PCR & Gel electrophoresis

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Gel electrophoresis

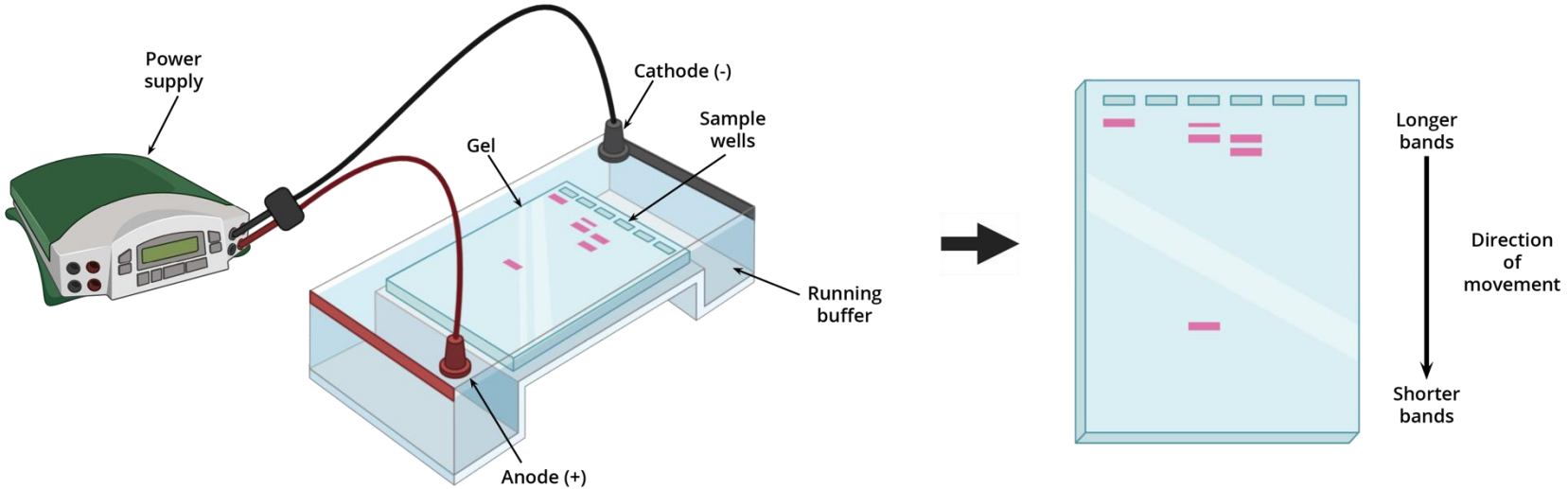
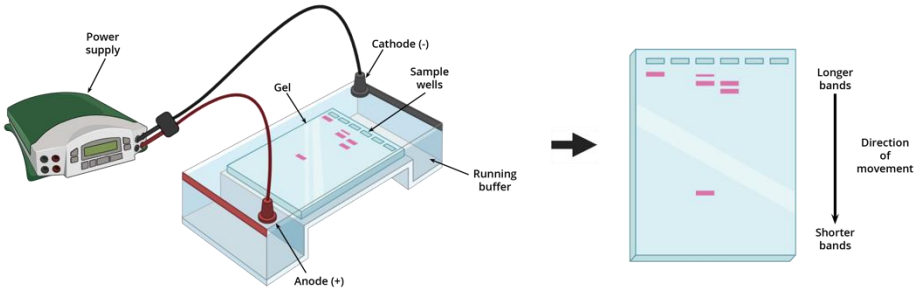


Figure 1. Schematic representation of Gel electrophoresis

Gel electrophoresis



The basic idea behind gel electrophoresis is to **separate molecules**, based on their charge, mass or shape **with an electric field**.

DNA is negatively charged; it will therefore **move towards the cathode.** Longer strands of DNA will encounter more resistance from the gel and move slower.

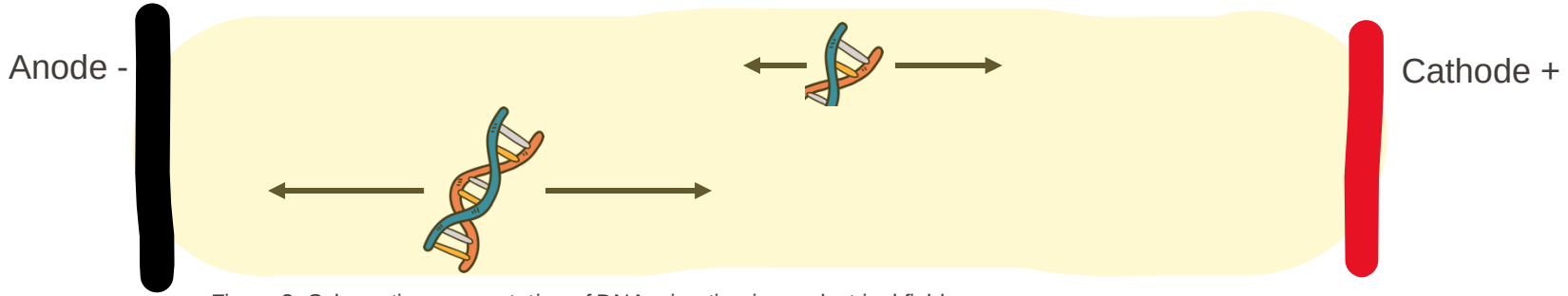


Figure 2. Schematic representation of DNA migration in an electrical field

Gel electrophoresis

Ladder :
A mix of known DNA lengths used as a calibration tool to determine the length of strands of interest

