INSTITUTE OF PLANT SCIENCES

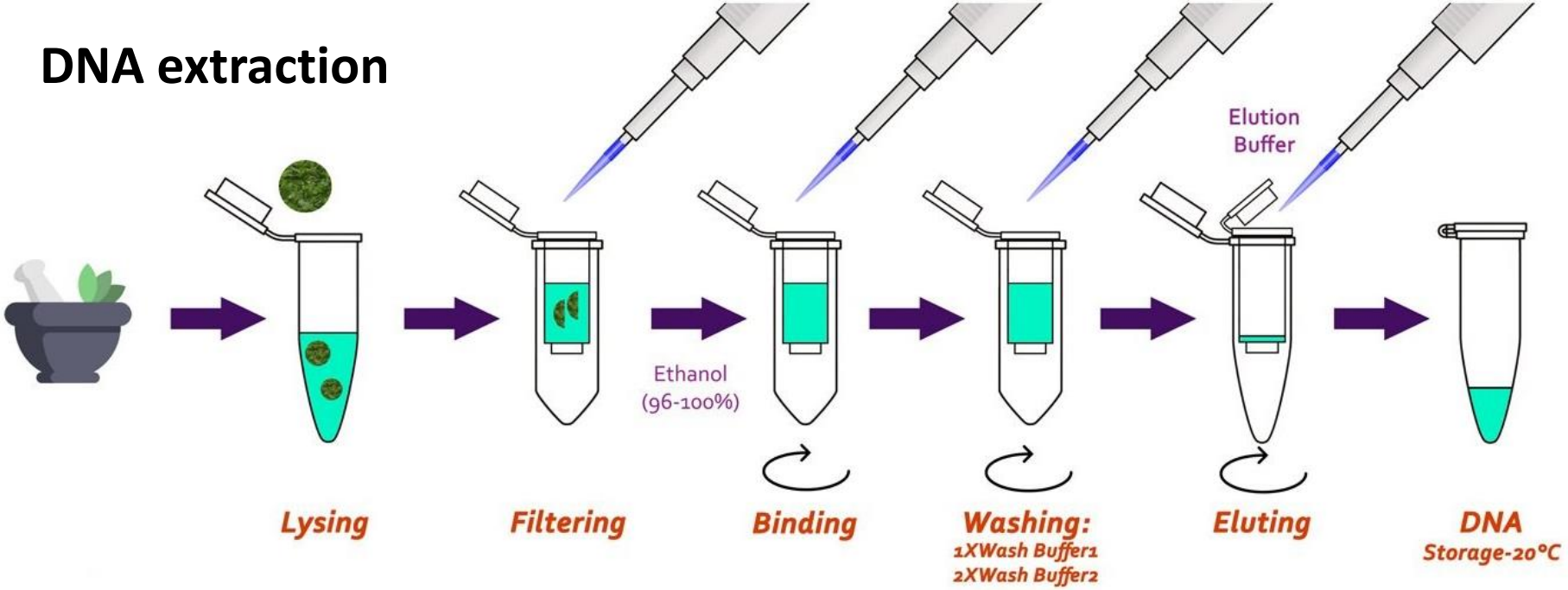
Sant'Anna
School of Advanced Studies – Pisa

# Polymerase Chain Reaction (PCR)

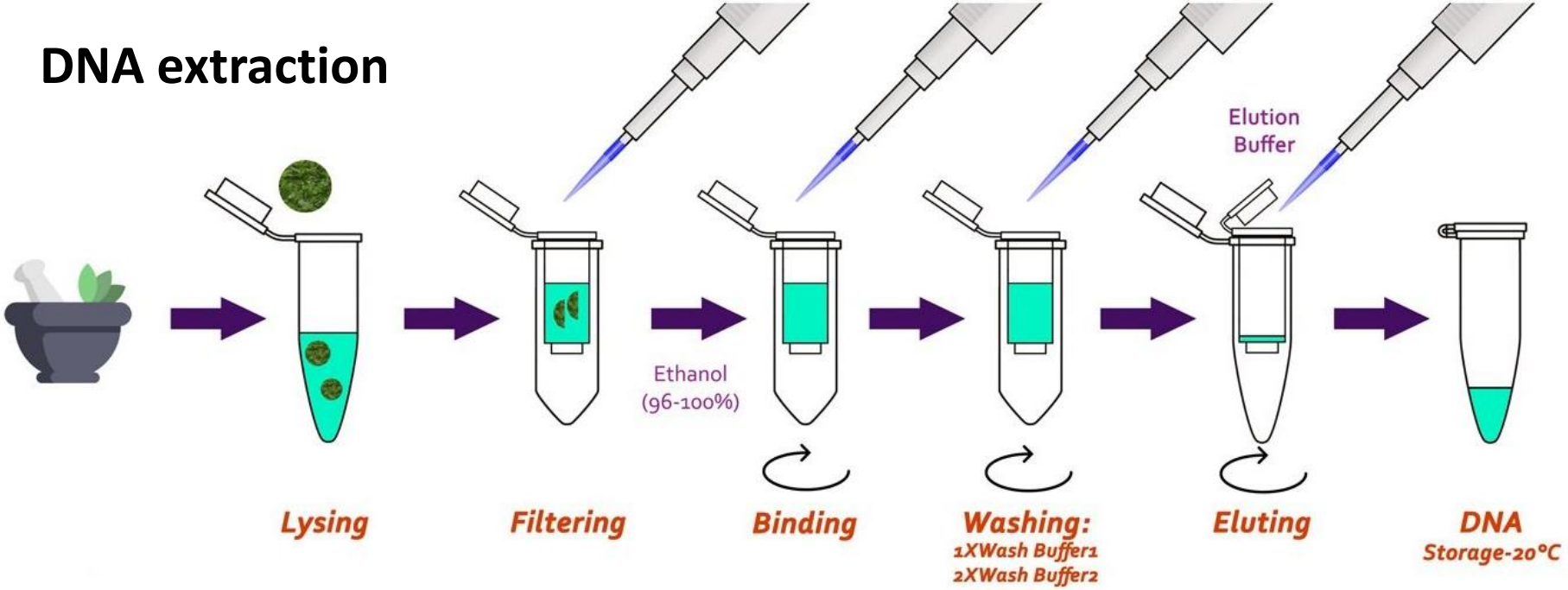
BREEDTECH Student Training Workshop on 5th and 9th May 2025, Pisa, Italy

