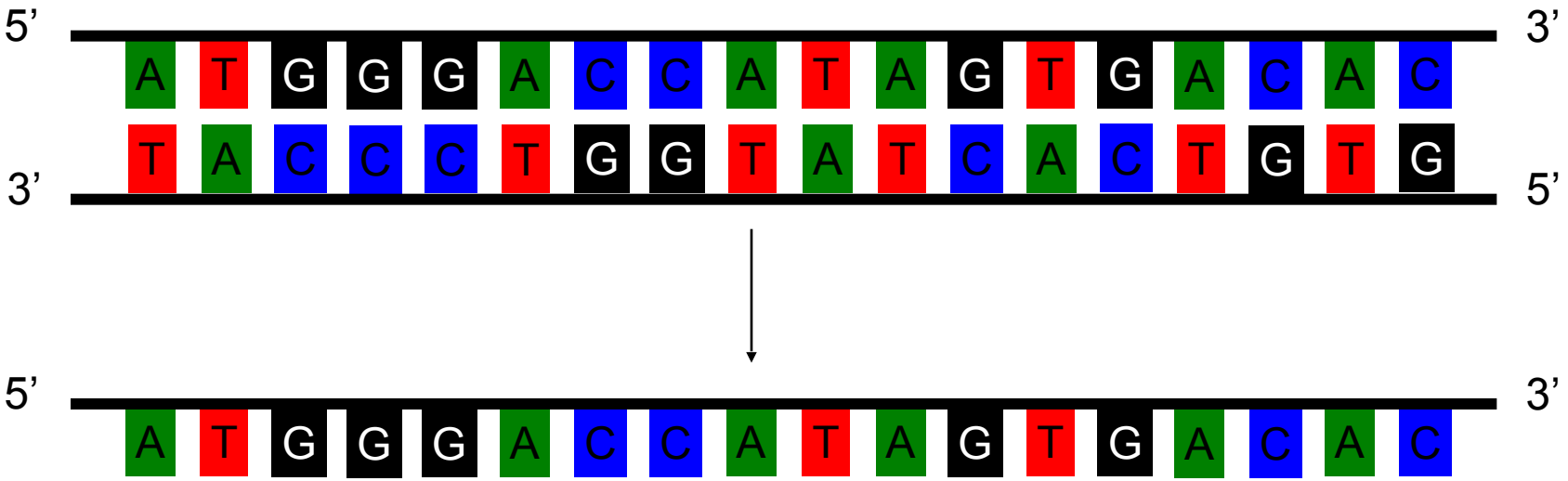
PCR is used:

1. To amplify small quantities of DNA.
2. For DNA quantification.
3. For genetic profile analyses:
    - RFLP
    - Microsatellite
    - Mitochondrial DNA genotyping and sequencing.
4. For sequencing small section of the genome or the genome.

What do we need to replicate (copy) DNA?

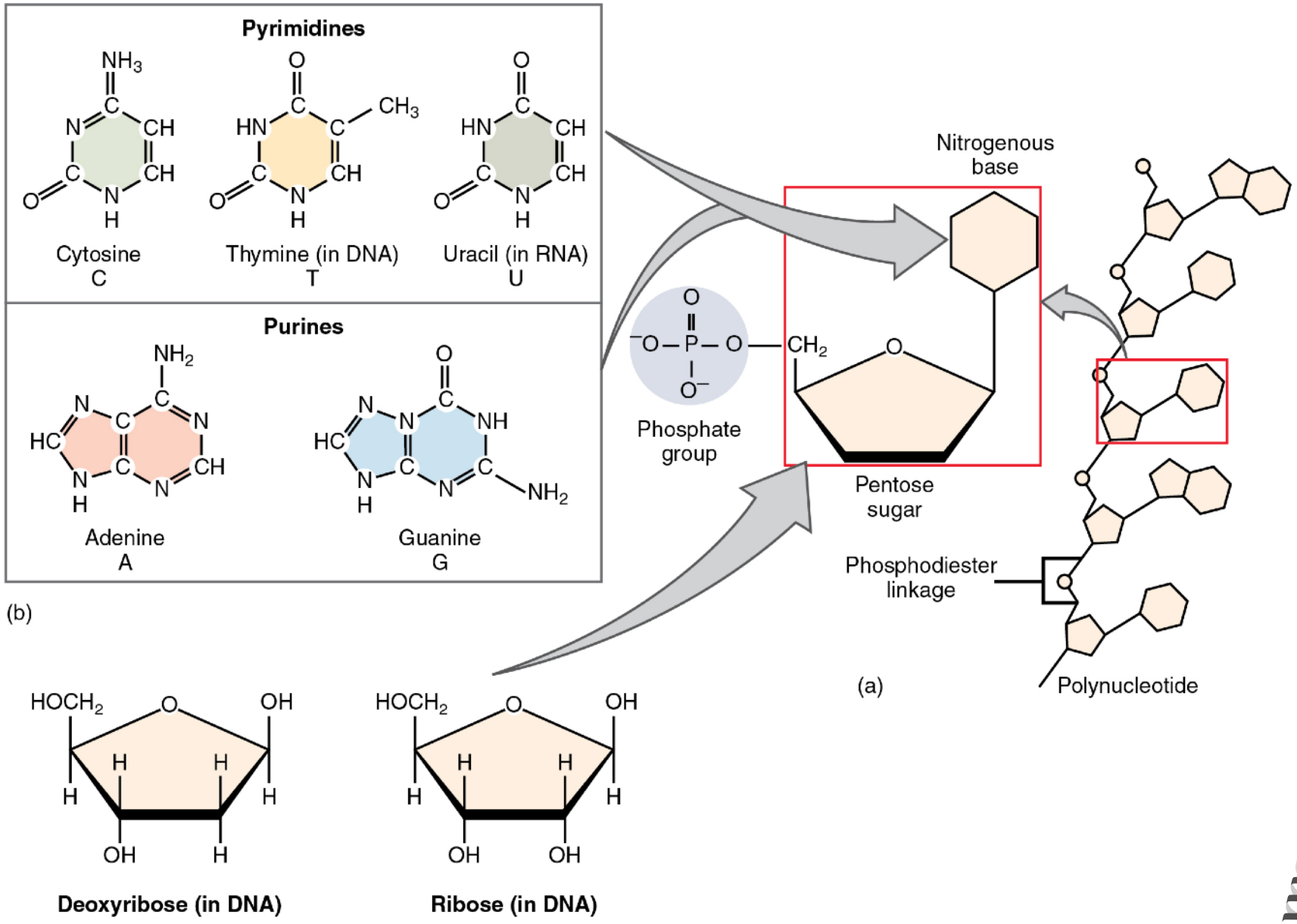
1. DNA template.
2. Building block of DNA (dNTPs).
3. DNA copier (an enzyme).
4. 3’OH (primer).