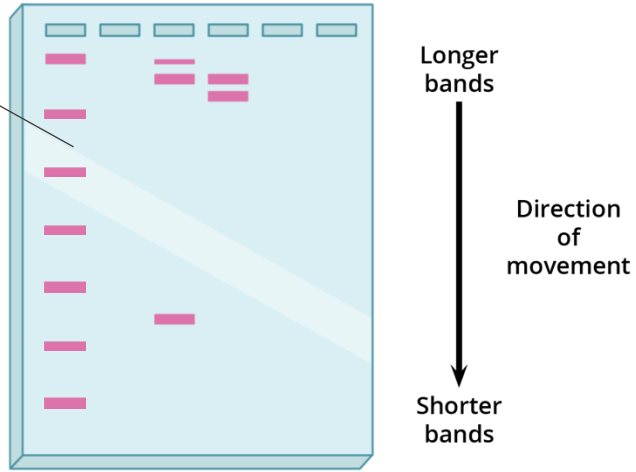


Figure 3. Schematic representation of DNA bands

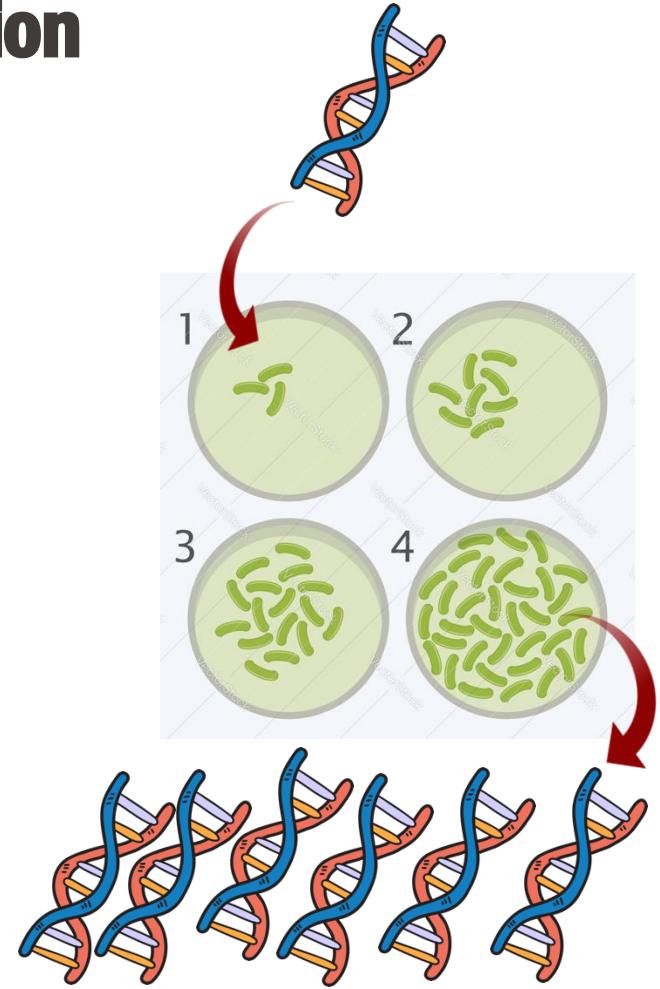
By adding known sections of the DNA in the wells, we can **compare** the **positions** of the bands we have with what is known in the literature.
Gel electrophoresis can therefore be used to confirm if PCR worked, and **DNA was amplified**.
One downside of this method is that it requires a lot of DNA.
How to get this DNA?

Motivations behind PCR invention

Motivation: To sequence and analyse DNA, a lot of it is needed.

Before PCR: DNA was inserted to a bacteria. Gowing the bacteria in the lab would replicate the DNA.

Need: Growing bacteria and extracting DNA is a long and tedious process, and the need to replicate DNA, in large orders of magnitude, fast was necessary to advance in forensics and genomics



Polymerase Chain Reaction

Key Idea from Mullis :

DNA is replicated by enzymes within each cells, why not do it directly in a test tube ?

Figure 13.17 (Campbell, 2017)

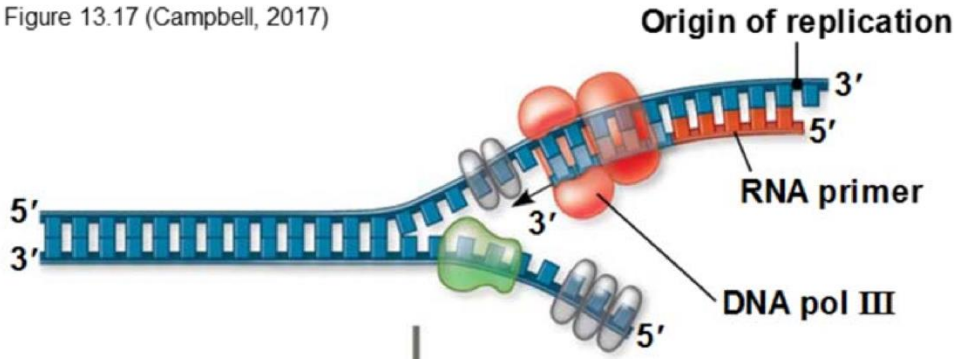
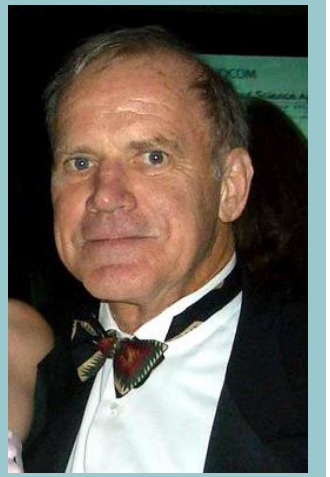


Figure 4. Schematic representation of DNA replication (Campbell, 2017)

For his invention of PCR in 1983, Kary Mullis received the 1993 Nobel Price in chemistry with Michael Smith.