Prepared by Ettore Riccucci

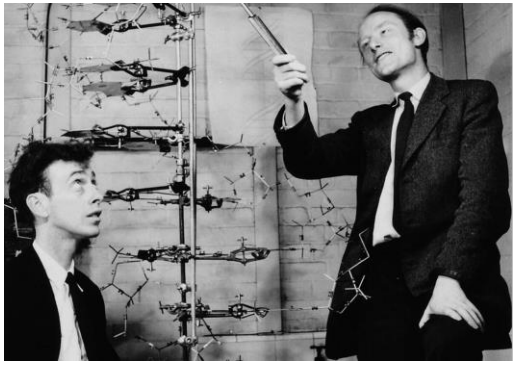
# DNA extraction

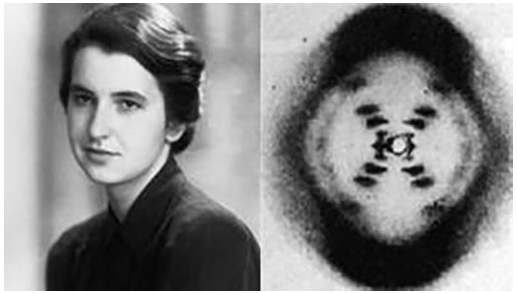


# DNA extraction




How to target and amplify a specific DNA sequence???





No. 4356 April 25, 1953 NATURE 737

equipment, and to Dr. G. E. R. Deacon and the ... captain and officers of R.R.S. *Discovery II* for their ... part in making the observations.

¹ Young, F. B., Gerrard, H., and Jevons, W., *Phil. Mag.*, **40**, 145 (1920).
² Longuet-Higgins, M. S., *Mon. Not. Roy. Astro. Soc., Geophys. Supp.*, **5**, 285 (1949).
³ Van Arx, W. S., Woods Hole Papers in Phys. Oceanog. Meteor., ... 13) (1956).
⁴ Ekman, V. W., *Arkiv. Mat. Astron. Fysik.* (Stockholm), **2** (11) (190...

## MOLECULAR STRUCTURE OF NUCLEIC ACIDS

### A Structure for Deoxyribose Nucleic Acid

WE wish to suggest a structure for the salt of deoxyribose nucleic acid (D.N.A.). This structure has novel features which are of considerable biological interest.

A structure for nucleic acid has already been proposed by Pauling and Corey[1]. They kindly made their manuscript available to us in advance of publication. Their model consists of three inter-twined chains, with the phosphates near the fibre axis, and the bases on the outside. In our opinion, this structure is unsatisfactory for two reasons: (1) We believe that the material which gives the X-ray diagrams is the salt, not the free acid. Without the acidic hydrogen atoms it is not clear what forces would hold the structure together, especially as the negatively charged phosphates near the axis will repel each other. (2) Some of the van der Waals distances appear to be too small.

Another three-chain structure has also been suggested by Fraser (in the press). In his model the phosphates are on the outside and the bases on the inside, linked together by hydrogen bonds. This structure as described is rather ill-defined, and for this reason we shall not comment on it.

We wish to put forward a radically different structure for the salt of deoxyribose nucleic acid. This structure has two helical chains each coiled round the same axis (see diagram). We have made the usual chemical assumptions, namely, that each chain consists of phosphate di-

diagrammatic. The two ribbons symbolize the two phosphate–sugar chains, and the horizontal rods the pairs of bases holding the chains together. The vertical line marks the fibre axis

of the sugar and the atoms near it is close to Furberg's 'standard configuration', the sugar being roughly perpendicular to the attached base. There

constant advice and criticism, especially on inter-atomic distances. We have also been stimulated by a knowledge of the general nature of the unpublished experimental results and ideas of Dr. M. H. F. Wilkins, Dr. R. E. Franklin and their co-workers at

738 NATURE April 25, 1953 Vol. 171

King's College, London. One of us (J. D. W.) has been aided by a fellowship from the National Foundation for Infantile Paralysis.

J. D. WATSON
F. H. C. CRICK

Medical Research Council Unit for the Study of the Molecular Structure of Biological Systems, Cavendish Laboratory, Cambridge.
April 2.

¹ Pauling, L., and Corey, R. B., *Nature*, **171**, 346 (1953); *Proc. U.S. Nat. Acad. Sci.*, **39**, 84 (1953).
² Furberg, S., *Acta Chem. Scand.*, **6**, 634 (1952).
³ Chargaff, E., for references see Zamenhof, S., Brawerman, G., and Chargaff, E., *Biochim. et Biophys. Acta*, **9**, 402 (1952).
⁴ Wyatt, G. R., *J. Gen. Physiol.*, **36**, 201 (1952).
⁵ Astbury, W. T., Symp. Soc. Exp. Biol. 1, Nucleic Acid, 66 (Camb. Univ. Press, 1947).
⁶ Wilkins, M. H. F., and Randall, J. T., *Biochim. et Biophys. Acta*, **10**, 192 (1953).

## Molecular Structure of Deoxypentose Nucleic Acids

WHILE the biological properties of deoxypentose nucleic acid suggest a molecular structure containing great complexity, X-ray diffraction studies described here (cf. Astbury[5]) show the basic molecular configuration has great simplicity. The purpose of this communication is to describe, in a preliminary way, some of the experimental evidence for the polynucleotide chain configuration being helical, and existing in this form when in the natural state. A fuller account of the work will be published shortly.