• The DNA sample you collect from a crime scene or the one under investigation is the DNA template.

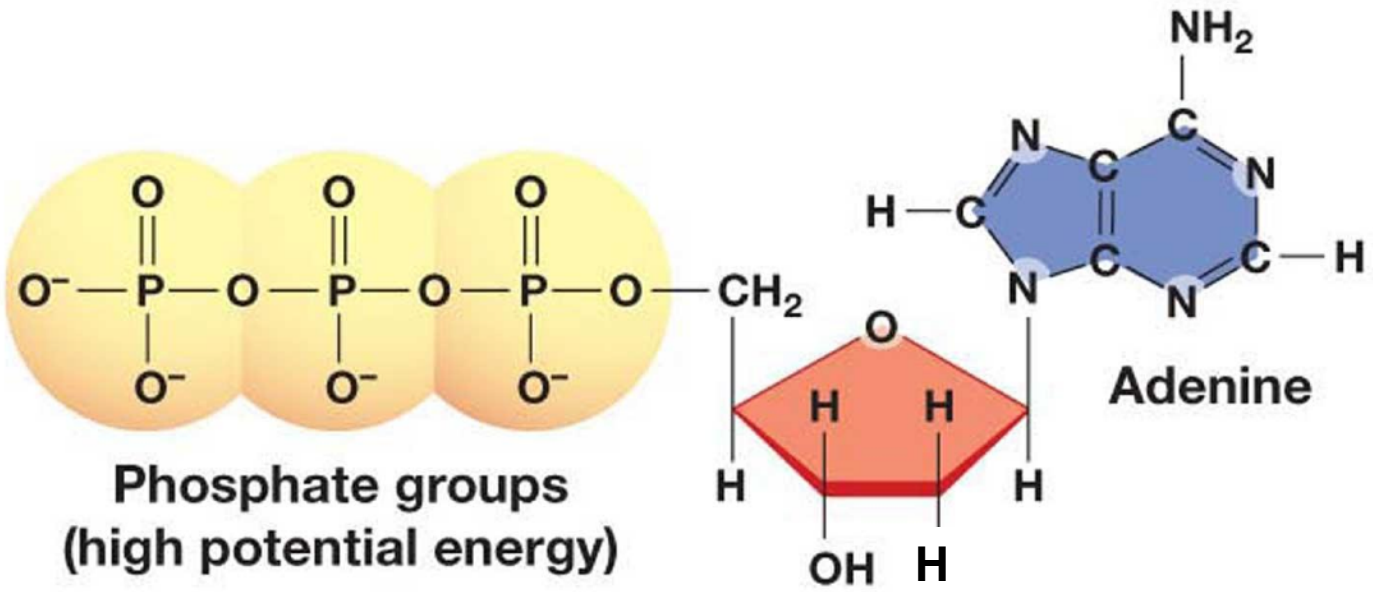


Each strand serves as a **template** for copying.

Remember complementary base-pairing!


Pyrimidines
Cytosine
C
Thymine (in DNA)
T
Uracil (in RNA)
U
Purines
Adenine
A
Guanine
G
(b)
Nitrogenous base
Phosphate group
Pentose sugar
Phosphodiester linkage
Polynucleotide
(a)
Deoxyribose (in DNA)
Ribose (in DNA)
(c)

Deoxyribonucleoside triphosphate (dNTP)

