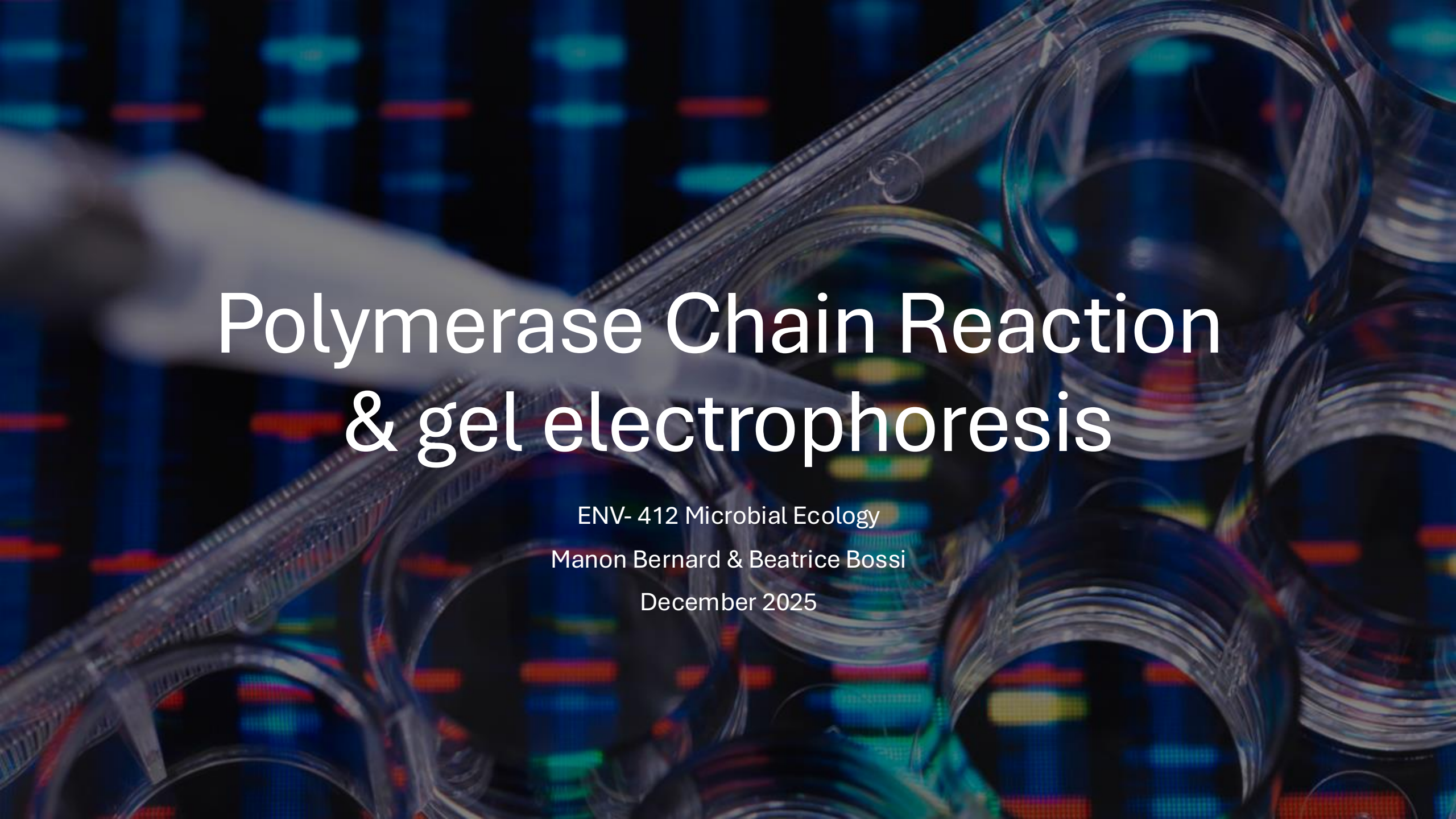


Polymerase Chain Reaction & gel electrophoresis

ENV- 412 Microbial Ecology

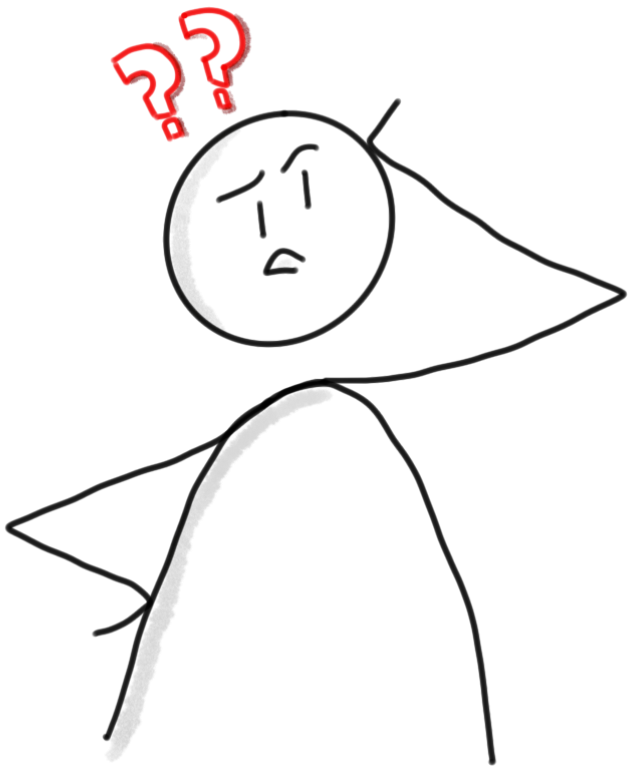
Manon Bernard & Beatrice Bossi

December 2025

Agenda

- Aim & uses
- History of PCR
- The PCR toolbox
- Procedure
- Gel electrophoresis
- The PCR family
- PCR at work

Aim & uses of PCR

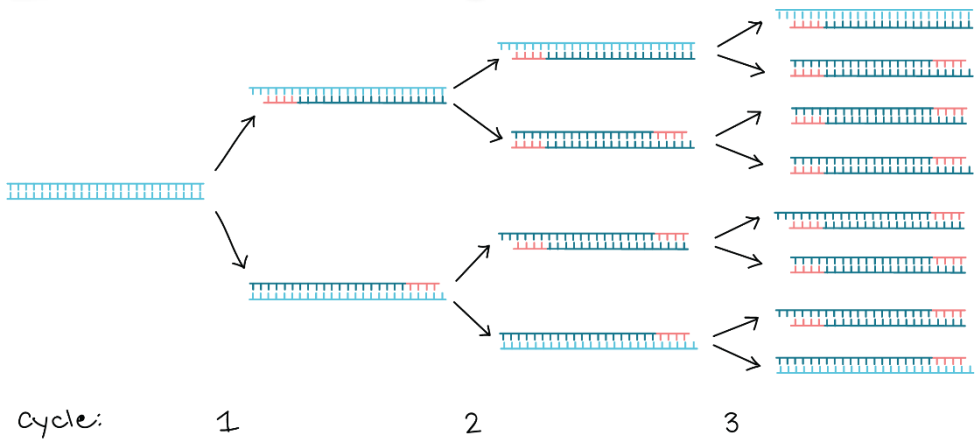


Enzymes which catalyse DNA synthesis

Polymerase

Chain

Reaction



Aim & uses of PCR

Polymerase
Chain
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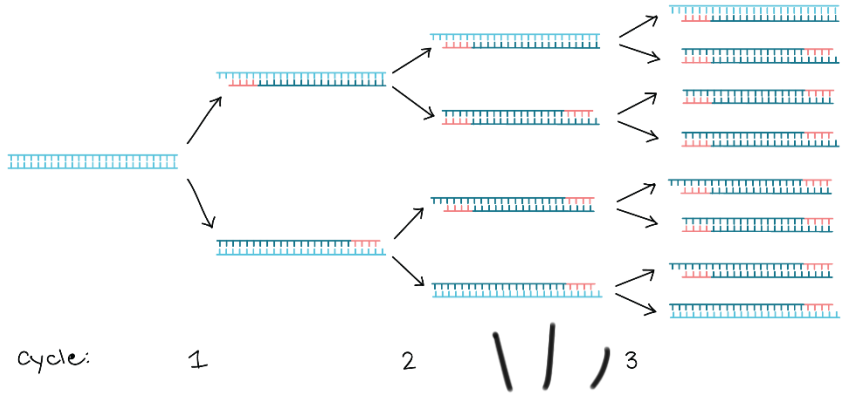
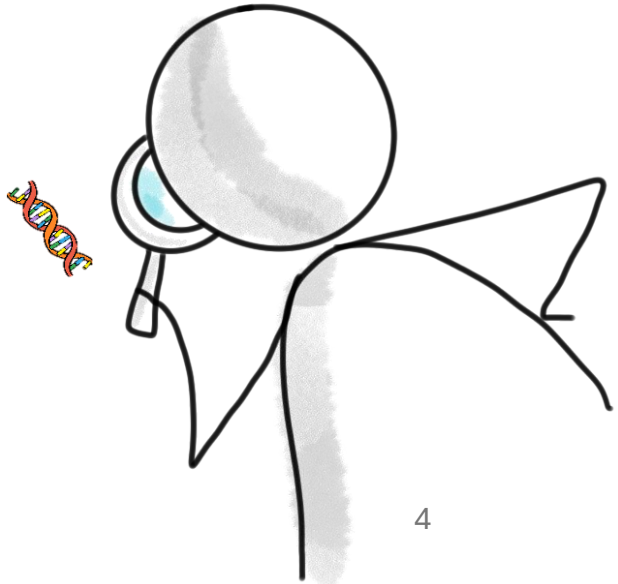
Enzymes which catalyse DNA synthesis