The structure of deoxypentose nucleic acid is the same in all species (although the nitrogen base ratios alter considerably) in nucleoprotein, extracted or in cells, and in purified nucleate. The same linear group of polynucleotide chains may pack together parallel in different ways to give crystalline[5,6], semi-crystalline or paracrystalline material. In all cases the X-ray diffraction photograph consists of two regions, one determined largely by the regular spacing of nucleotides along the chain, and the other by the longer spacings of the chain configuration. The sequence of different nitrogen bases along the chain is not made visible.

Oriented paracrystalline deoxypentose nucleic acid (structure *B*[5] in the following communication by Franklin and Gosling) gives a fibre diagram as shown in Fig. 1 (cf. ref. 4). Astbury suggested that the strong 3·4-A. reflexion corresponded to the inter-

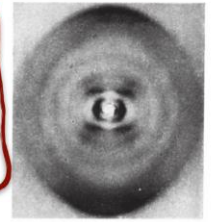


Fig. 1. Fibre diagram of deoxypentose nucleic acid from *B. coli*. Fibre axis vertical

the innermost maxima of each Bessel function and the origin. The angle this line makes with the equator is roughly equal to the angle between an element of the helix and the helix axis. If a unit repeats *n* times along the helix there will be a meridional reflexion ($J_n$) on the *n*th layer line. The helical configuration produces side-bands on this fundamental frequency, the effect[5] being to reproduce the intensity distribution about the origin around the new origin, on the *n*th layer line, corresponding to *C* in Fig. 2.

We will now briefly analyse in physical terms some of the effects of the shape and size of the repeat unit or nucleotide on the diffraction pattern. First, if the nucleotide consists of a unit having circular symmetry about an axis parallel to the helix axis, the whole diffraction pattern is modified by the form factor of the nucleotide. Second, if the nucleotide consists of a series of points on a radius at right-angles to the helix axis, the phases of radiation scattered by the helices of different diameter passing through each point are the same. Summation of the corresponding Bessel functions gives reinforcement for the inner-

continuous helix gives a series of layer lines of spacing corresponding to the helix pitch, the intensity distribution along the *n*th layer line being proportional to the square of $J_n$, the *n*th order Bessel function. A straight line may be drawn approximately through

Fig. 2. Diffraction pattern of system of helices corresponding to structure of deoxypentose nucleic acid. The sequences of Bessel functions are plotted about 0 on the equator and on the first, second, third and fifth layer lines for half of the nucleotide mass at 20 Å. diameter and remainder distributed along a radius, the mass at a given radius being proportional to the radius. About *C* on the tenth layer line similar functions are plotted for an outer diameter of 12 Å.

**It has not escaped our notice that the specific pairing we have postulated immediately suggests a possible copying mechanism for the genetic material.**

Watson & Crick, Nature, April 1953.

Polymerase Chain Reaction

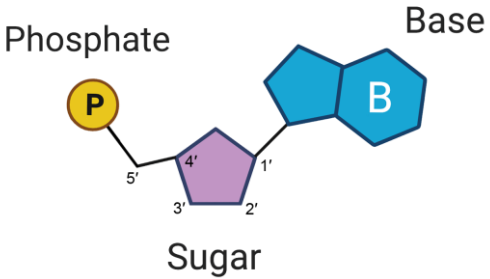
Enzymic synthesis of deoxyribonucleic acid (1956)
_ Nobel Prize in Physiology or Medicine 1959

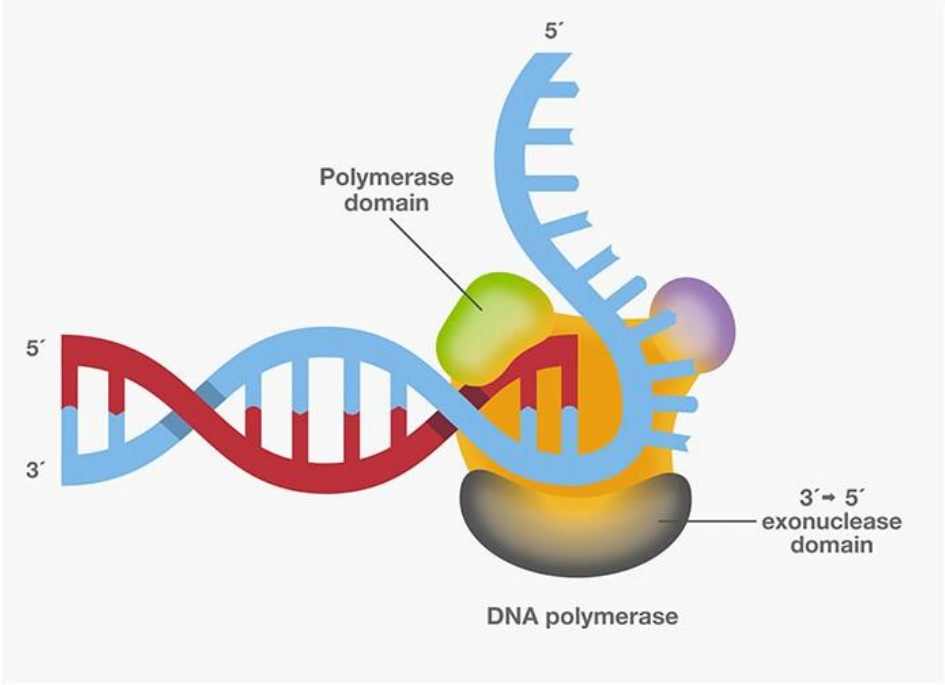
Arthur Kornberg compared DNA to a tape recording of instructions that can be copied over and over