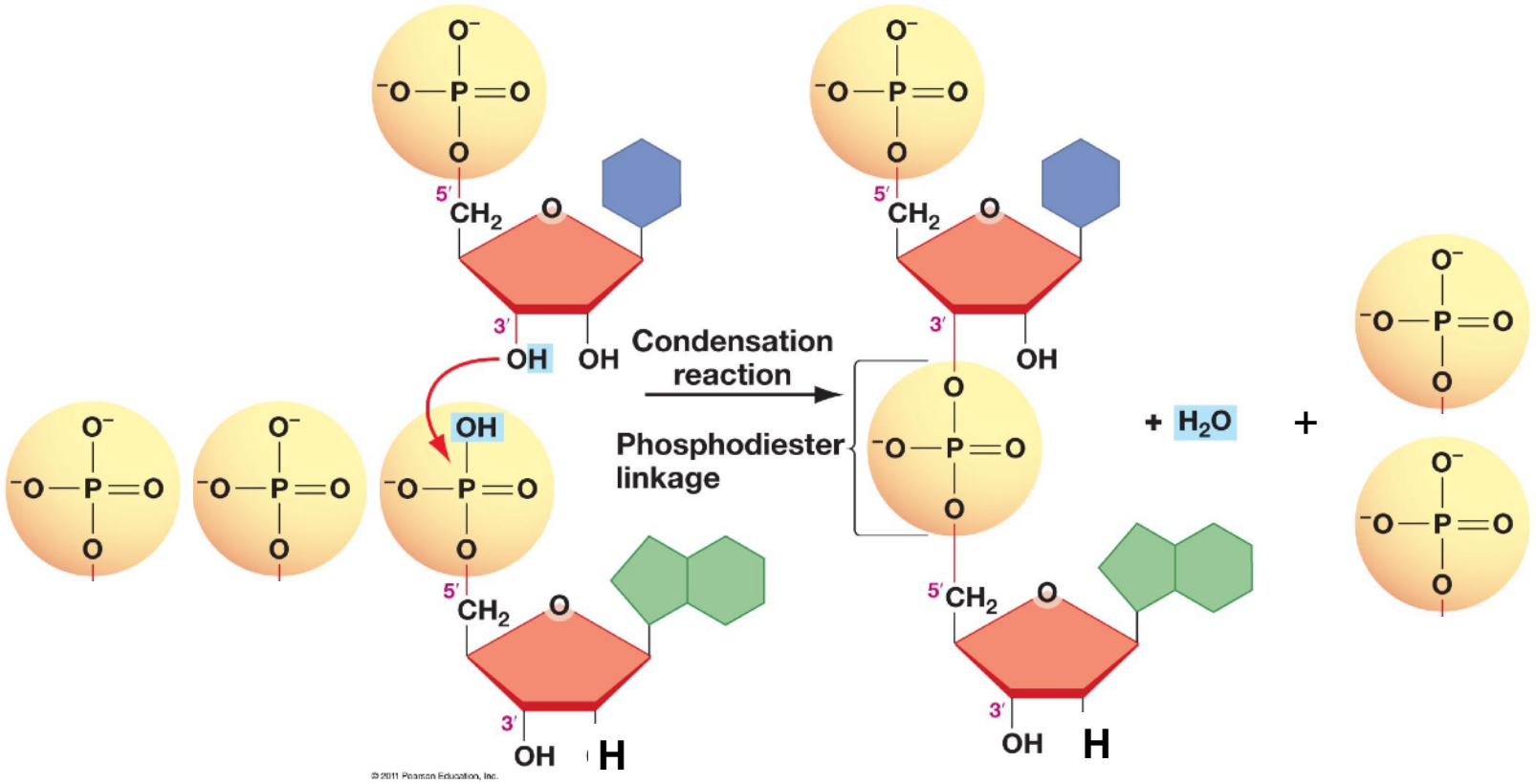


Four dNTPs serve as the building blocks of DNA
(dATP, dTTP, dGTP, dCTP)

Deoxyribonucleoside triphosphate (dNTP)

Why triphosphate?

For the energy required to for the phosphodiester bond

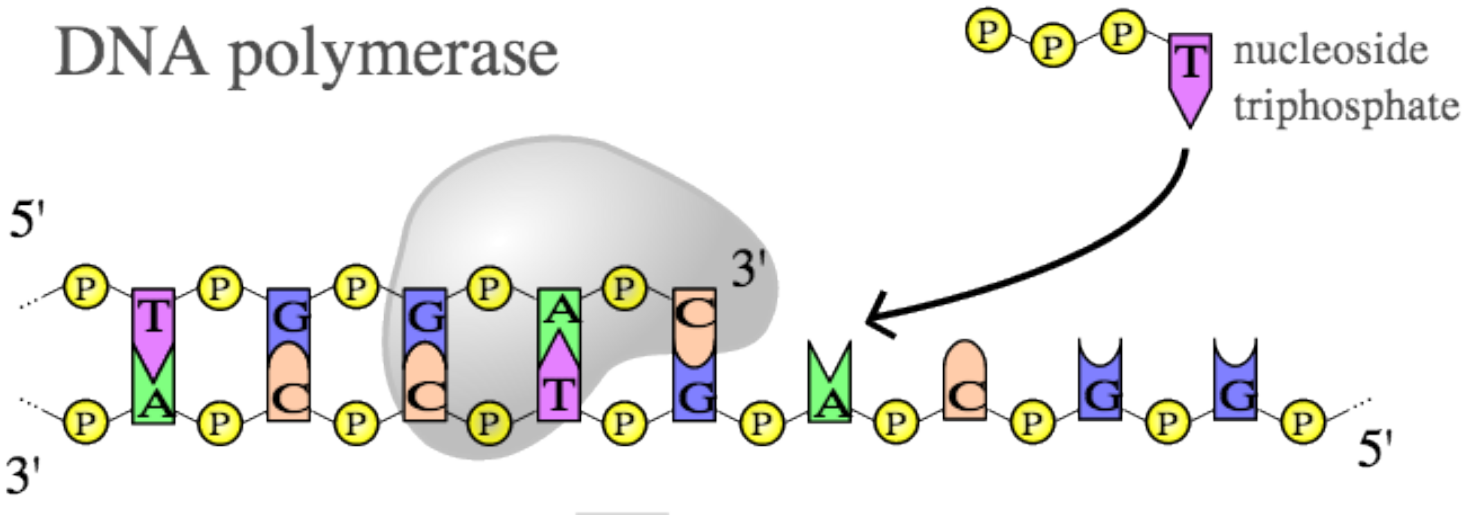


- DNA polymerase is the DNA copier in the cell.