Kary Mullis

THEORY

Polymerase Chain Reaction

Science behind it

PCR

DNA is mixed in a buffer solution with DNA polymerase, primers, and DNA building blocks (dNTP)

- **Denaturation:** heat splits apart the two strands of DNA
- **Annealing:** primers stick to the target bases and mark the boundaries of the zone that will be replicated
- **Extension:** DNA polymerase moves from the primers to the end of the strand, fastening complementary bases

DNA is doubled for each cycle, making it a chain reaction

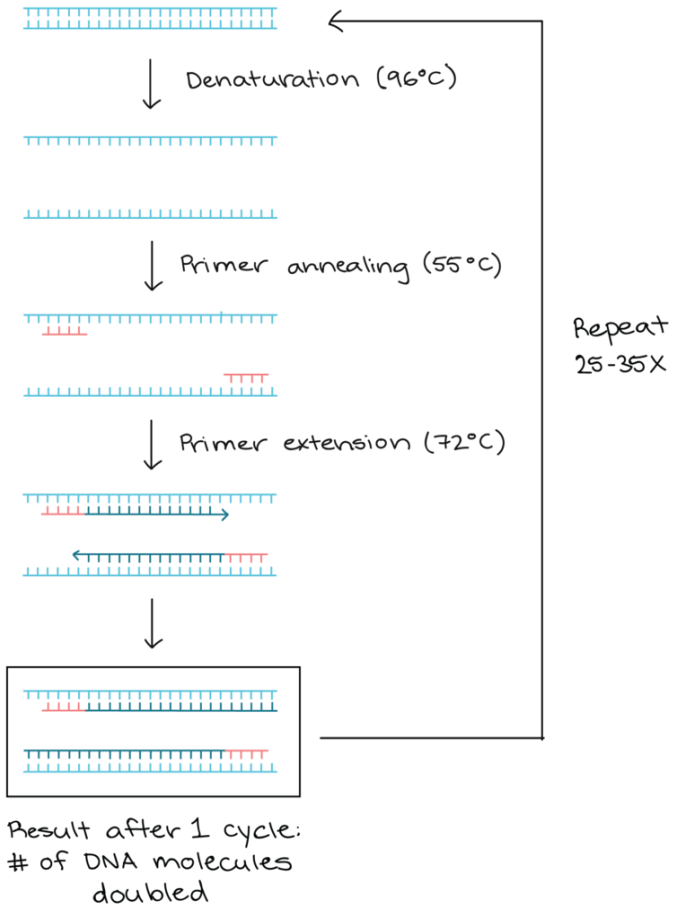
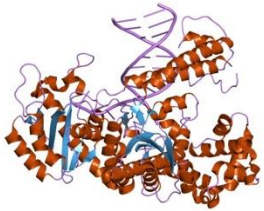


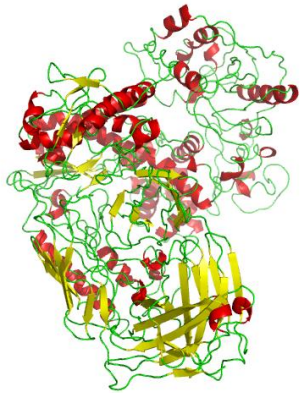
Figure 5. Schematic representation of PCR

DNA Polymerase

The denaturation process damages regular DNA polymerase. At first DNA pol had to be added after each cycle, until a **thermostable DNA pol** was discovered. This allowed the automation and **continuous run of PCR** cycles.

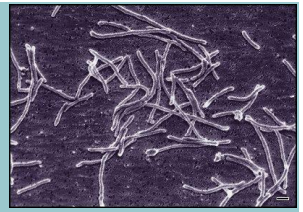


DNA polymerase I



Taq Polymerase

Thermus Aquaticus



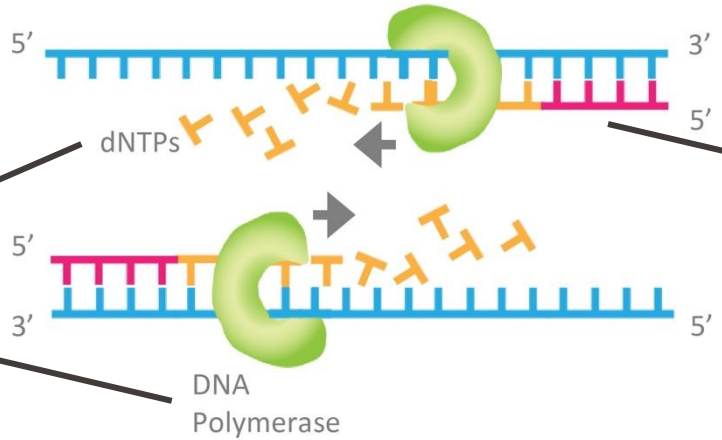
Is a thermophilic bacteria, first discovered in a hot spring of Yellowstone park.

This chemotroph grows optimally at 70°C thanks to its thermo-stable enzymes.

Being able to withstand temperatures of 95°C ($t_{1/2}$ 40min), the Taq polymerase is especially useful for PCR.

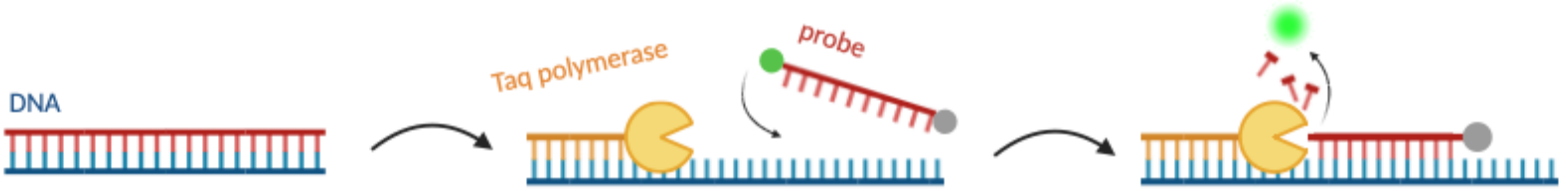
PCR lab


FIREPol
Mix Read
with 7.5
Cat
Lot
Expiry Dat
Storage
For Research
SOLIS
BIODYNE


5’
3’
dNTPs
5’
5’
3’
5’
DNA
Polymerase


Bacterial
1492R Prim
10uM
Bacterial 16
Primer
10uM

qPCR



- Produces **quantitative** results
- Monitors amplification in **real-time**
- Fluorescence is emitted when DNA is copied
- Standards are used to obtain n° of copies