- DNA sequence rapid amplification technique
  - Uses a small amount of DNA
  - Replication cycles towards large quantities
- Uses
  - Selective DNA isolation & DNA sequencing
  - Amplification & quantification of target DNA regions

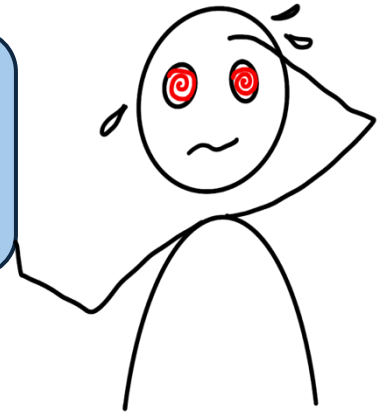


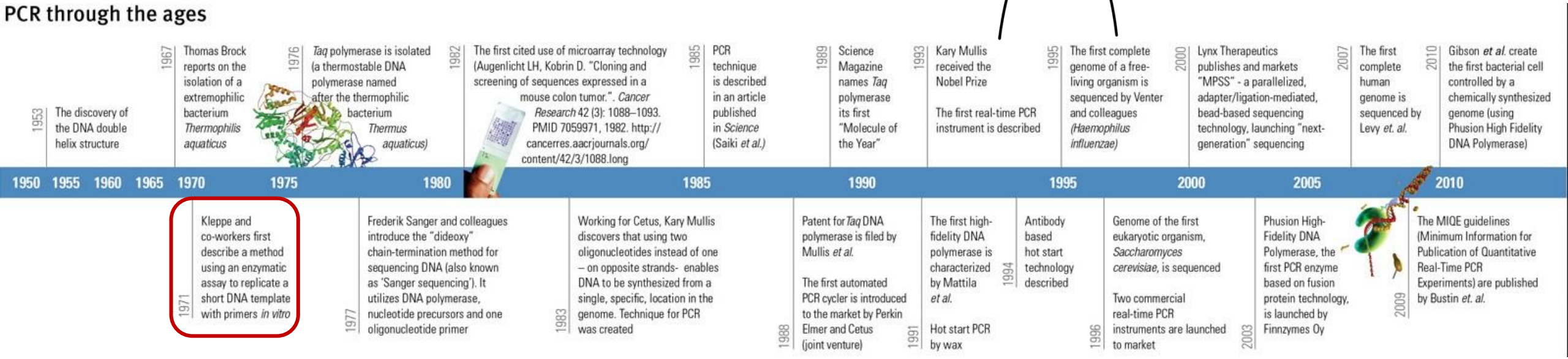
(Khehra et al., 2025)

A brief history of PCR development

- **1971** first description of a method using enzymatic assay to replicate a short DNA template with primers in vitro

Thermosensitive enzymes: clumps, issues with replication
Manual process: tedious, time consuming
Hazardous: early stages of research with hybridization of radioactive-labelled oligonucleotides





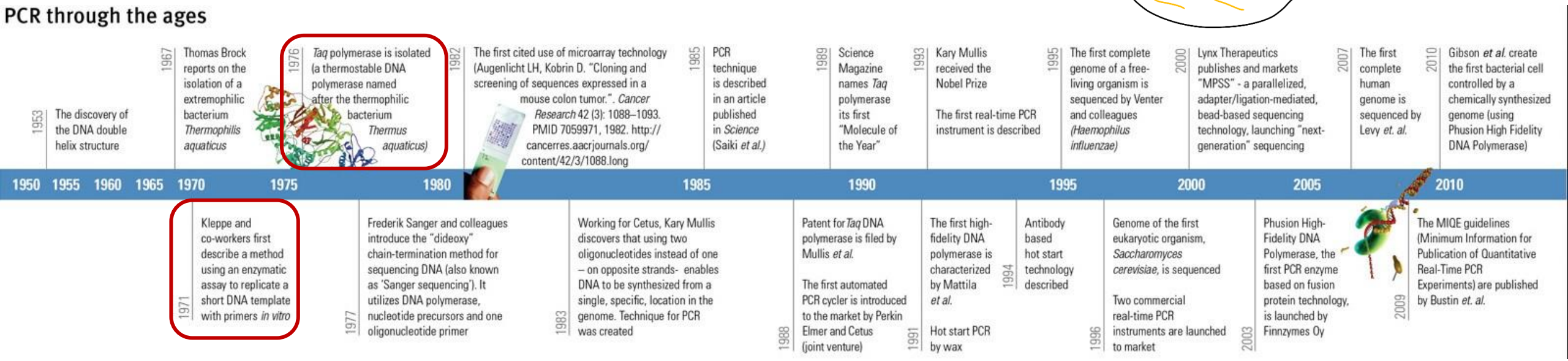
In vitro : process taking place in a test tube ≠ in vivo

(*The History of PCR | Thermo Fisher Scientific - US*, n.d.)

A brief history of PCR development

- **1971** first description of a method using enzymatic assay to replicate a short DNA template with primers in vitro
- **1976** isolation of **Taq DNA polymerase enzyme**

Thermostable enzyme isolated from *Thermus aquaticus*



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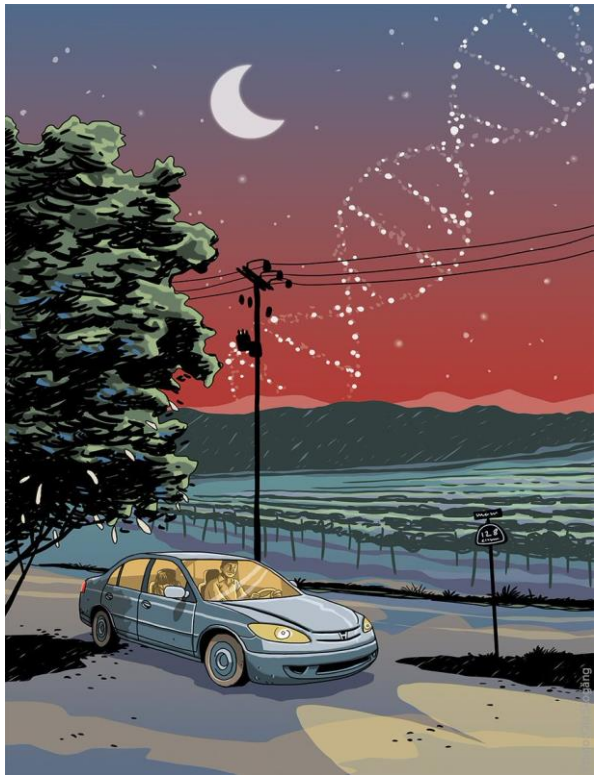
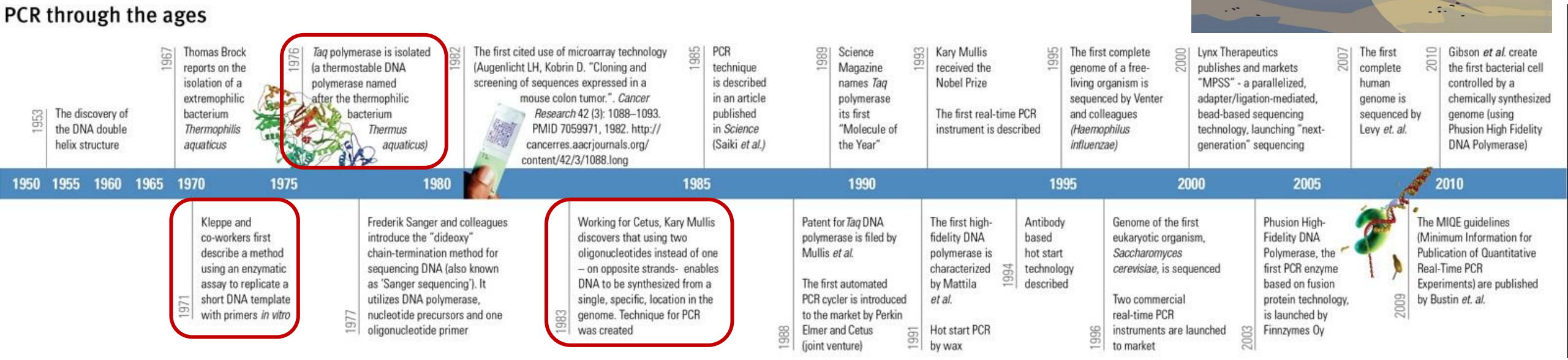
(*The History of PCR | Thermo Fisher Scientific - US*, n.d.)

A brief history of PCR development

- **1971** first description of a method using enzymatic assay to replicate a short DNA template with primers in
- **1976** isolation of Taq DNA polymerase enzyme
- **1983** Kary Mullis develops the first PCR technique





In vitro : process taking place in a test tube ≠ in vivo

(*The History of PCR | Thermo Fisher Scientific - US*, n.d.)

A brief history of PCR development

Enzymatic amplification of beta-globin genomic sequences and restriction site analysis for diagnosis of sickle cell anemia

R K Saiki, S Scharf, F Faloona, K B Mullis, G T Horn, H A Erlich, N Arnheim

PMID: 2999980 DOI: 10.1126/science.2999980

A third author being given the Nobel Prize ?

Kary B. Mullis

Facts

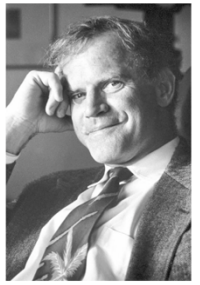


Photo from the Nobel Foundation archive.