**How do cells make these near-perfect copies?**

## DNA polymerase

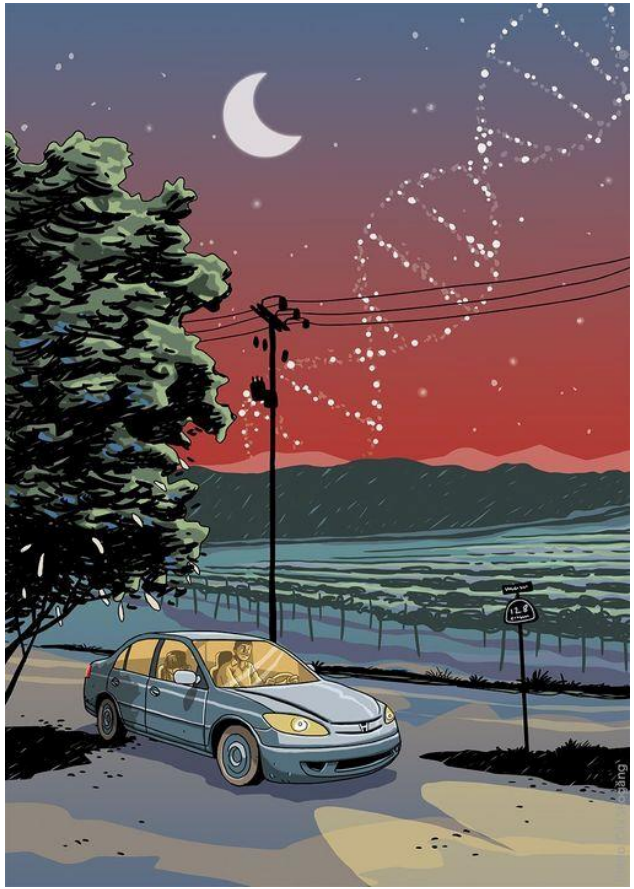
- Can synthesise only in the 5’ to 3’ direction
- Needs:
  1. ssDNA (single strand) as a template
  2. 3’-OH group to add new nucleotides to



How to target and amplify a specific DNA sequence???

*“Sometimes a good idea comes to you when you are not looking for it … such a revolution came to me one Friday night in April, 1983, as I gripped the steering wheel of my car and snaked along a moonlit mountain road into northern California’s redwood country. That was how I strumbled across a process that could make* ***unlimited copies of genes***”

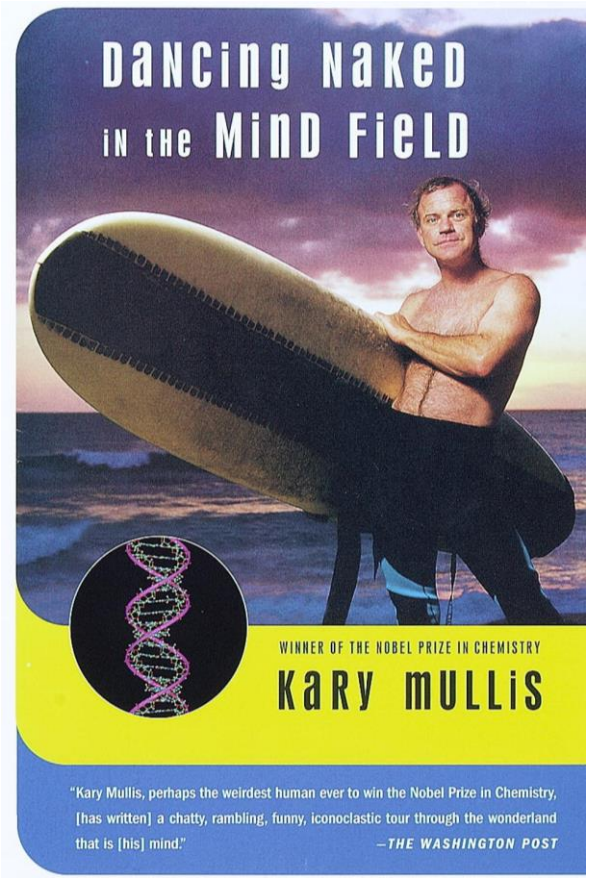


# Kary Mullis

**Nobel Prize in Chemistry** (1993)
for creating **polymerase chain reaction** (**PCR**)

Questionable opinions about:
- Link between AIDS and HIV
- Human-made global warming
- Astrology

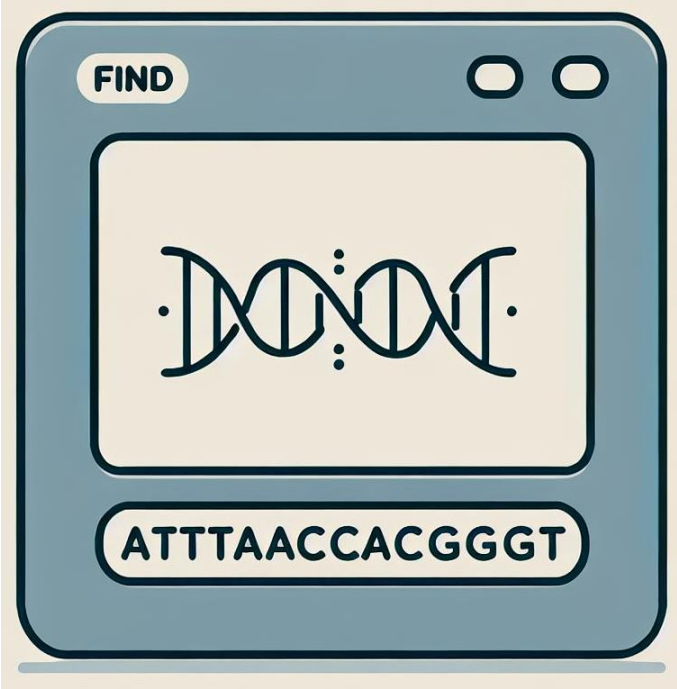
**Surf lover**



K. Mullis was working as DNA chemist for the synthesis of **oligonucleotide probes**

*“Like a “FIND” sequence in a computer search, a short string of nucleotides in a synthetic molecule might be able to define a position along a very much longer natural DNA molecule”*