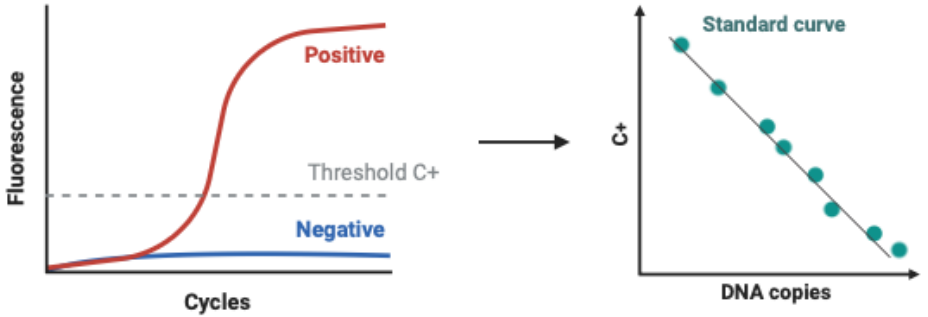
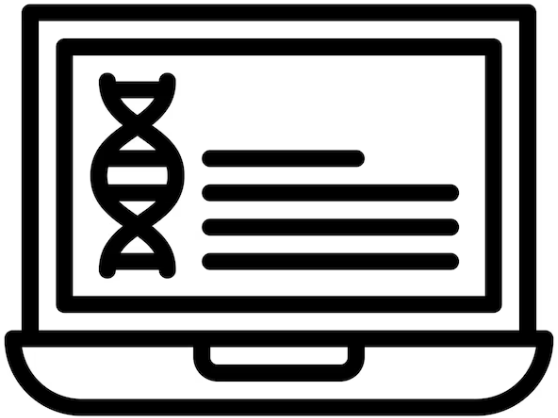


Figure 6. Schematic representation of qPCR standards



Data & applications

What information can provide us?

qPCR is used in many fields

Research

Medicine

Forensics

- Detection of **viruses** and **bacteria** since it allows many targets in one PCR
- It gives information about the **abundance** and **diversity** of microorganisms
- Gene expression levels
- It can **use specific primers** to target genes of interest

qPCR in research

The ISME Journal (2014) 8, 660–674

www.nature.com/ismej

ORIGINAL ARTICLE

Linking N_2O emissions from biochar-amended soil to the structure and function of the N-cycling microbial community

Johannes Harter[1,4], Hans-Martin Krause[1,4], Stefanie Schuettler[1], Reiner Ruser[2], Markus Fromme[3], Thomas Scholten[3], Andreas Kappler[1] and Sebastian Behrens[1]
[1]*Geomicrobiology and Microbial Ecology, Center for Applied Geosciences, University of Tuebingen, Tuebingen, Germany;* [2]*Fertilisation and Soil Matter Dynamics, Institute of Crop Science, University of Hohenheim, Stuttgart, Germany and* [3]*Soil Science and Geomorphology, Department of Geography, University of Tuebingen, Tuebingen, Germany*

Nitrous oxide (N_2O) contributes 8% to global greenhouse gas emissions. Agricultural sources represent about 60% of anthropogenic N_2O emissions. Most agricultural N_2O emissions are due to increased fertilizer application. A considerable fraction of nitrogen fertilizers are converted to N_2O by microbiological processes (that is, nitrification and denitrification). Soil amended with biochar (charcoal created by pyrolysis of biomass) has been demonstrated to increase crop yield, improve soil quality and affect greenhouse gas emissions, for example, reduce N_2O emissions. Despite several studies on variations in the general microbial community structure due to soil biochar amendment, hitherto the specific role of the nitrogen cycling microbial community in mitigating soil N_2O emissions has not been subject of systematic investigation. We performed a microcosm study with a water-saturated soil amended with different amounts (0%, 2% and 10% (w/w)) of high-temperature biochar. By quantifying the abundance and activity of functional marker genes of microbial nitrogen fixation (*nifH*), nitrification (*amoA*) and denitrification (*nirK*, *nirS* and *nosZ*) using quantitative PCR we found that biochar addition enhanced microbial nitrous oxide reduction and increased the abundance of microorganisms capable of N_2-fixation. Soil biochar amendment increased the relative gene and transcript copy numbers of the *nosZ*-encoded bacterial N_2O reductase, suggesting a mechanistic link to the observed reduction in N_2O emissions. Our findings contribute to a better understanding of the impact of biochar on the nitrogen cycling microbial community and the consequences of soil biochar amendment for microbial nitrogen transformation processes and N_2O emissions from soil.
The ISME Journal (2014) **8,** 660–674; doi:10.1038/ismej.2013.160; published online 26 September 2013
Subject Category: Geomicrobiology and microbial contributions to geochemical cycles
Keywords: nitrogen cycle; biochar; denitrification; nitrification; nitrous oxide; *nosZ*; N_2O emission; greenhouse gas; soil microbial community

Motivation: Understand the specific role of the nitrogen cycling microbial community in mitigating soil N_2O emissions

Objective: Quantify responses of functional genes to soil biochar amendments; Using functional genes as a proxy of N_2O emissions