- Uses the dNTPs (DNA building blocks) to make a complementary strand to the template.

- Uses the available 3’-OH of a previous nucleotide and 5’phsphate from dNTP to form a phosphodiester bond.

• Each time DNA Pol finds the correct complementary dNTP and catalyzes the reaction linking the new nucleotide.



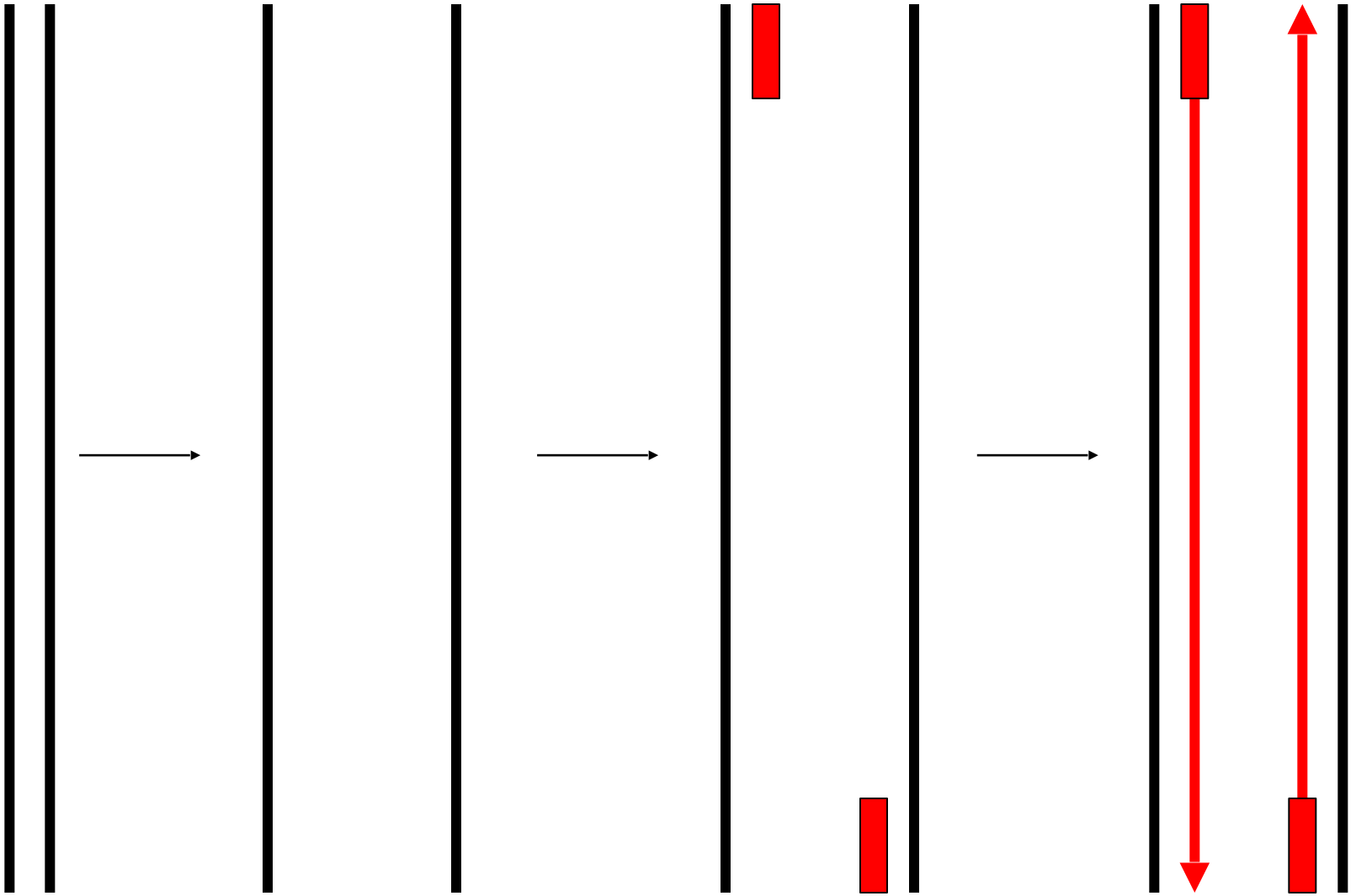
Primers are short piece of polynucleotide

In order for the DNA copying machine to work and add nucleotides,

a 3’-OH needs to be available to form a phosphodiester bond!