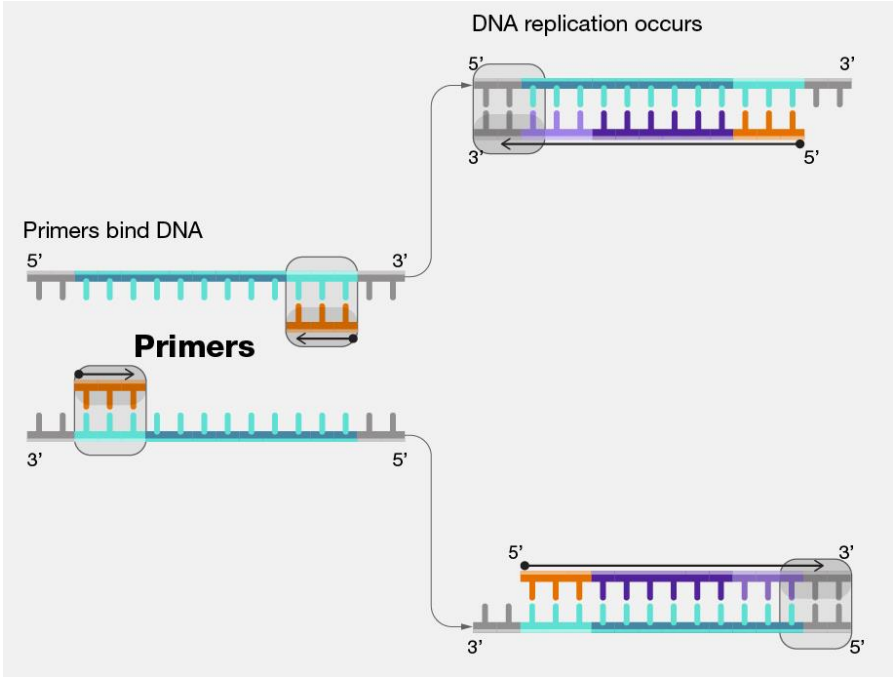
→ **Primers:** synthetic DNA oligonucleotides (15–30 bp) designed to bind to sequences that flank the region of interest in the template DNA

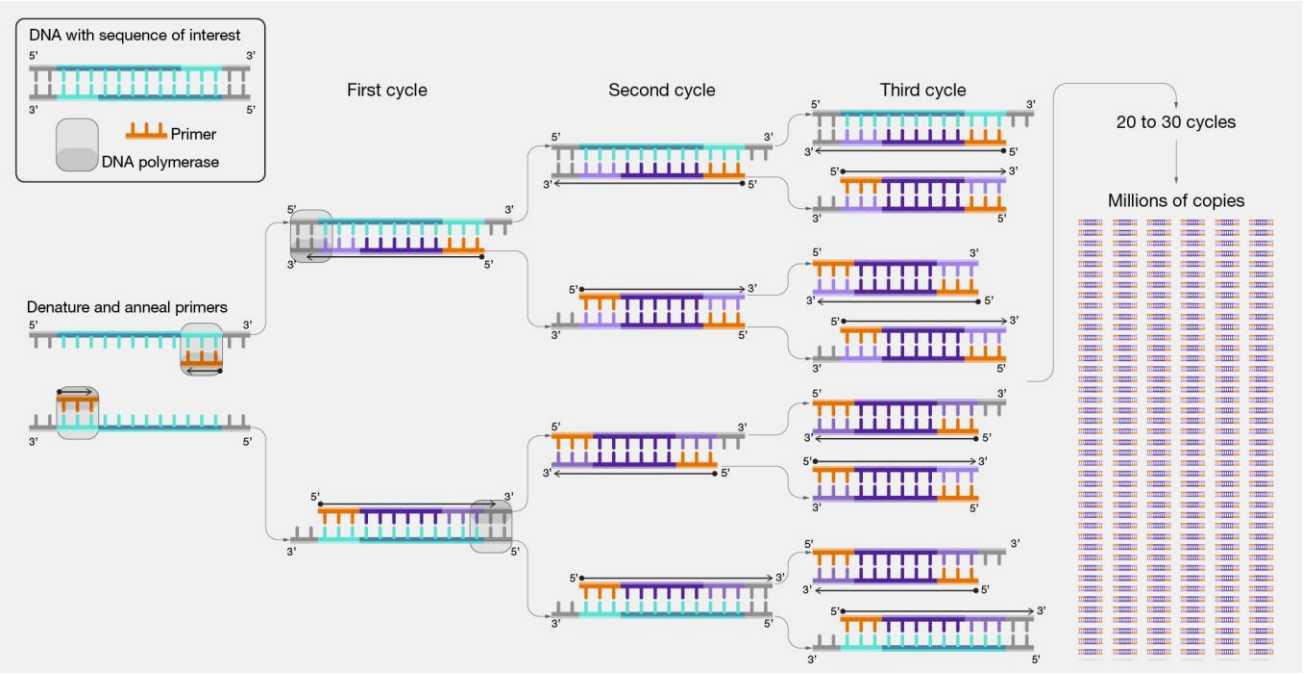


*"If I could locate a thousand sequences out of billions with one short piece of DNA, I could use another short piece to narrow the search"*