Methodology: qPCR to determine the abundance of key functional marker genes (*amoA*, *nirS, nirK, nosZ and nifH*)

qPCR in research

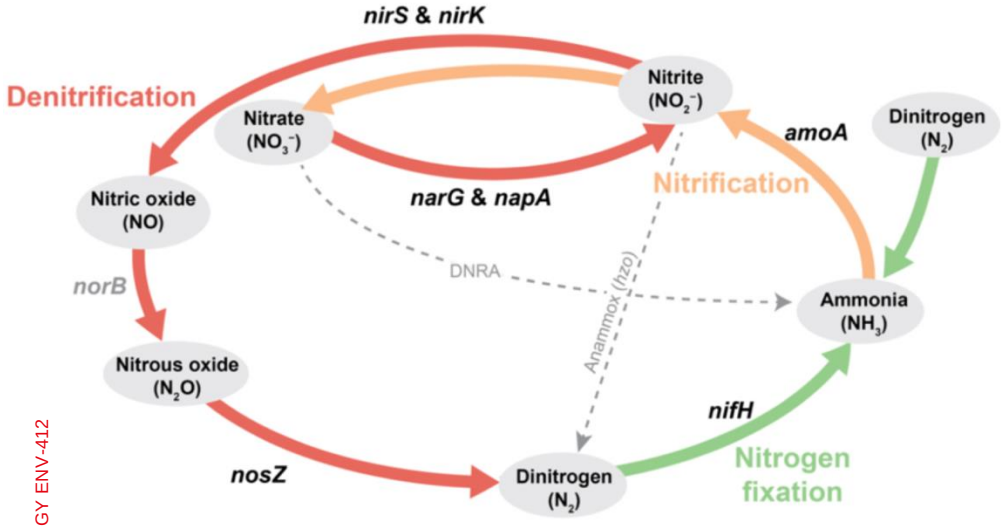


Figure 7. Schematic representation of N-cycle and functional genes involved (PhD thesis Griffith., 2016)

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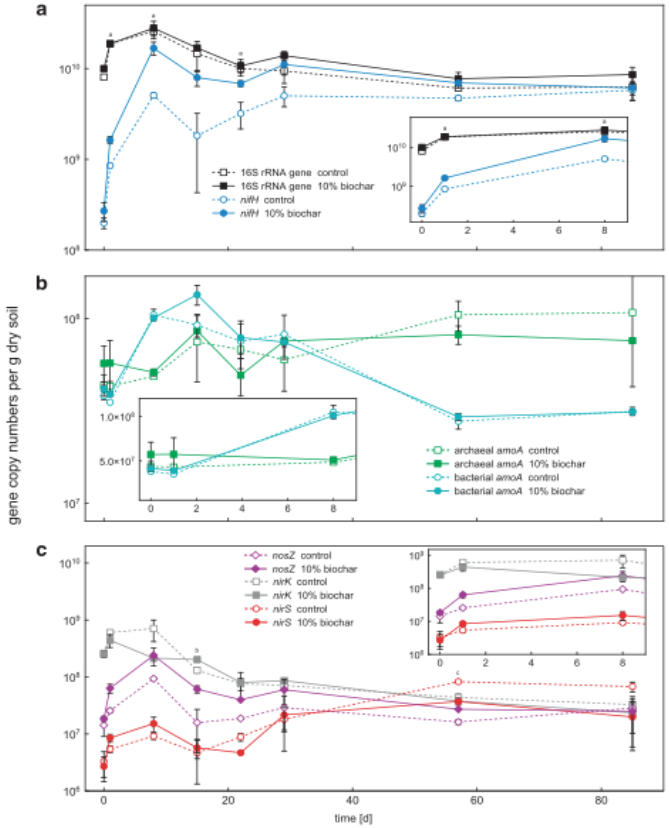
qPCR in research



nifH gene copy n° were consistently higher in the treatment microcosms compared with the control

No significant differences between control and biochar-amended for the archaeal and bacterial ***amoA*** gene data

nirS and ***nirK*** did not show significant differences between treatment and control

Significantly higher ***nosZ*** gene copy n° in treatment compared to control were quantified at day 15

Figure 8. Gene copy numbers per gram dry soil over time for various key genes of microbial nitrogen transformation processes (Harter et al., 2013, The ISME Journal (2014) 8, 660–674)