Kary B. Mullis
Nobel Prize in Chemistry 1993

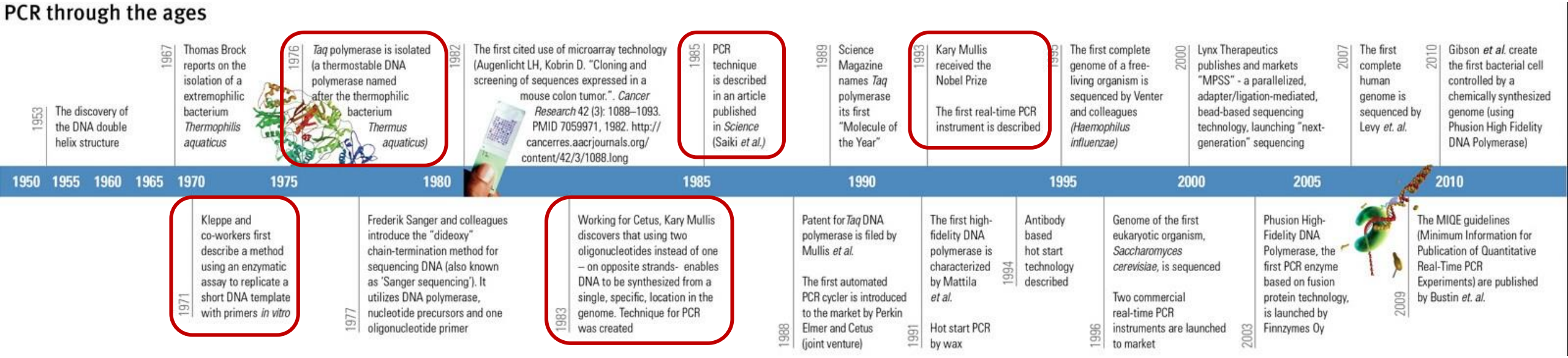
Born: 28 December 1944, Lenoir, NC, USA

Died: 7 August 2019, Newport Beach, CA, USA

Prize motivation: "for his invention of the polymerase chain reaction (PCR) method"

Prize share: 1/2

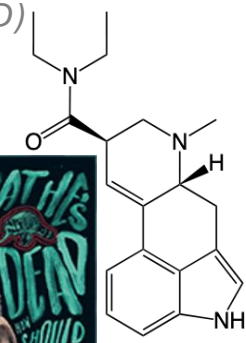
PCR through the ages



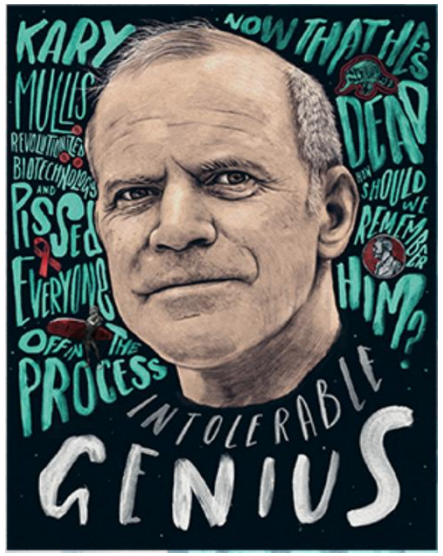
Sickle cell anemia: inherited disorder; red blood cells are distorted and may cause premature destruction of small blood vessels

(Saiki et al., 1985)
(*The History of PCR | Thermo Fisher Scientific - US*, n.d.)

Lysergic acid diethylamide (LSD)



Mullis: the good, the bad and the ugly

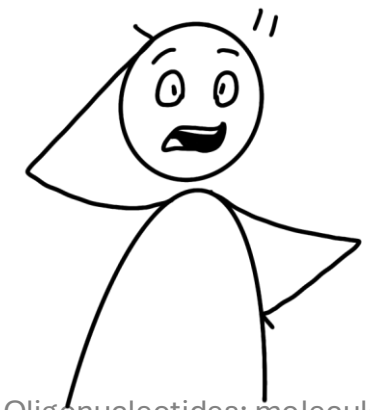


- Excellent **synthetic chemist…** *trained by producing his own drugs*
- **Conceptualized** the idea of **attaching two oligonucleotides to a split strand of DNA** to isolate it, and with the **addition of polymerase create copies** by repeating the steps to improve DNA reading & fought for its development despite skeptics **=PCR**

He **helped persuade** the South African president Mebeki that **AIDS is *not* caused by HIV**:
35,000 babies were born with HIV and **330,000 South Africans died of AIDS** due to the country's lack of action against AIDS during the 90s epidemic

Erlich & Saiki were two colleagues of Mullis who **actively participated in PCR development**:

- ✓ Thanks to their rigor, knowledge and perseverance **PCR was proved effective**
- ✓ **Wrote the first article** describing the technique before other companies would steal the patent **and further developed the devices**



→ Mullis got the spark and the Nobel, yet ***we owe PCR to the efforts of a team of scientists developing the technique***.

Oligonucleotides: molecules allowing isolation of natural DNA

(Cal Alumni Association, 2022)

Components and steps of PCR

- **DNA or RNA sample**: contains the segment to be amplified.

(Powledge, 2004)

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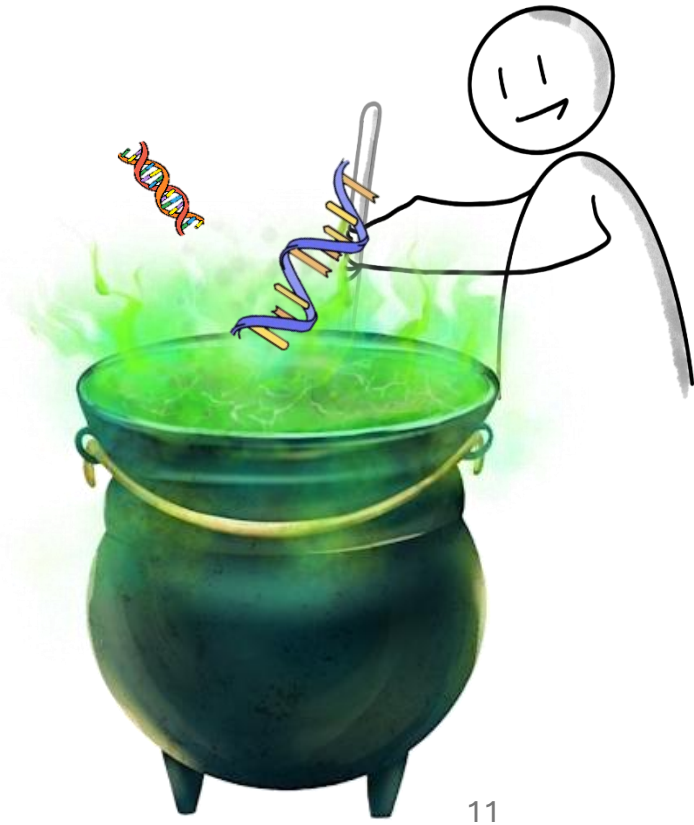

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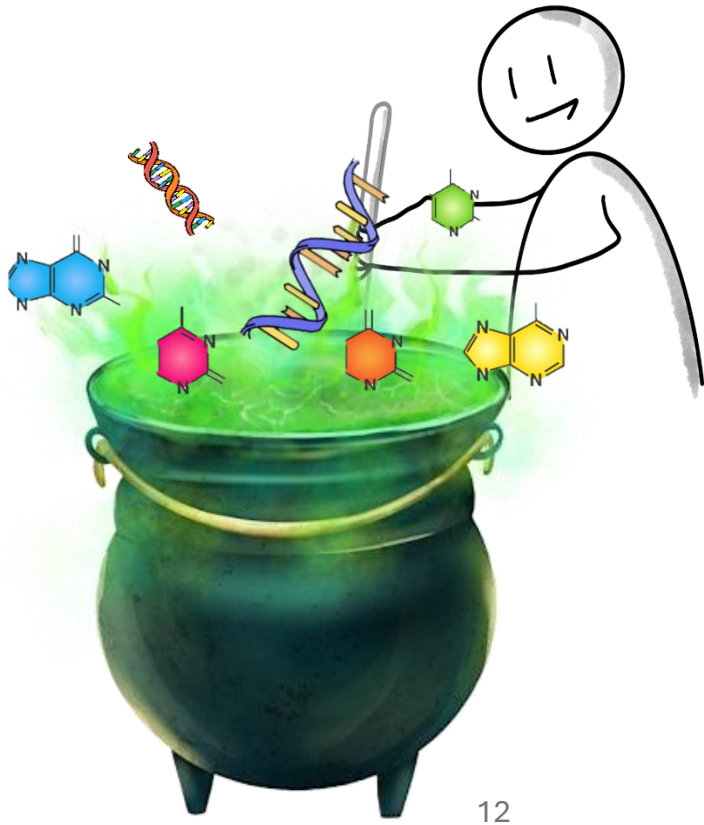