→ **'Forward' primer** : binds to the 3' end of the antisense DNA strand

→ **'Reverse' primer**: binds to the sense DNA strand



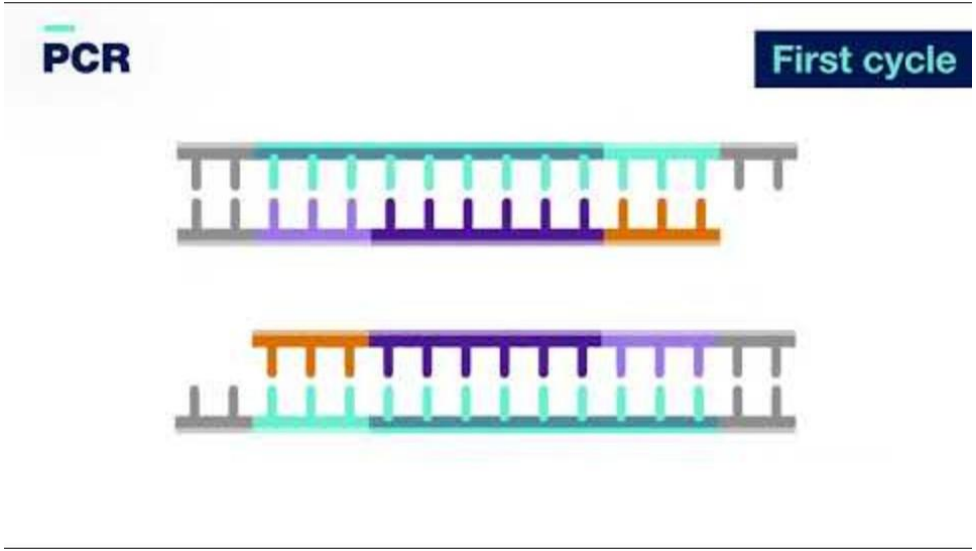



*“ Not only could I make a zillion copies, but they would always be the same size … This simple technique would make* ***as many copies as I wanted of any DNA sequence I chose***”

**PCR**: a series of temperature cycles enable the replication of DNA segments, making it possible to generate millions of copies of a target DNA region

PCR setup:

- Template DNA
- DNA polymerase
- Primers
- Deoxynucleoside triphosphates (dNTPs)
- Magnesium ion
- Buffer



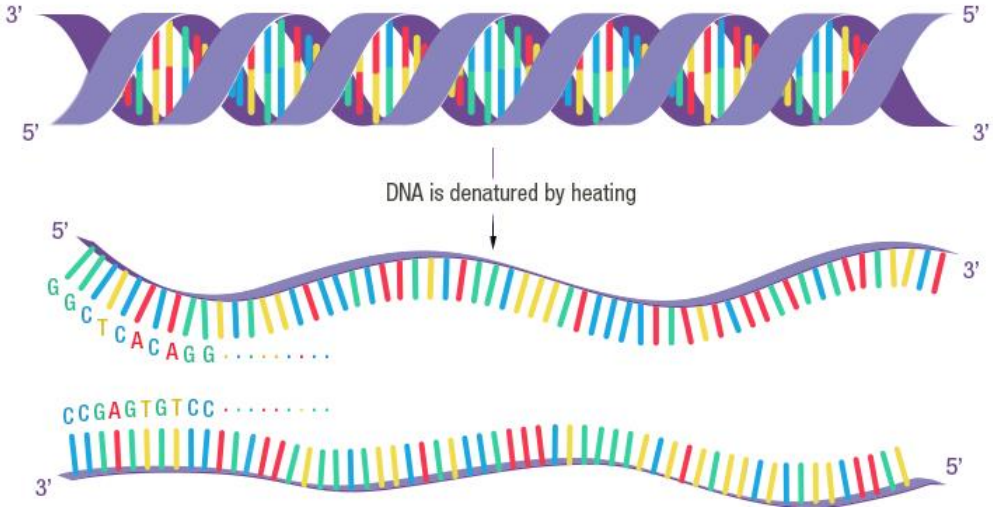
## Main steps in PCR

### 1. DENATURATION

- 94–98°C for 1–3 minutes

- Separate the double-stranded template DNA into single strands

- Long and/or GC-rich DNA targets may benefit from a prolonged incubation and/or a higher temperature



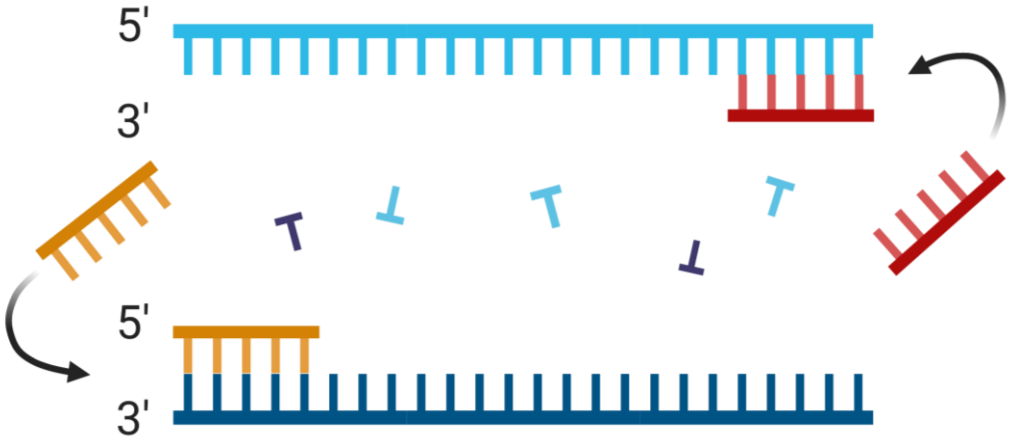
## Main steps in PCR

### 2. PRIMERS ANNEALING

→ Provide the 3'-OH starting point for DNA synthesis

**Annealing temperature**: 50 - 65°C determined by the melting temperature ($T_m$) of the selected primers for PCR amplification.