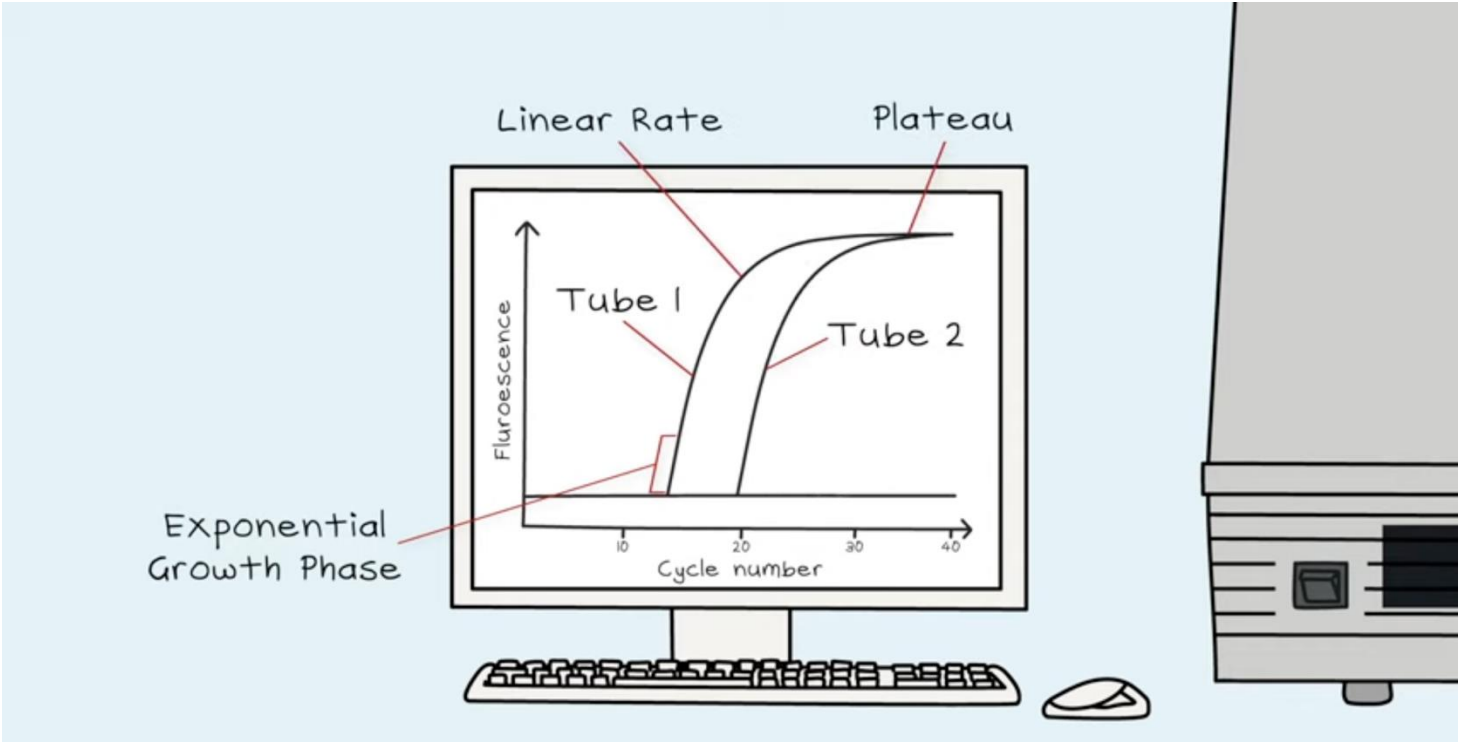
qPCR in research

Conclusions

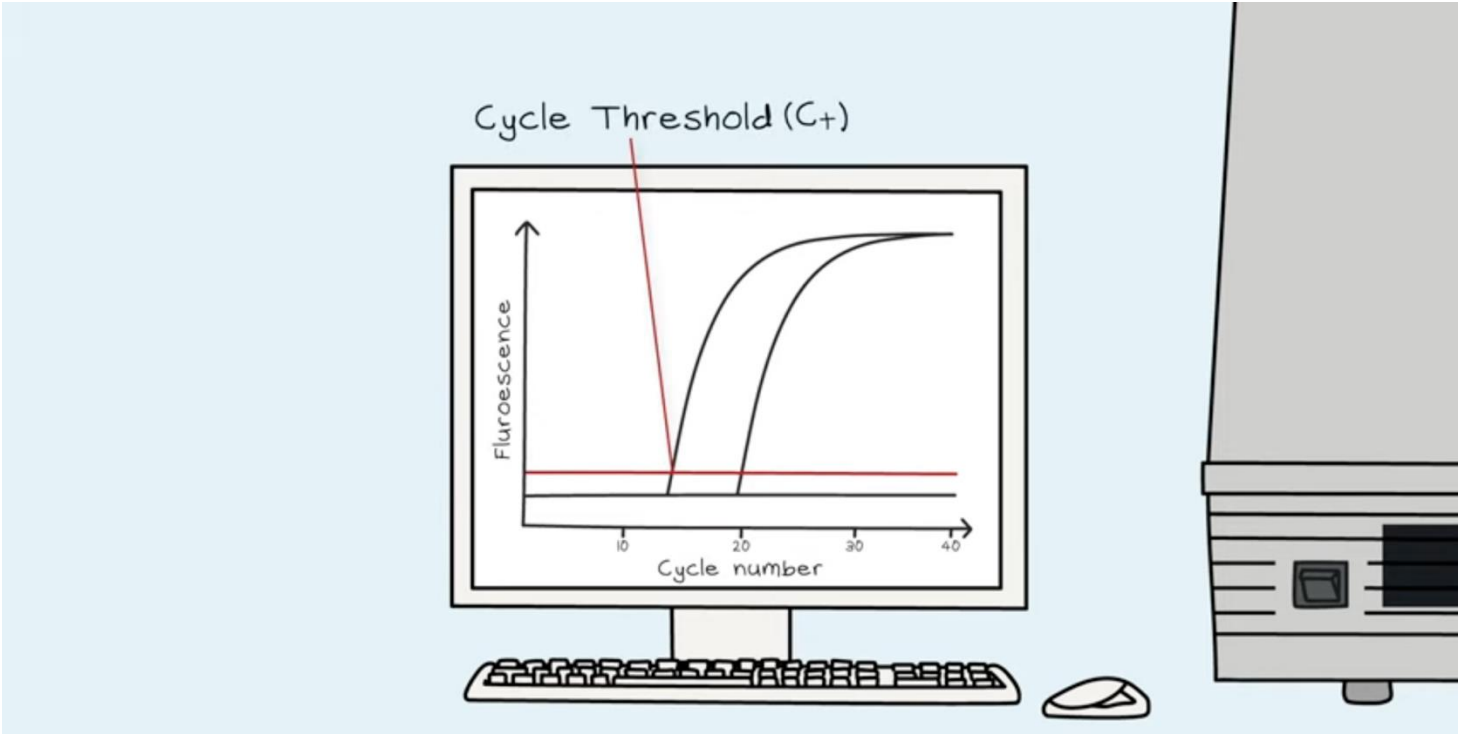
- Biochar changed the **denitrifier microbial community composition** by promoting the growth
- qPCR demonstrates that these **functional genes** could be used as a **proxy of N_2O** emissions from biochar-amended soil
- Results are valid under **controlled conditions**, which differ from those in the **field**.