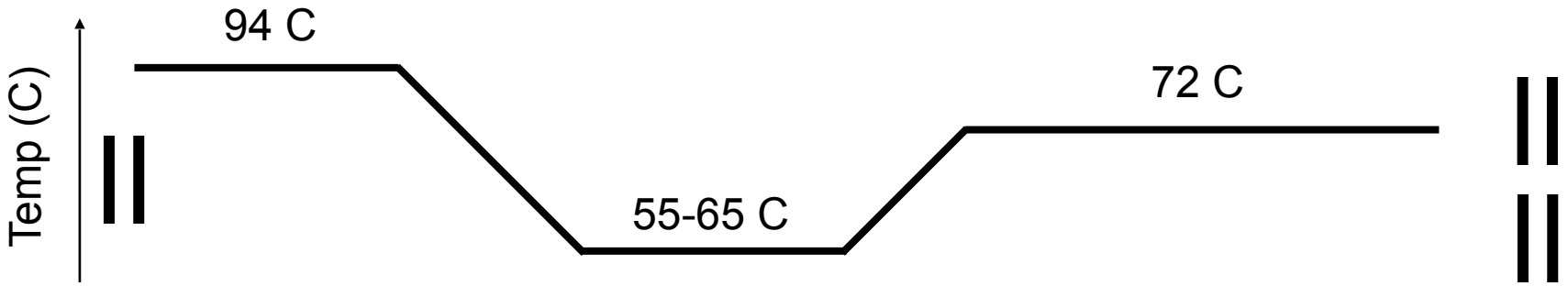
- Three steps are involved in PCR:

    1. **DNA template denaturation:** separation of the two strands of DNA.
    2. **Primers annealing:** small oligonucleotide attaches to each separated strand providing the 3’OH for DNA polymerase.
    3. **DNA polymerization (extension):** DNA polymerase extends the primers on both strands and adds nucleotides.



1. DNA denaturation
2. Primers annealing
3. DNA extension

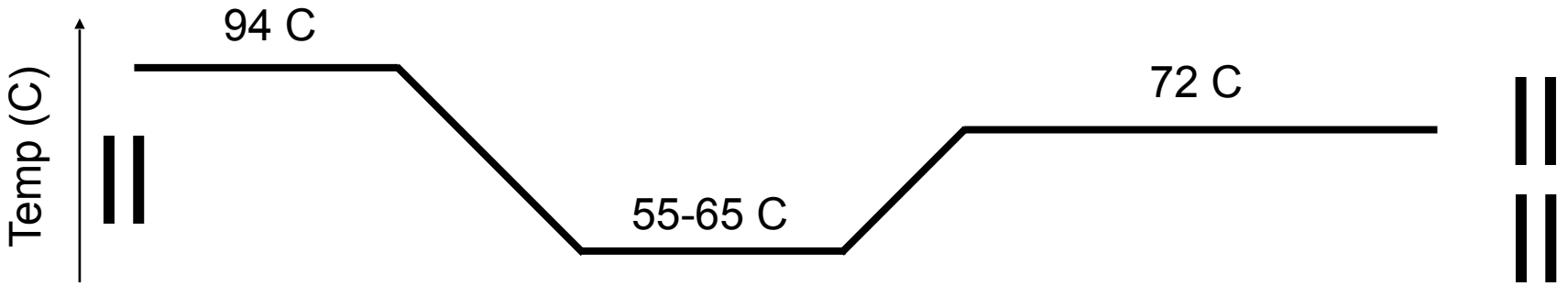
PCR cycles



1. DNA denaturation 2. Primers annealing 3. DNA extension

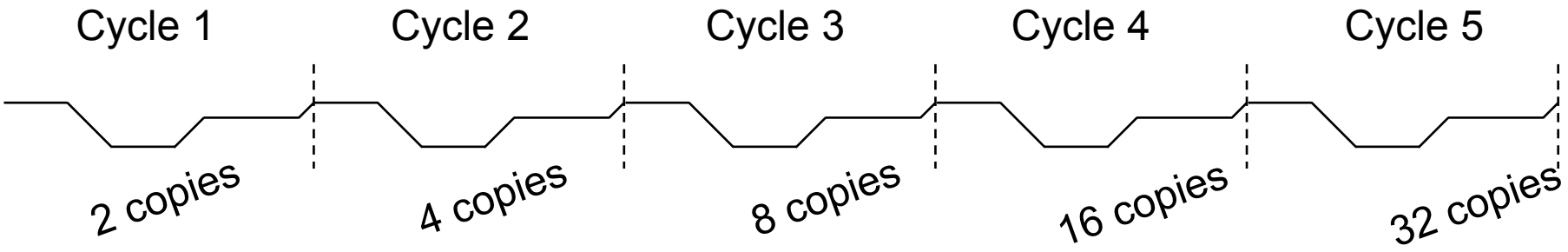
What happens if we repeat this cycle many times?

PCR cycles



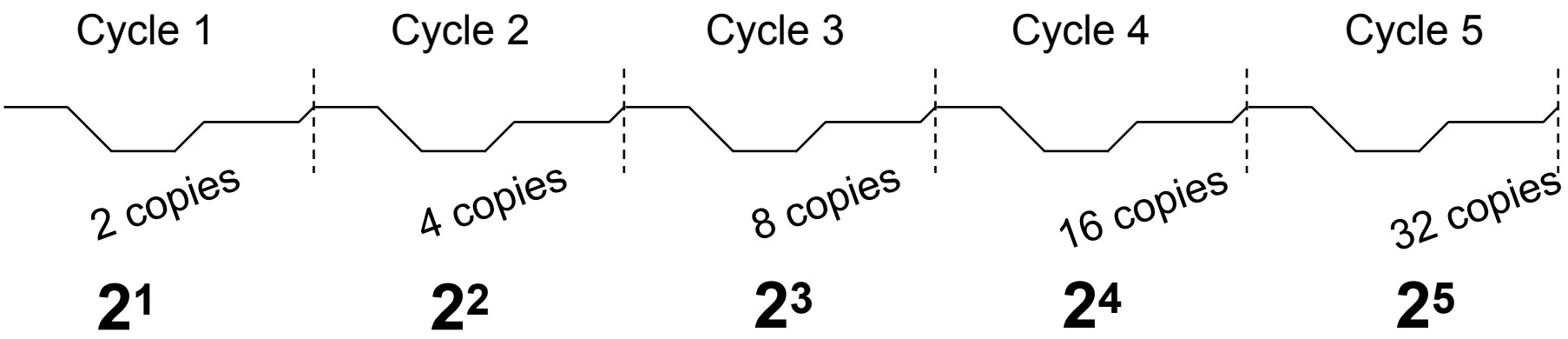
1. DNA denaturation 2. Primers annealing 3. DNA extension