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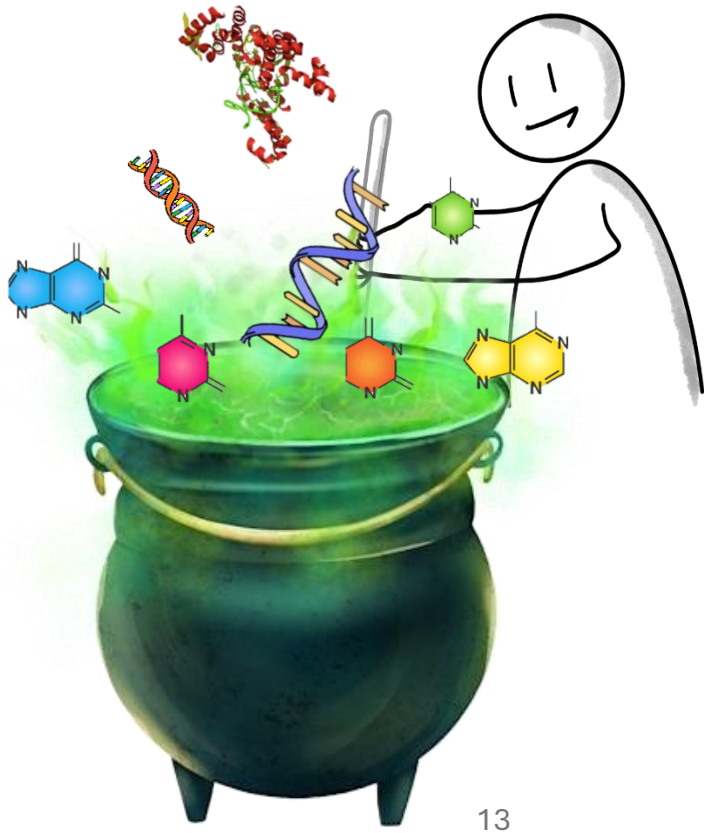

(Powledge, 2004)

Components and steps of PCR

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- **Primers:** short pieces of single-stranded DNA, complementary to target sequence (one per DNA strand).
- **Nucleotides:** A, T, G, C needed to create new DNA strands.
- ***Taq* DNA polymerase:** enzyme that synthesizes new DNA strands.
- **Buffer solution:** maintains the optimal conditions for the reaction.

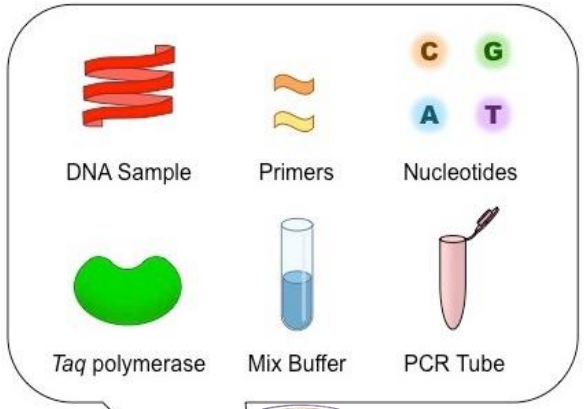
Master Mix

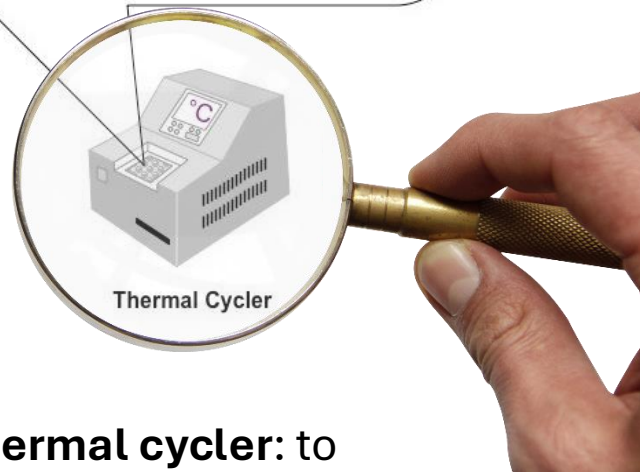
± 100 CHF/mL !

(Powledge, 2004)

Components and steps of PCR

- **DNA or RNA sample**
- **Primers**
- **Nucleotides**
- ***Taq* DNA polymerase**
- **Buffer solution**

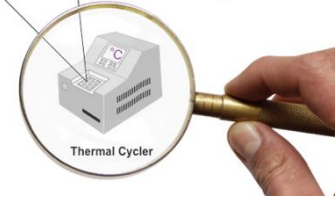




Micro-test tube: where all of the reactants are combined, and the reaction occurs.

Thermal cycler: to control temperature cycle.

(Powledge, 2004)



Extracted nucleic acid sample (DNA or RNA) ~ 1-100 ng

1

Denaturation

High T breaks the H-bonds between complementary bases, separating DNA into two single strands

(National Ataxia Foundation, 2022)

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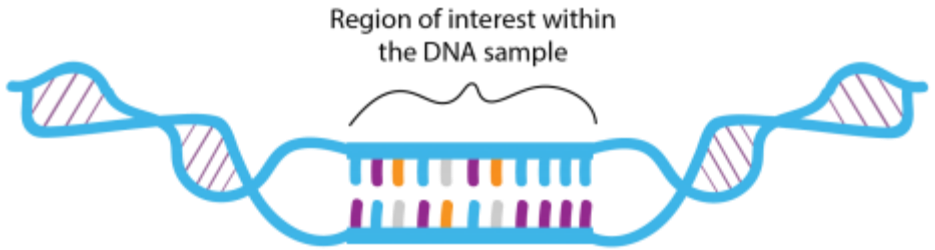

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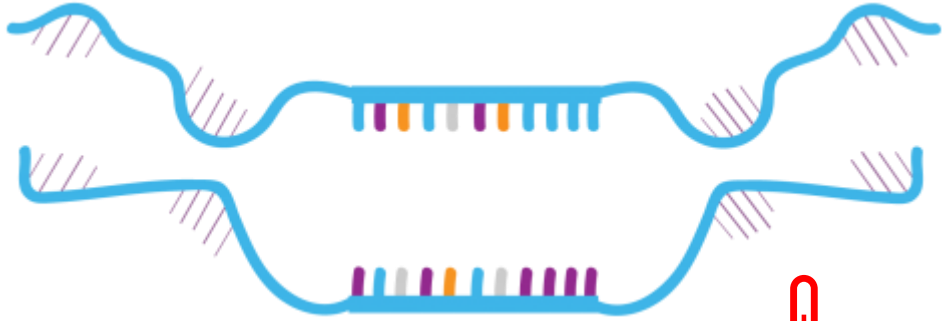
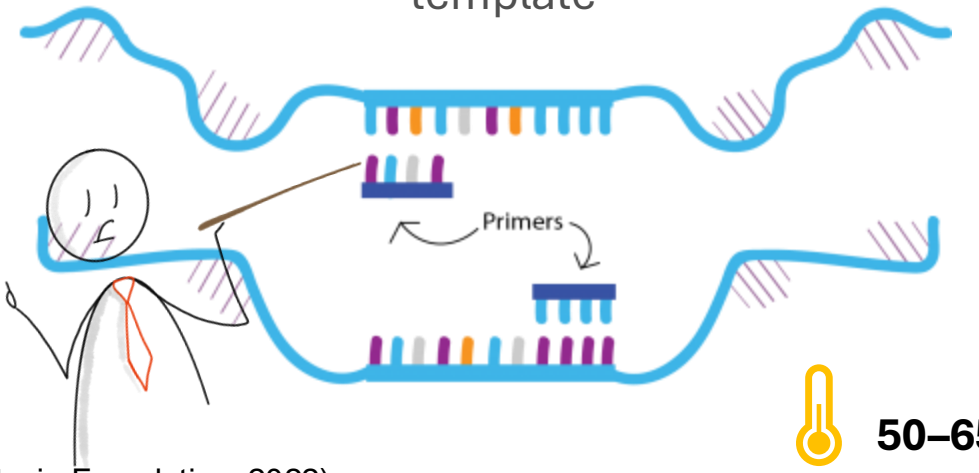


95°C

2

Annealing

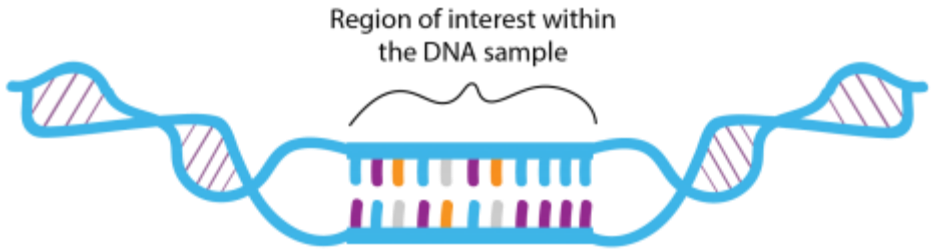
Primers bind (anneal) to the complementary sequences of the template



50–65°C

(National Ataxia Foundation, 2022)

Extracted nucleic acid sample (DNA or RNA) ~ 1-100 ng



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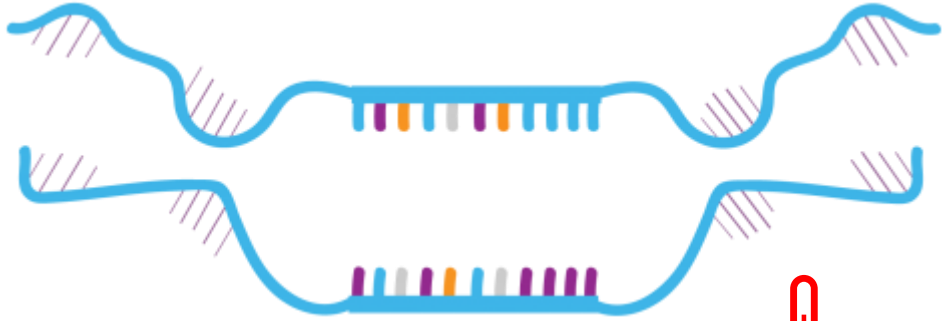