Thank you!

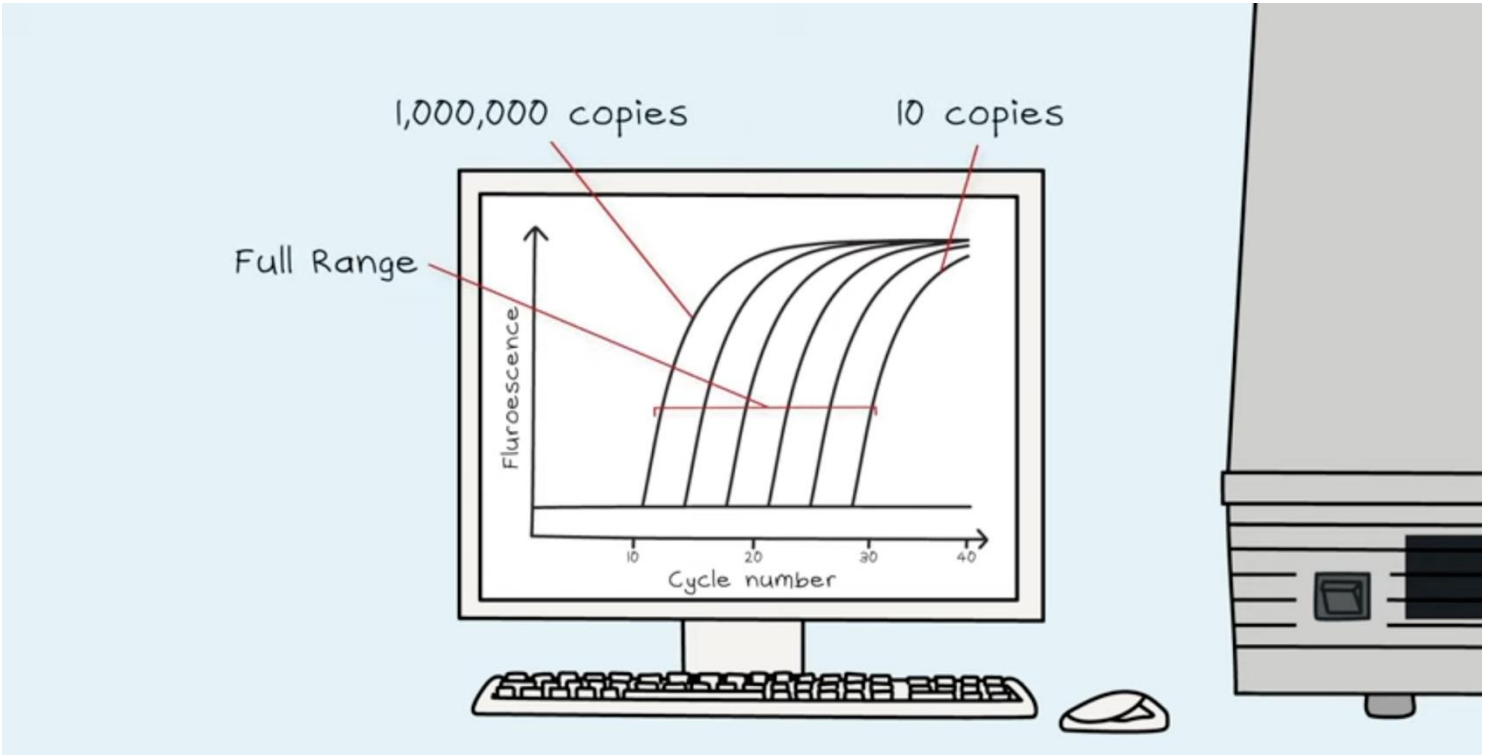
qPCR


Linear Rate
Plateau
Tube 1
Tube 2
Fluroescence
Exponential
Growth Phase
10
20
30
40
Cycle number