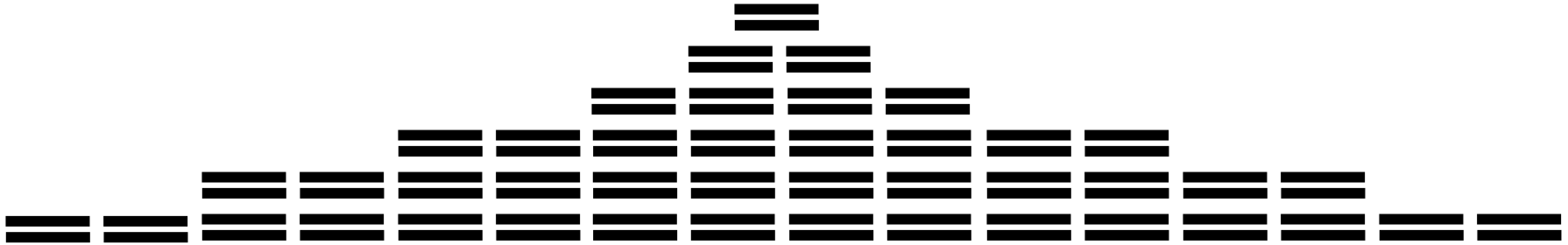
What happens if we repeat this cycle many times?

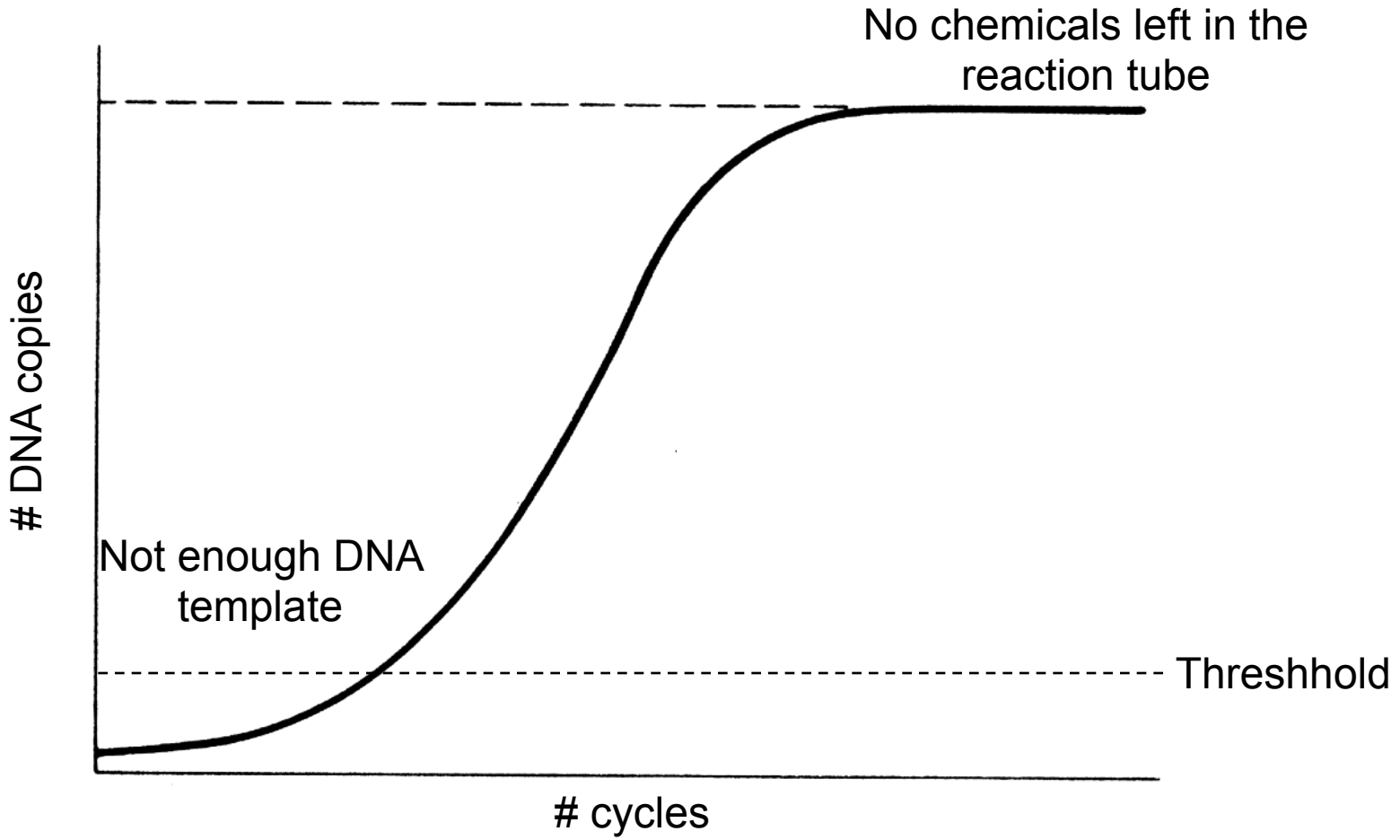


Exponential growth in the number of copies generated.