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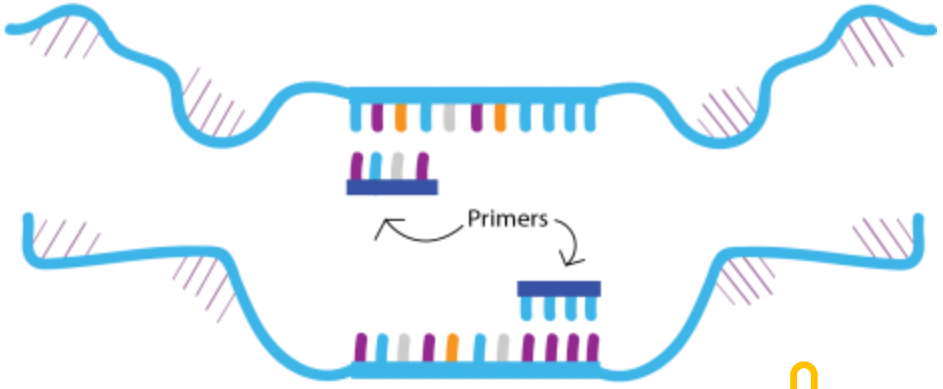
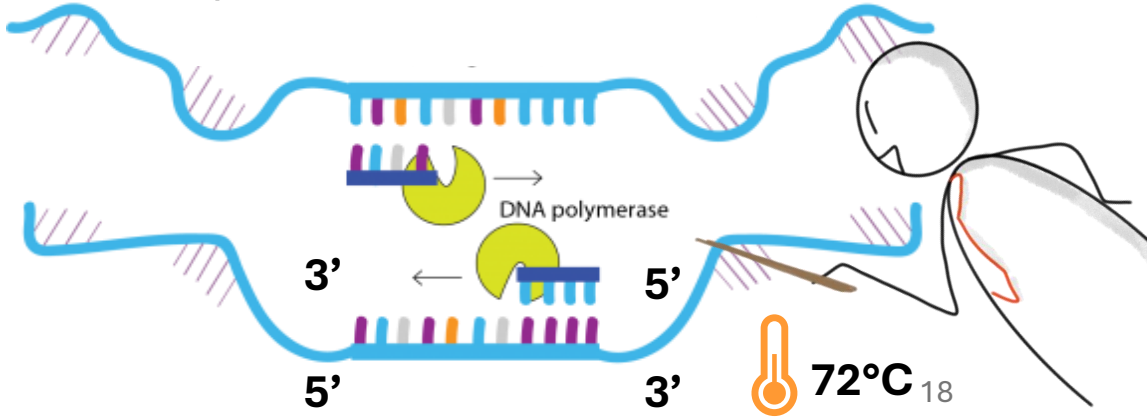


50–65°C

3

Extension

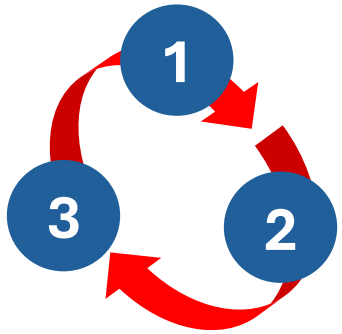
Taq polymerase synthesizes new strands, adding nucleotides complementary to the template strand in direction 5’→ 3’



72°C

(National Ataxia Foundation, 2022)

Amplification of target sequence



Steps **1-2-3** are repeated in cycles for **25-30 times**, depending on DNA input & desired yield.

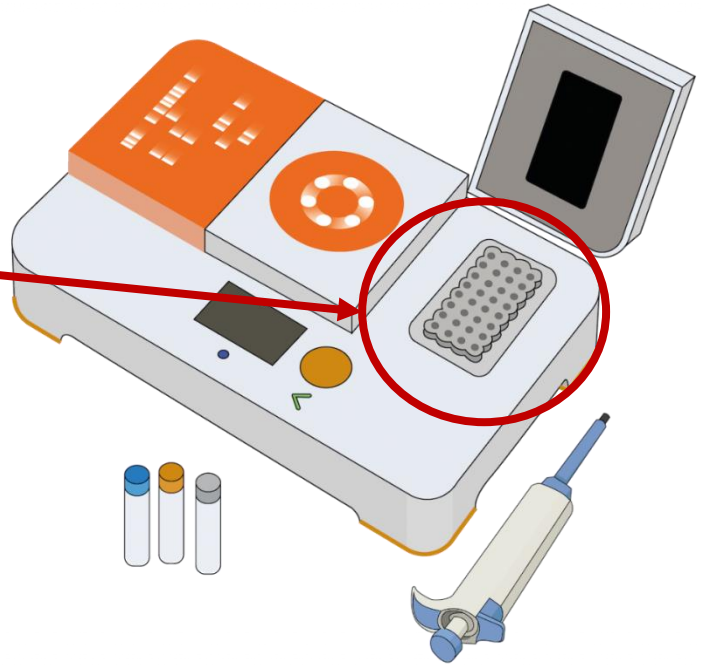
PCR produces copies of the target sequence equal to:

$$2^n$$

n = number of cycles

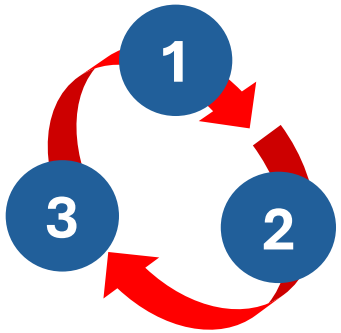
Remember the cute little machine used in the lab last month ?

where the magic happens



(*PCR Cycling Parameters—Six Key Considerations for Success | Thermo Fisher Scientific - US*, n.d.)

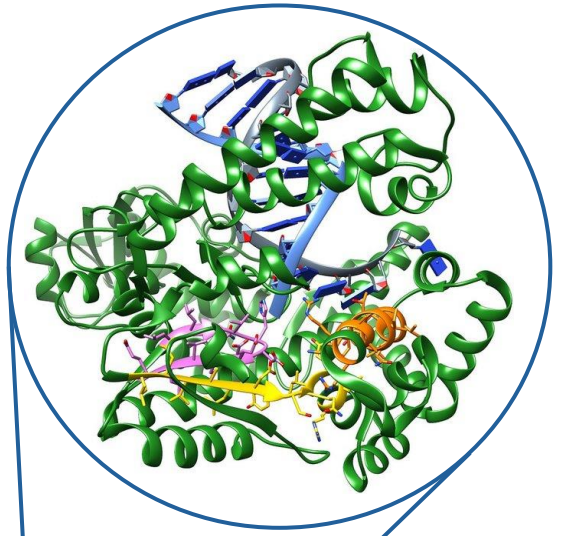
Relevance of Taq polymerase



Steps **1-2-3** are repeated in cycles for **25-30 times**, depending on DNA input & desired yield.

→possible thanks to the use of thermoresistant enzyme **Taq** polymerase from the thermophilic bacterium ***Thermus aquaticus***, found in hot springs