$T_m$ depends on: primers length, GC / AT content and salt concentration ($Na^+$)



## Main steps in PCR

### 3. EXTENSION

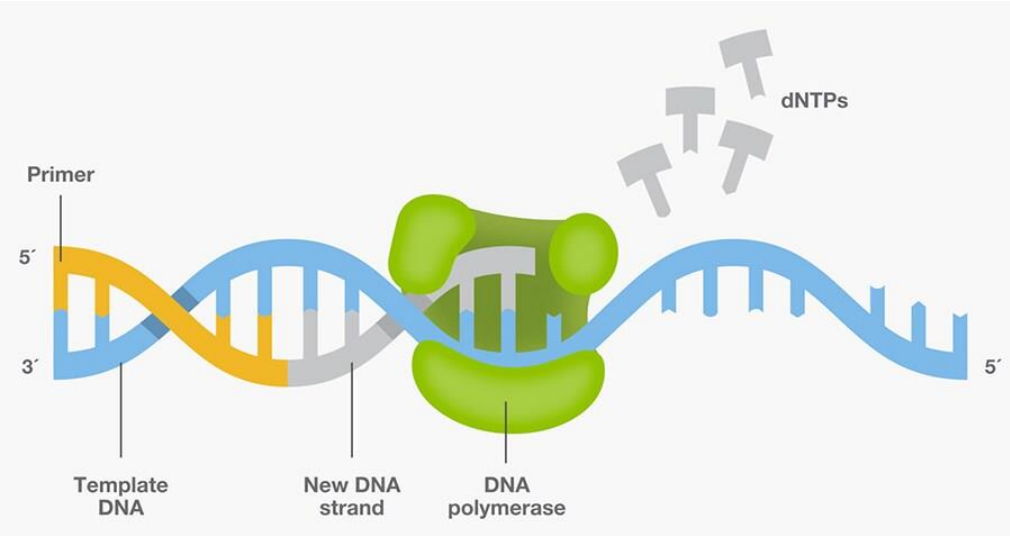
Temperature: 75-80°C

Thomas D. Brock, 1969 → discovery of *Thermus aquaticus* from the hot springs of Yellowston national park
→ thermostable **Taq DNA polymerase**