The number of copies you get at the end of your PCR will be $2^{\#cycles}$ ($2^{36\ cycles}$ = 68 billion copies)






No chemicals left in the reaction tube
DNA copies
Not enough DNA template
Threshhold
cycles

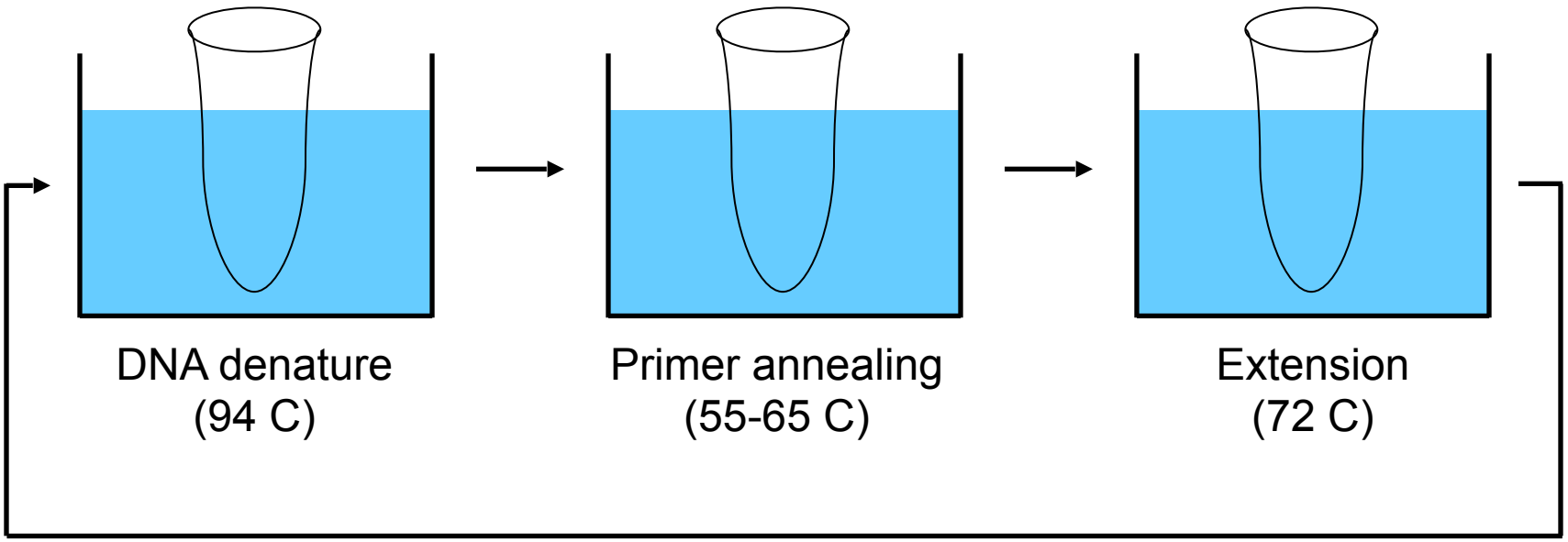
Problems!

There were some difficulties with this system:

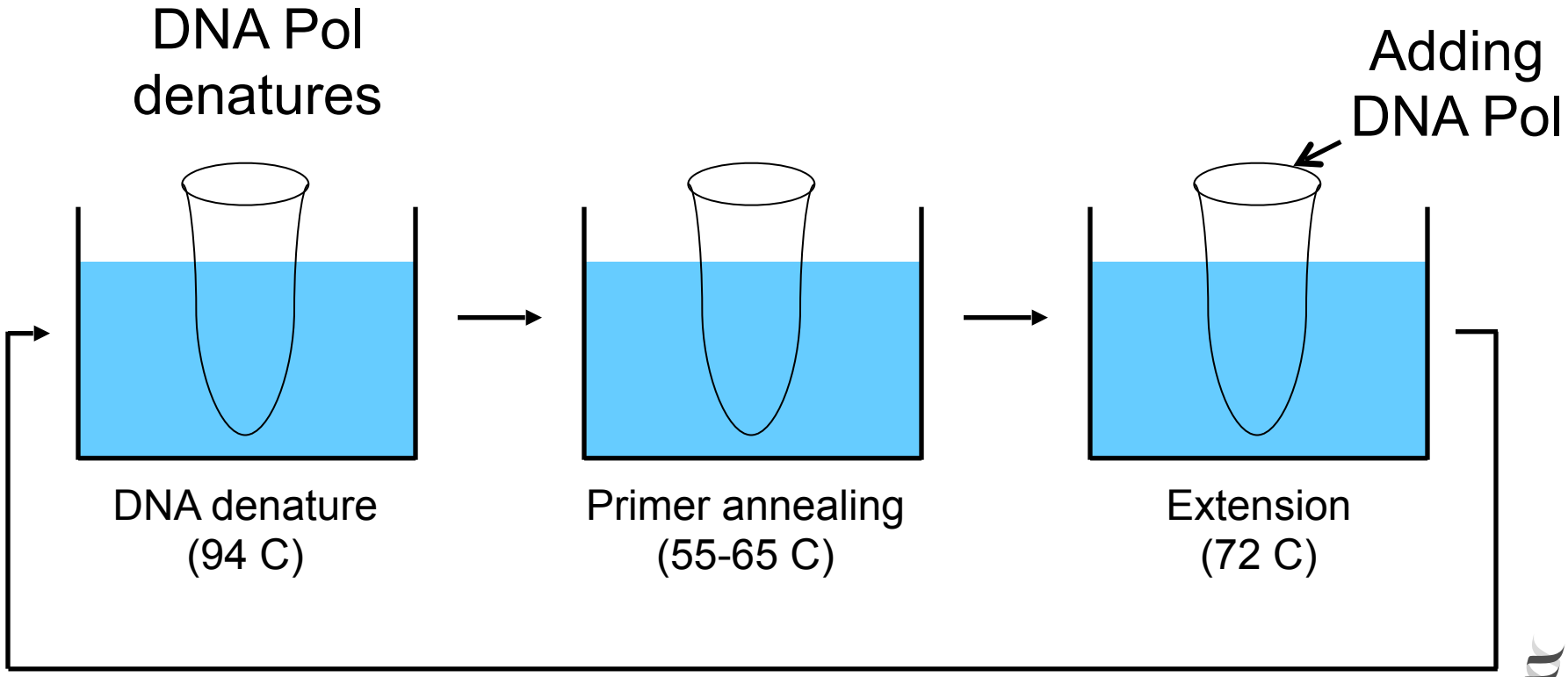
1. Three water-baths with three different temperature.