→ Regular DNA polymerases would be denatured after each cycle



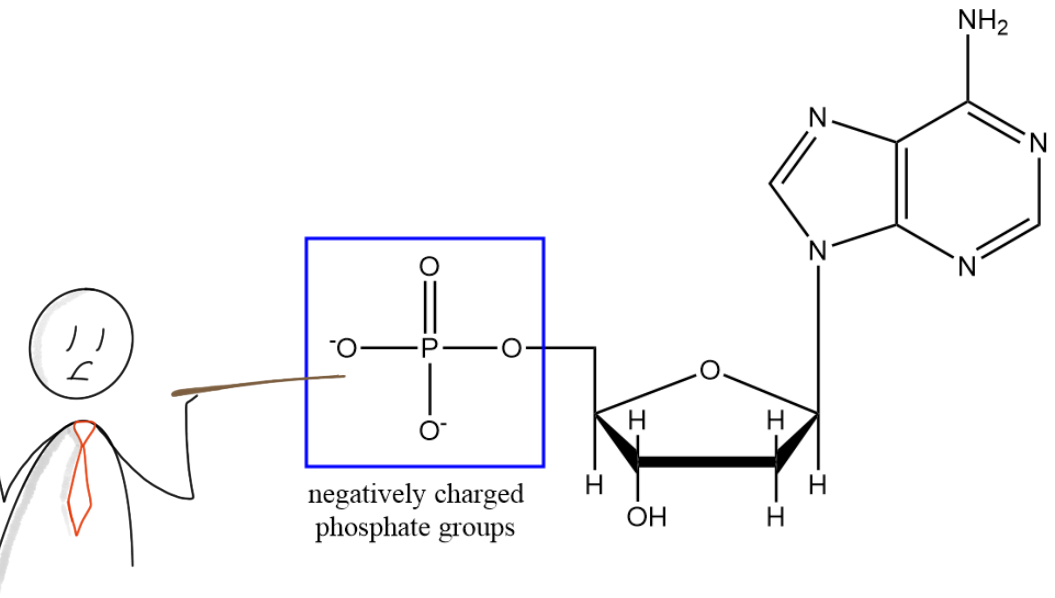
Structure of Taq polymerase

Thermus aquaticus

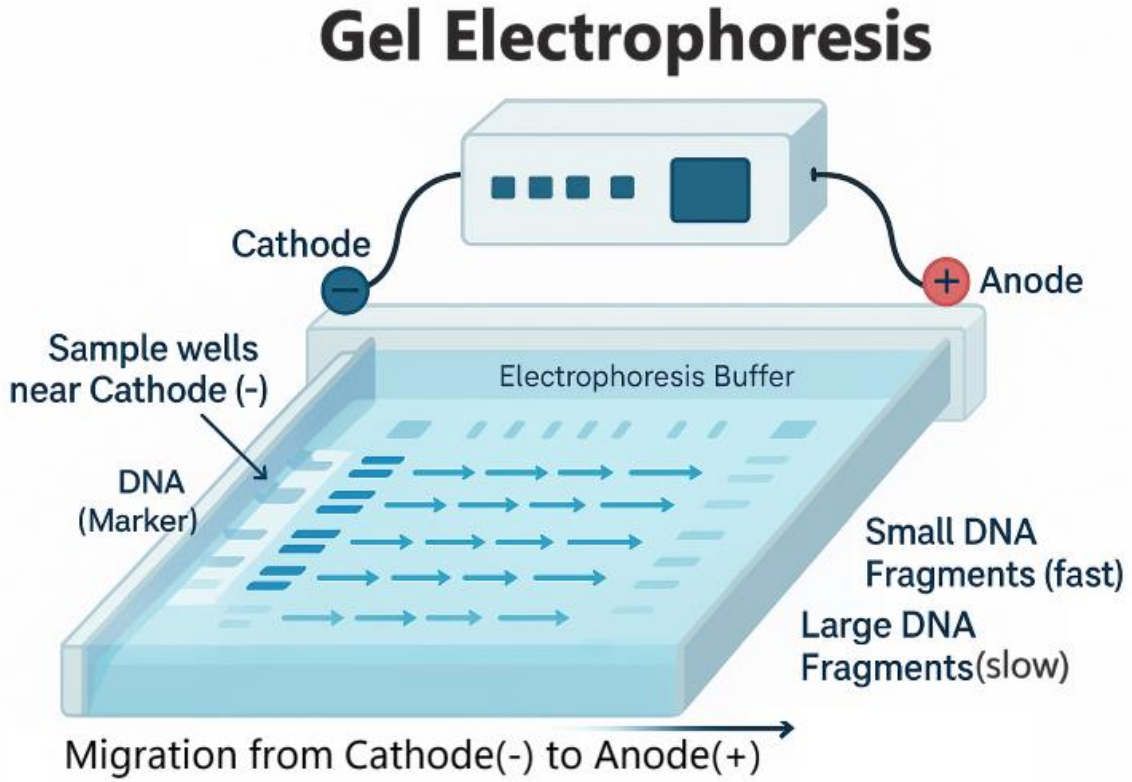
(Parsaeimehr et al., 2013; Laos et al., 2014)

Gel electrophoresis

Laboratory technique used to separate DNA, RNA, or protein segments based on **size** and **charge**. An electric field is applied to an agarose gel, causing charged molecules to move through the gel. Smaller molecules will move more quickly.

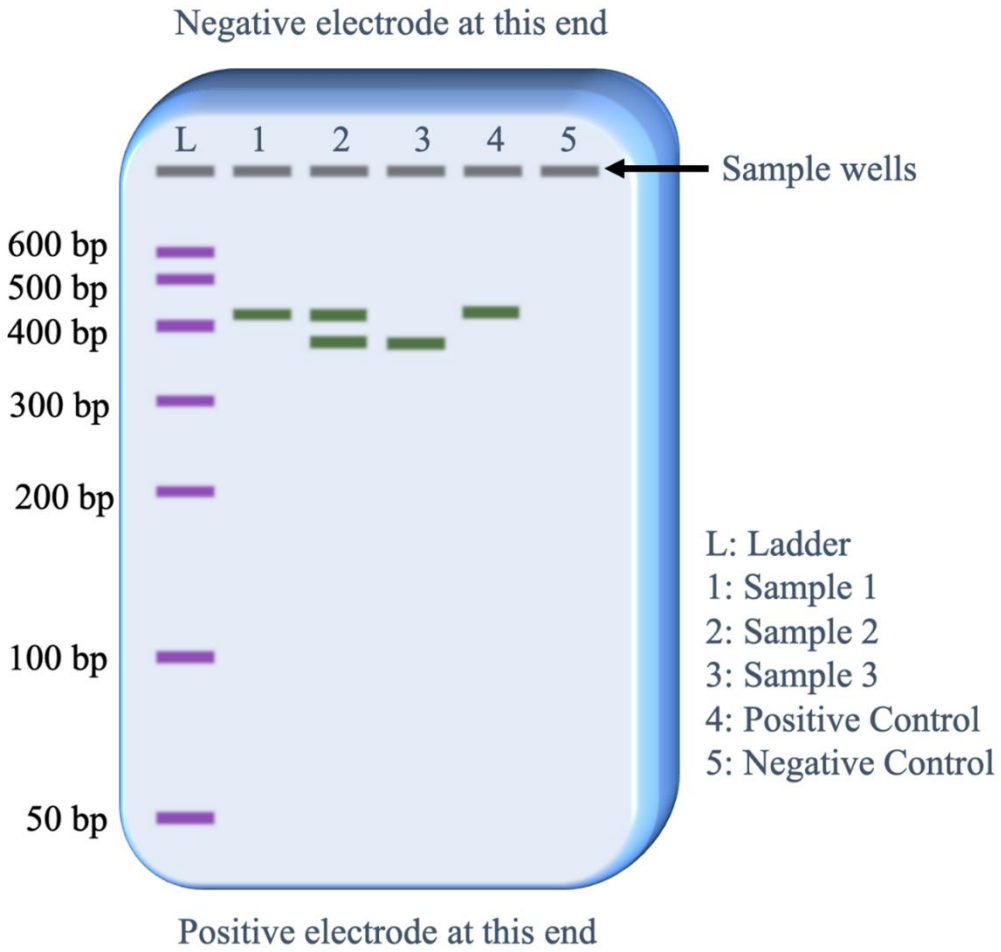


(*Capillary Vs. Gel Electrophoresis: Differences, Advantages, and Applications*, n.d.)



DNA molecules are negatively charged due to the **phosphate groups** attached to the 5' C → travel towards the anode

Interpreting gel electrophoresis results



After the gel is run, DNA fragments are stained with a dye visible with **UV light**

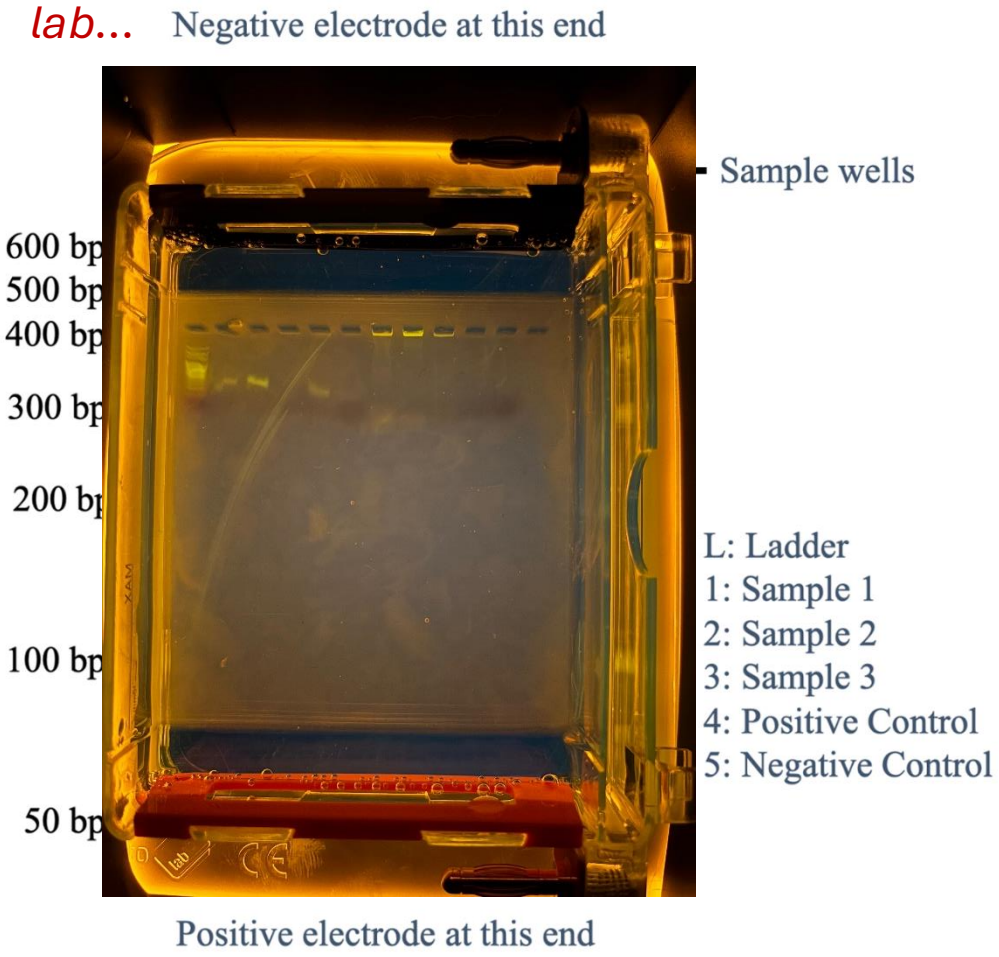
→ This allows to visualise **bands** – containing **DNA fragments** of the **same size,** measured in base pairs **(bp)**