## Main steps in PCR

### 3. EXTENSION

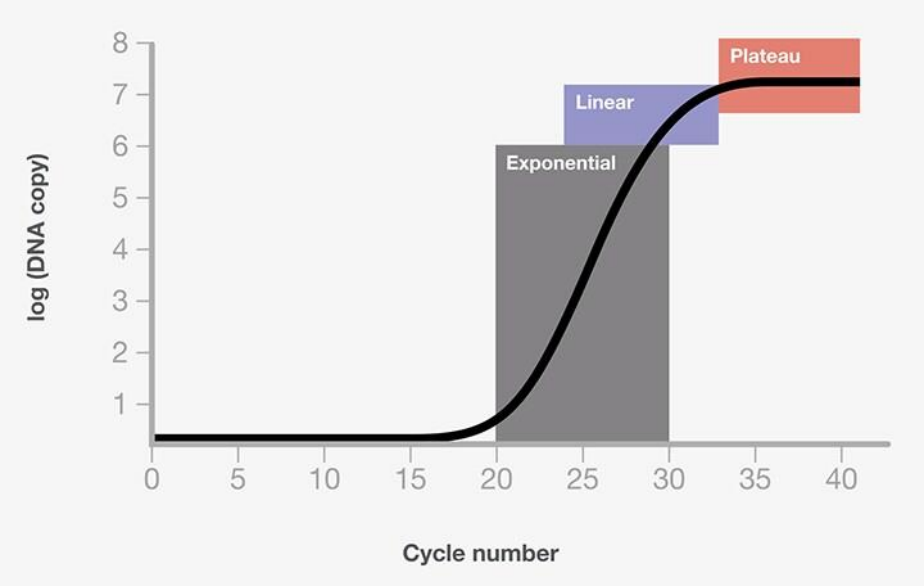
Temperature: 75-80°C



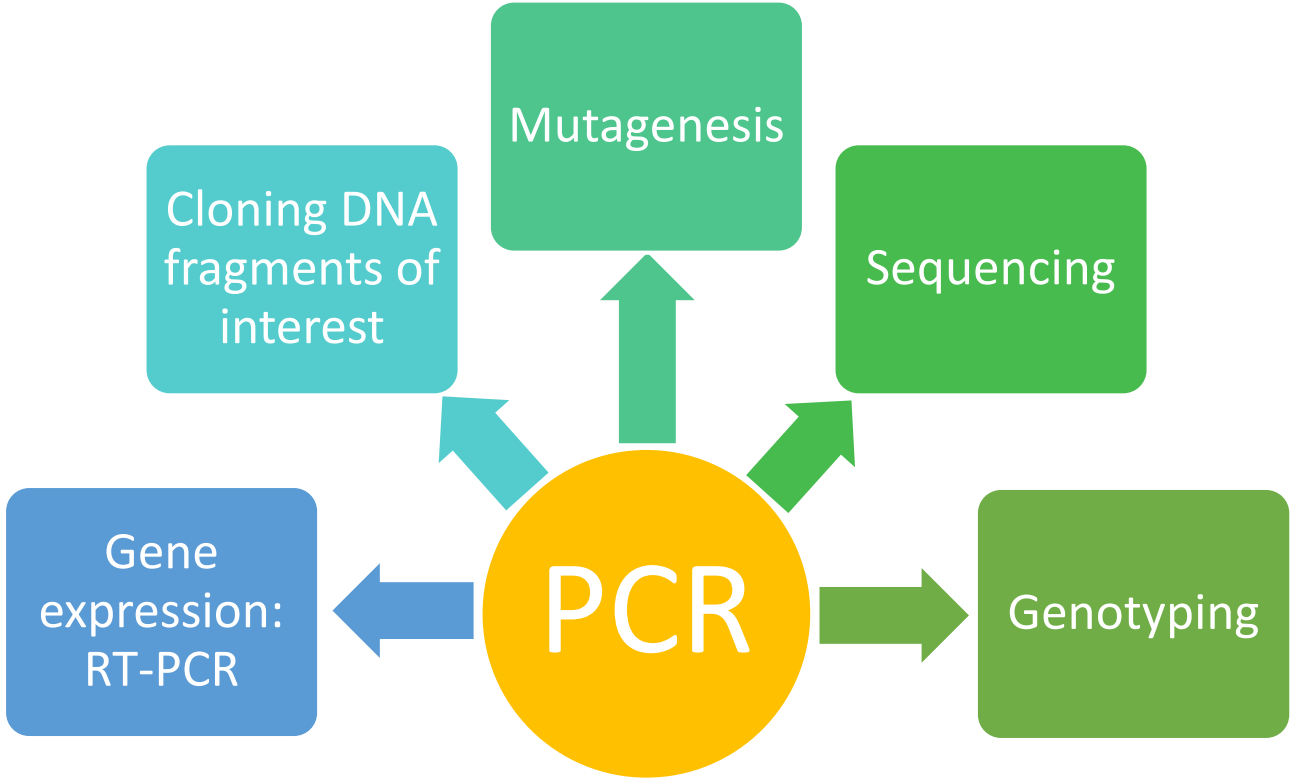

5′→ 3′ **polymerase activity** of the DNA polymerase incorporates dNTPs and synthesizes the daughter strands

# CYCLING

- Steps 1 - 3 are repeated 25–35 times
- Nonspecific bands start to appear with numbers of cycles higher than 45
- Accumulation of by-products and depletion of reaction components →lower PCR efficiency
- Low cycle numbers for unbiased amplification (next-generation sequencing) and accurate replication of target DNA (cloning)



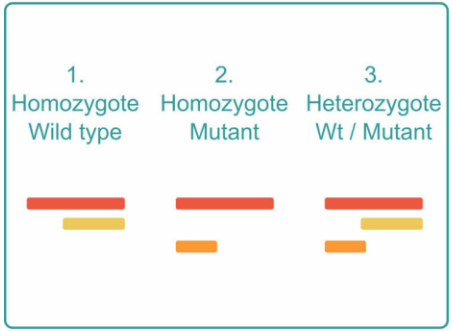
## PCR applications

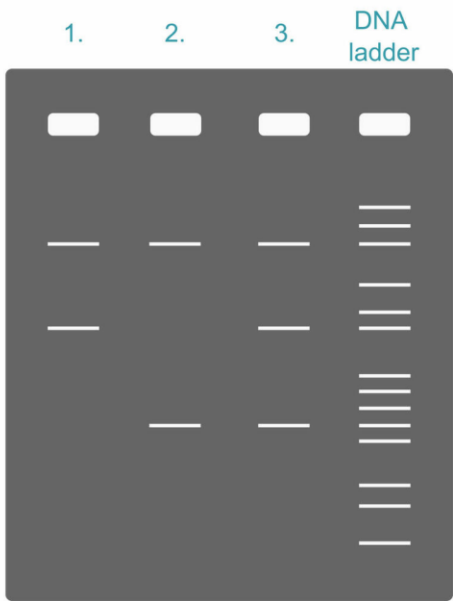


# Genotyping

Detect sequence variations in alleles in specific cells or organisms:

- Primers designed to flank regions of interest
- Presence / absence of an amplicon → genetic variations

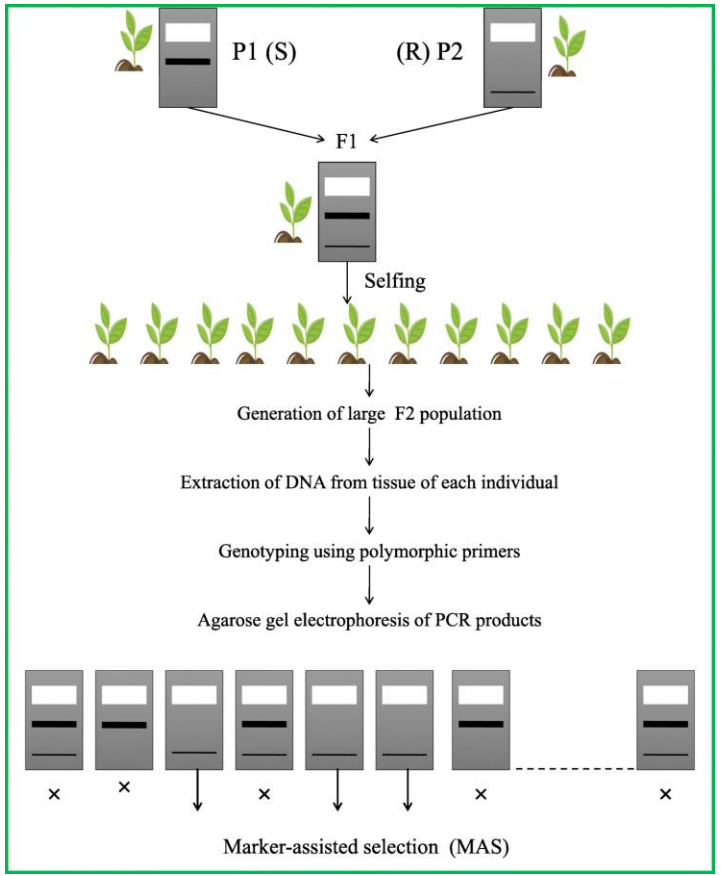


# Genotyping

Detect sequence variations in alleles in specific cells or organisms:

- Primers designed to flank regions of interest
- Presence / absence of an amplicon → genetic variations



# THANK YOU!