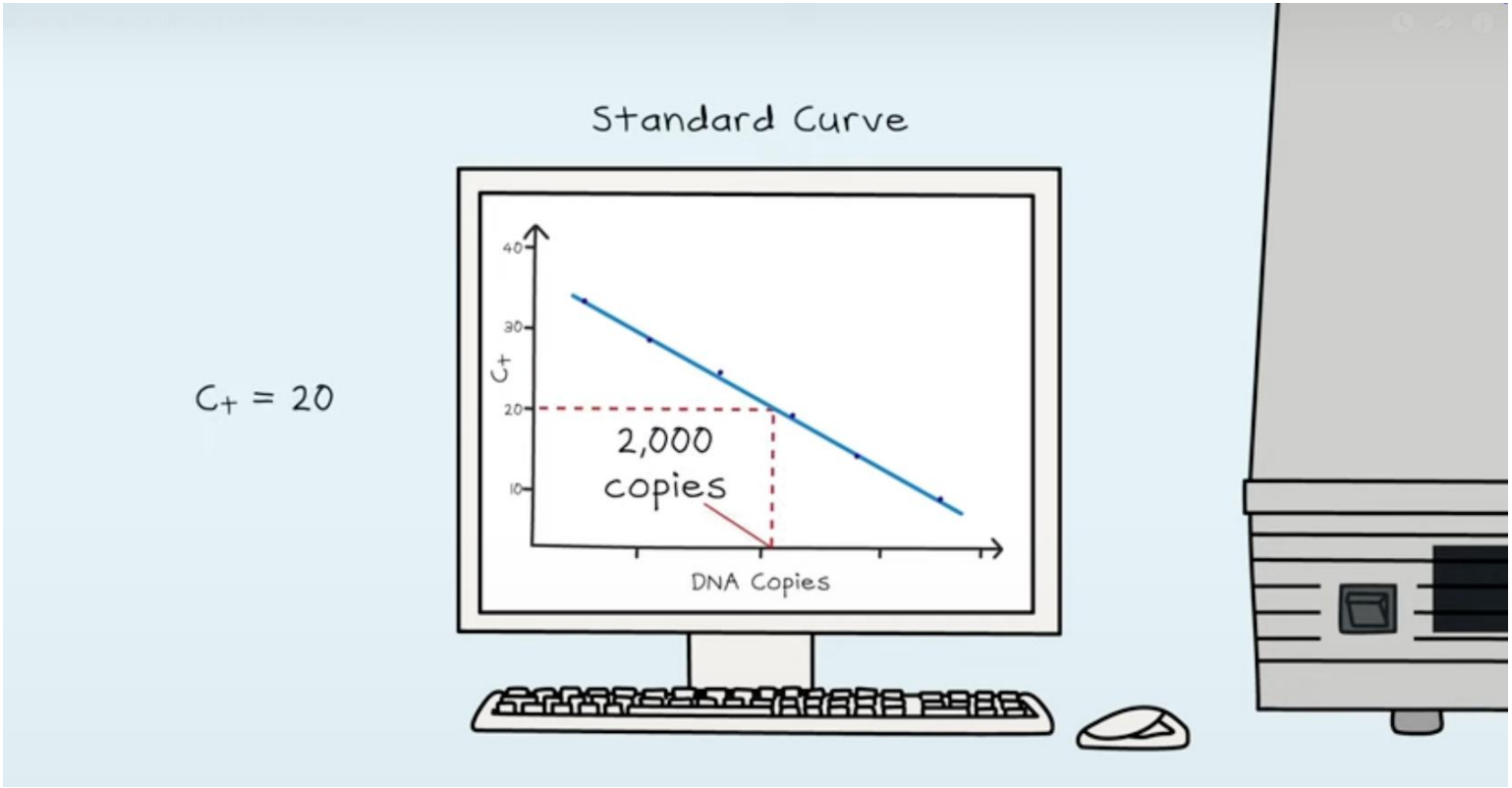
qPCR



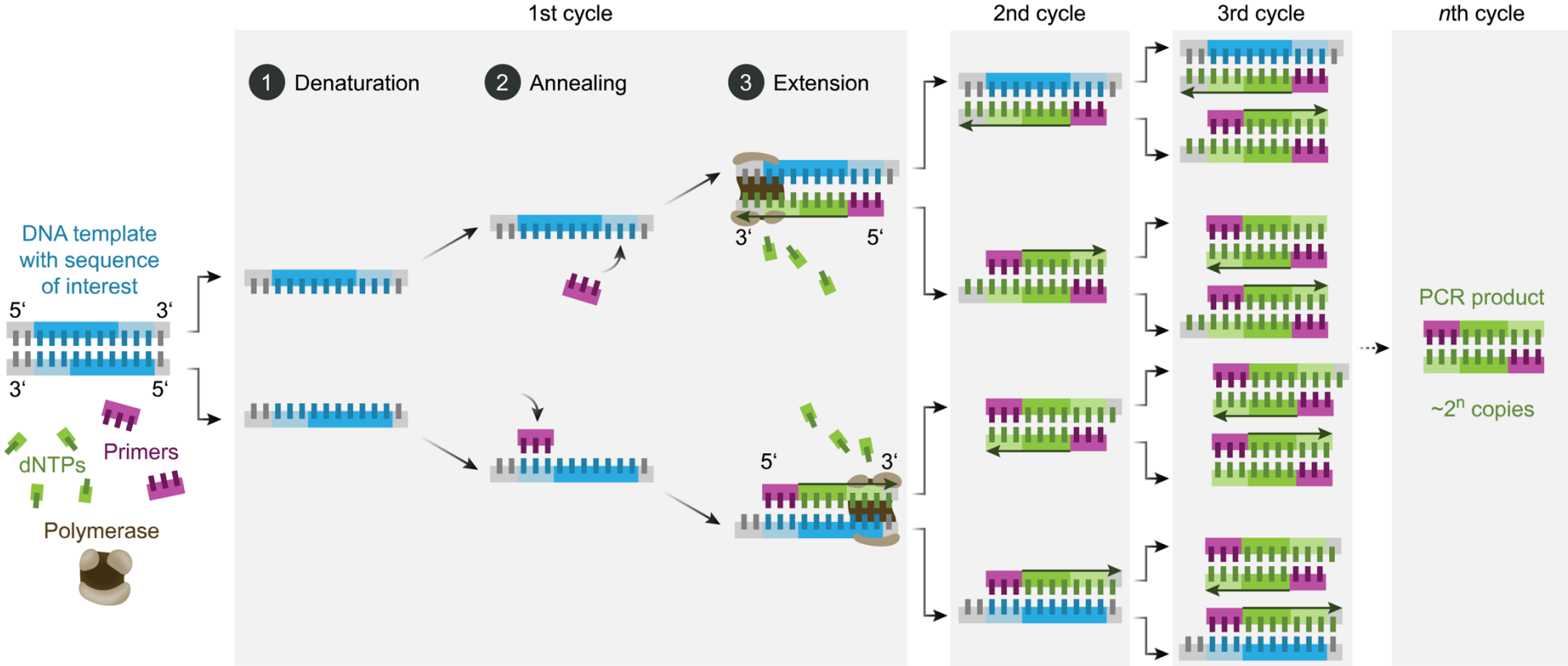
qPCR


1,000,000 copies
10 copies
Full Range
Fluroescence
10
20
30
40
Cycle number

qPCR



qPCR


1st cycle
2nd cycle
3rd cycle
nth cycle
1 Denaturation
2 Annealing
3 Extension
DNA template with sequence of interest
5‘
3‘
3‘
5‘
dNTPs
Primers
Polymerase
3‘
5‘
5‘
3‘
PCR product
~2n copies