- A **ladder (L)** = reference for the size of DNA fragments contained in the bands of sample lanes
- To assess quality of PCR, include:
  - **+ control:** template of known sequence and length
  - **– control:** PCR reagents but no template

(Suza et al., 2021)

Interpreting gel electrophoresis results

Remember a few weeks ago in the lab…



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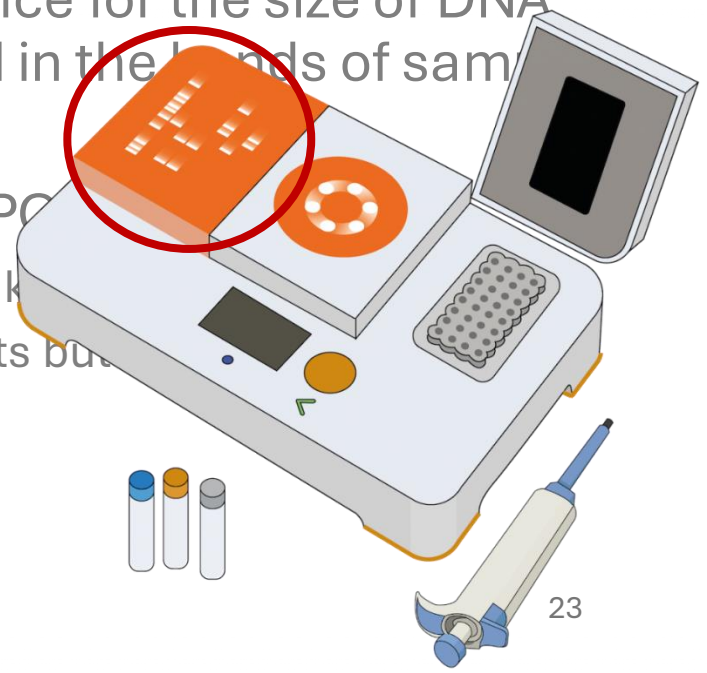
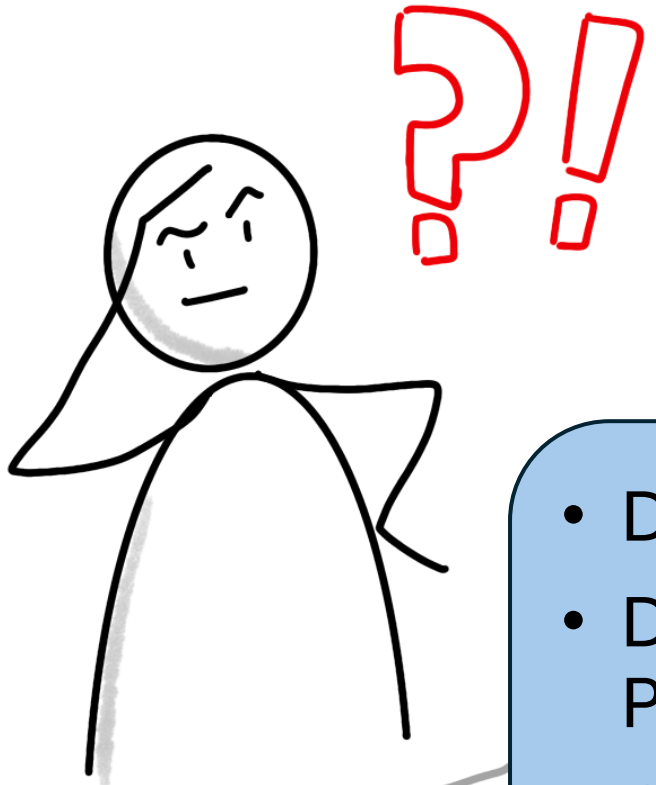


Large family of PCR

- Multiplex PCR
- Long-range PCR
- Single cell PCR
- Fast Cycling CPR
- Methylation- specific PCR
- **qPCR**
- **RT-PCR**
- End point PCR



- Digital PCR
- Droplet-based digital PCR
- Chip based digital PCR
- Isothermal amplification

(Zhu et al., 2020)

Large family of PCR

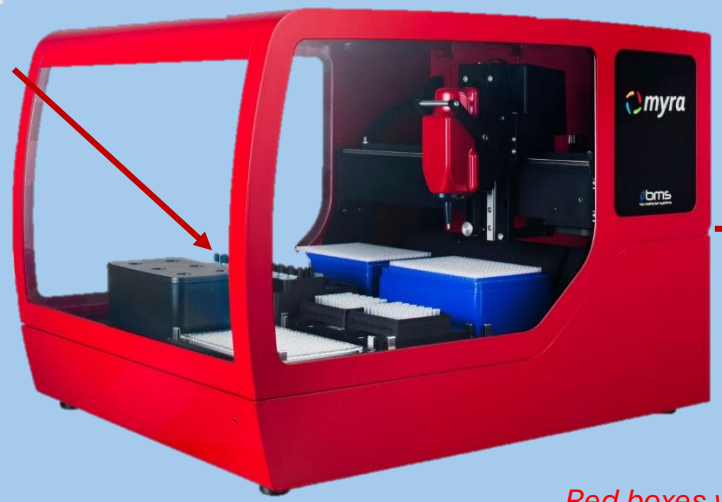
Quantitative PCR

PCR-based technique: **amplification a target DNA sequence & quantification of its concentration**

→ Allows for **back calculation of initial template concentration**

→ Evaluation of DNA copy number, viral load…

SAMPLES



Robot preparing the samples for PCR: mixing of template DNA, primers, buffers…

Looks like a little centrifuge where the PCR cycles occur with real-time quantification using fluorescence

Red boxes where the magic happens

(Dymond, 2013)

Large family of PCR

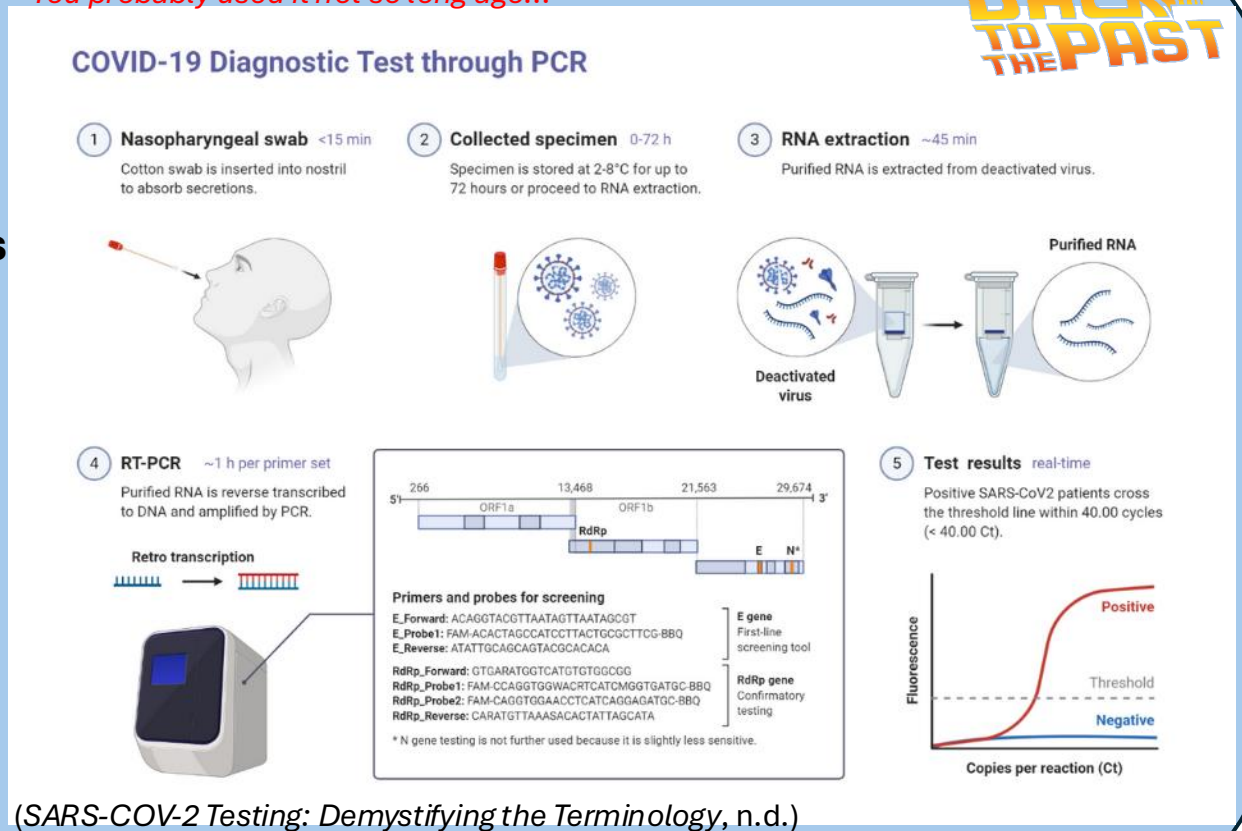
RT-PCR

Reverse **T**ranscription PCR: **reverse transcription** of **RNA into complementary DNA** & **amplification of this cDNA**

→ Use of reverse transcriptase enzyme

→ Mainly for genetic diseases and characterization of gene expression in tissues or cells over developmental time courses

You probably used it not so long ago…



(*SARS-COV-2 Testing: Demystifying the Terminology*, n.d.)