2. DNA polymerase denatures at 94 C.

- The sample has to be transferred into multiple water baths to accommodate the needed temperature.

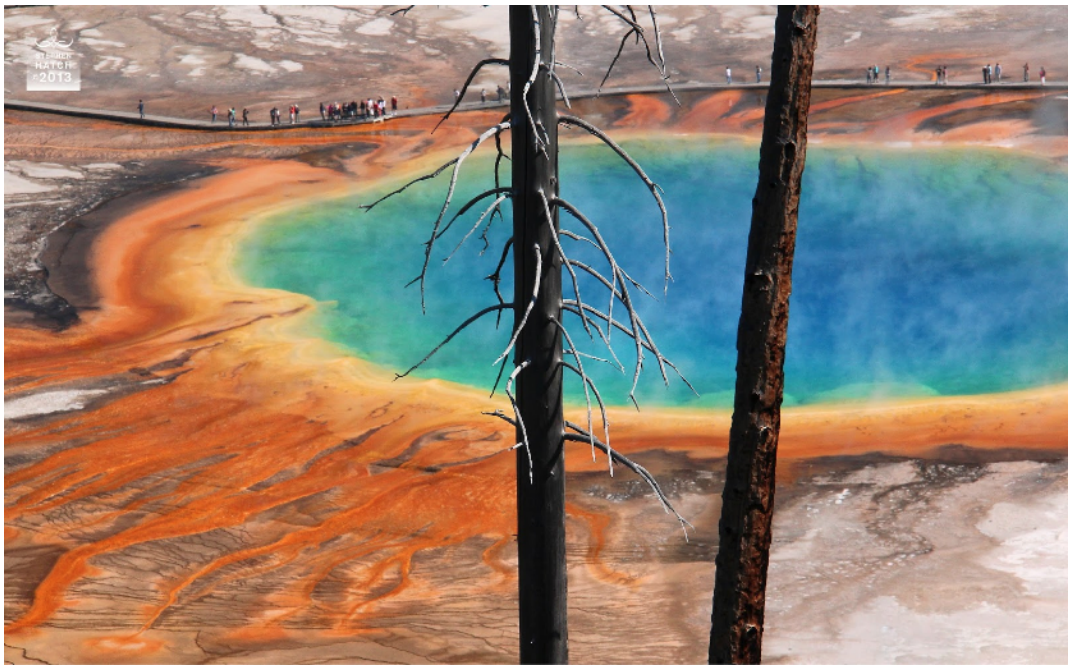


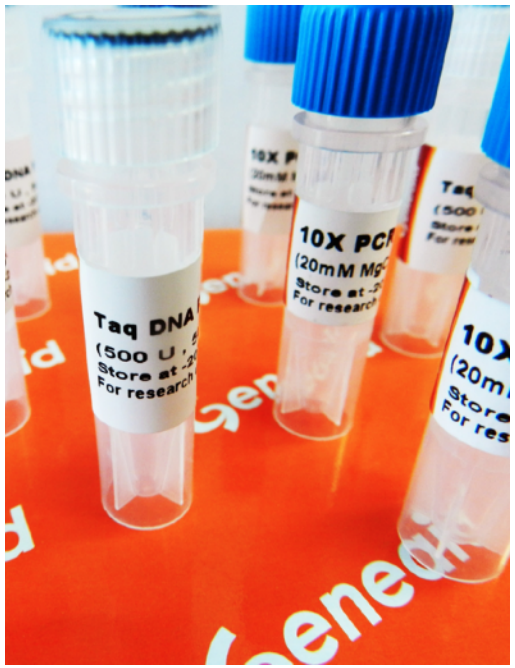
- DNA polymerase needs to be added in every cycle because DNA polymerase denatures at high temperature.



Improvement 1

- Using *Thermus aquaticus* (Taq) polymerase.
- Taq polymerase is heat stable and the cycles can take place without the polymerase being destroyed during the denaturation phase





Improvement 2

Replacing old machine (water baths) with a thermocycler

To consider

- Length and GC content of your primer.
- Compatibility of your forward and reverse primers.
- Primer's sequences do not complement each other (primer dimer).
- Annealing temperature of both primers should be the same.
- Length of the target DNA piece (the longer the target the longer the extension time).
- DNA polymerase, primers and other chemicals' concentration should be precisely calculated.

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