(Bachman, 2013,Dutta et al.,2022)

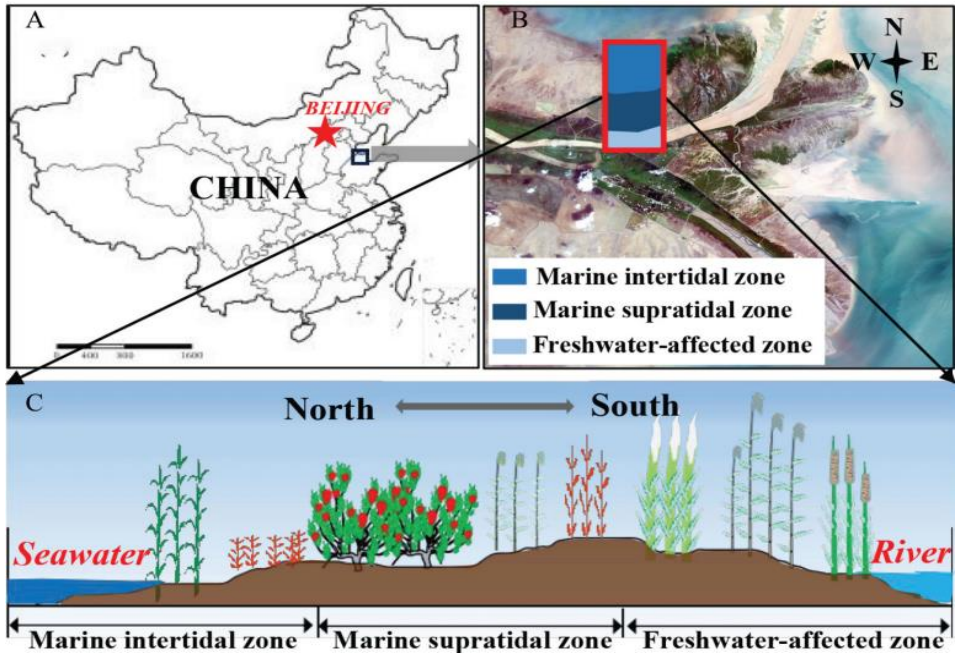
Applications

- **Forensics**:
  Thanks to its sensitivity, PCR detects very small amounts of template DNA (e.g., from few skin/hair cells) – *DNA fingerprinting*.
- **Medicine**:
  Study of genetic diseases, pathogen detection, and characterization of viruses.
- **Paternity testing**:
  DNA fingerprinting to match DNA of an individual to that of their suspected children, siblings, parents.
- **Environmental Microbiology**:
  Detect indicator bacteria of specific microbial communities (e.g. biodegradation and bioremediation).
  Detect and monitor environmental contamination (e.g. with pathogens).

(News-Medical, 2018)

Salinity controls soil microbial community structure and function in coastal estuarine wetlands (2021)

Guangliang Zhang , Junhong Bai , Christoph C. Tebbe , Qingqing Zhao, Jia Jia, Wei Wang, Xin Wang and Lu Yu

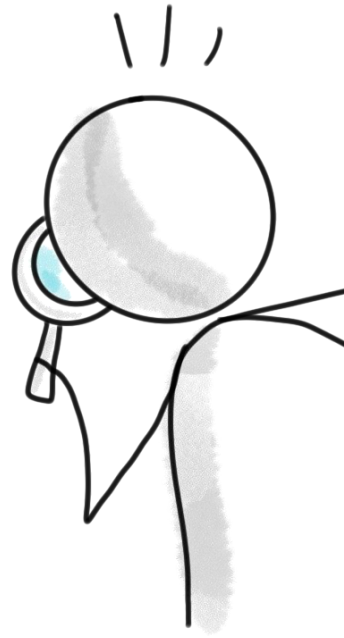


How will the increase in levels of salinity in freshwater ecosystems, due to sea level rise, affect microbial communities' composition and functions?

(Yoshimura et al., 2025)

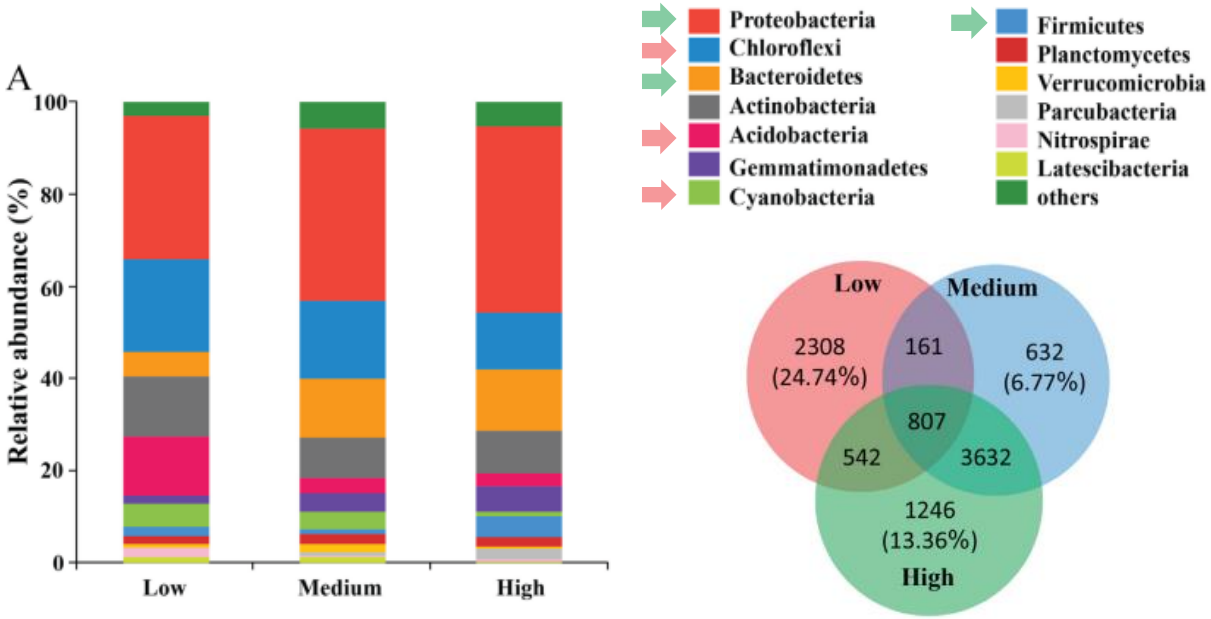
Experimental methods:

- 30 samples from coastal estuarine wetland of the **Yellow River**: from oligohaline to hyperhaline
- DNA extraction from soil samples
- **16s rRNA** amplification with **PCR** (V3-V4):
  1) **Denaturation:** 95°C – 30s
  2) **Annealing:** 55°C – 30s
  3) **Elongation:** 72°C – 45s
  4) **Final extension:** 72°C – 10 min
- PCR products separated on 2% agarose gel **electrophoresis**
- 16s rRNA quantification and sequencing

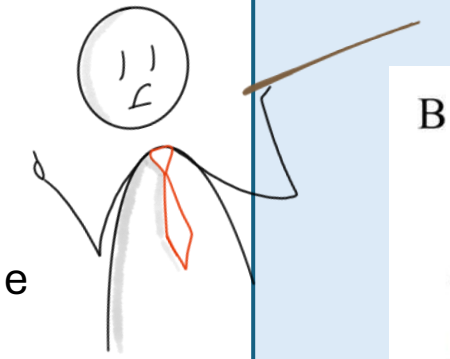


Result 1: soil salinity strongly influences the composition of microbial communities (9328 OTUs)

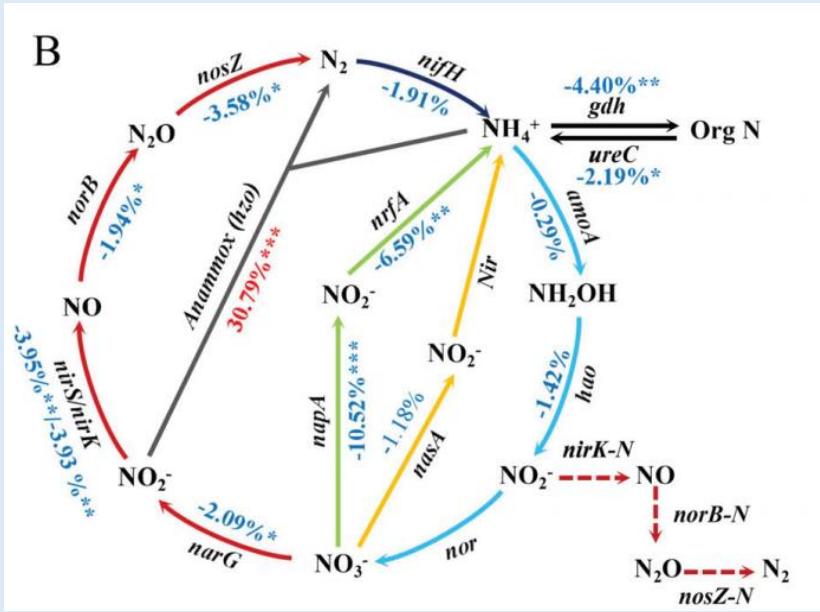
- **More unique OTUs** in **meso/hyperhaline** soils;
- **Higher bacterial OTU** numbers **at low salinity**;
- Highest phylogenetic diversity (PD) in mesohaline conditions (evolutionary history)
- Different **relative abundances dominant phyla**



(Yoshimura et al., 2025)



Result 2: soil salinity significantly altered the microbial functional genes along the salinity gradient



Abundance of most **functional genes** for **C, N, P, and S-cycling** **decreased** with increasing **salinity**:

→ salinity inhibits soil biogeochemical processes in estuary ecosystems, exception: ***hzo*** gene (**annamox**)

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