Metabarcoding : the main steps - part 2

Clarisse Lemonnier

Summary

Sampling

DNA extraction

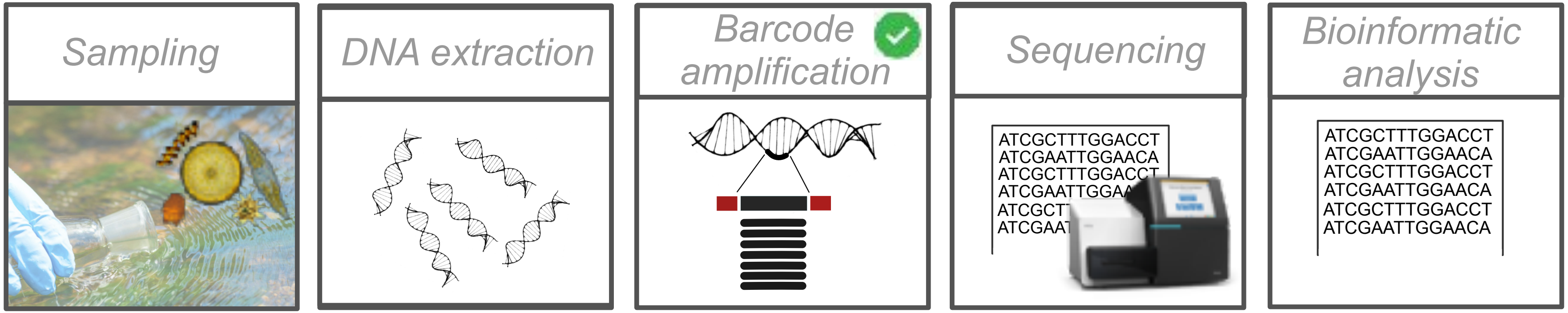
PCR

Sequencing

Bioinformatic analysis

Taxonomic assignation

Metabarcoding steps



Sampling

DNA extraction

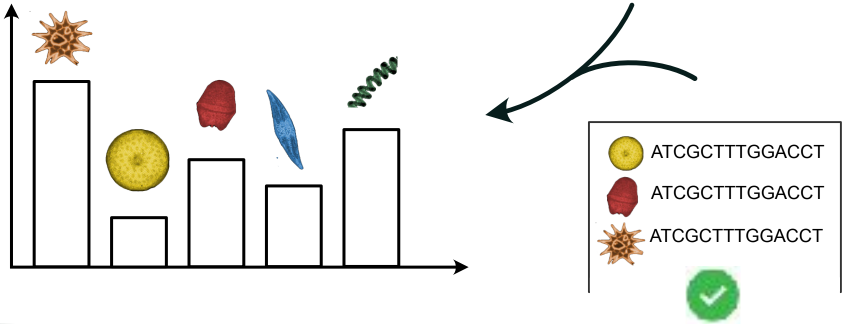
Barcode amplification ✓

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT

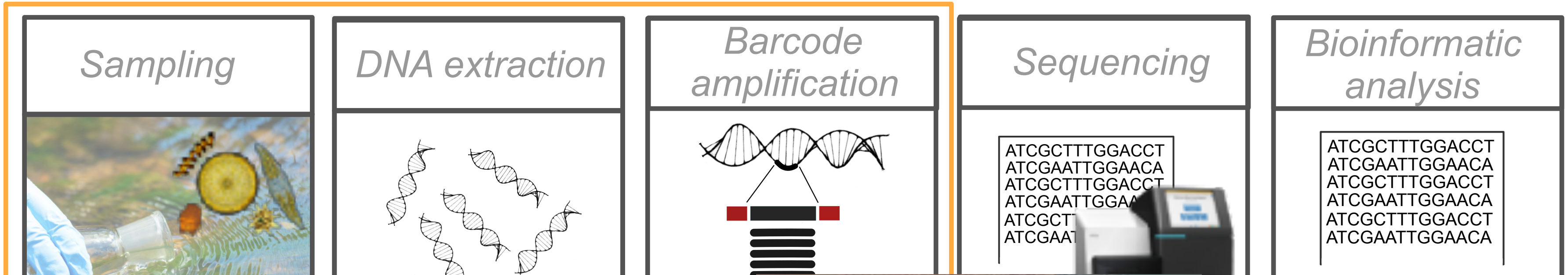
Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA



ATCGCTTTGGACCT
ATCGCTTTGGACCT
ATCGCTTTGGACCT
✓

Metabarcoding steps

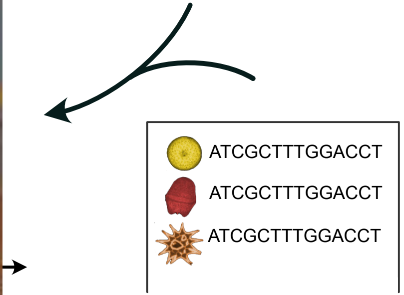


ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

« Clean » conditions

Avoid contaminations



ATCGCTTTGGACCT
ATCGCTTTGGACCT
ATCGCTTTGGACCT

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Metabarcoding steps

Diatom biofilm :
thousands of cells/cm2

Ancient DNA:
A very few/m2

Sampling

DN

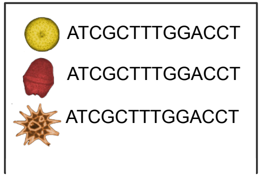
de
ation

rmatic
lysis

TGGACCT
TGGAACA
TGGACCT
TGGAACA
TGGACCT
TGGAACA

« Clean » conditions

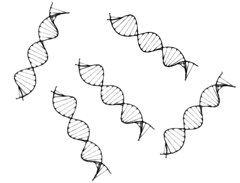
Avoid contaminations



ATCGCTTTGGACCT
ATCGCTTTGGACCT
ATCGCTTTGGACCT

« Clean » conditions

DNA extraction



Clean material

« Sterile » environment

Negative controls

Sampling

Sampling

Clean dishes.
Use of hydrogen peroxide, HCl, bleach, autoclave, sterile devices …
Add negative controls

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Sampling

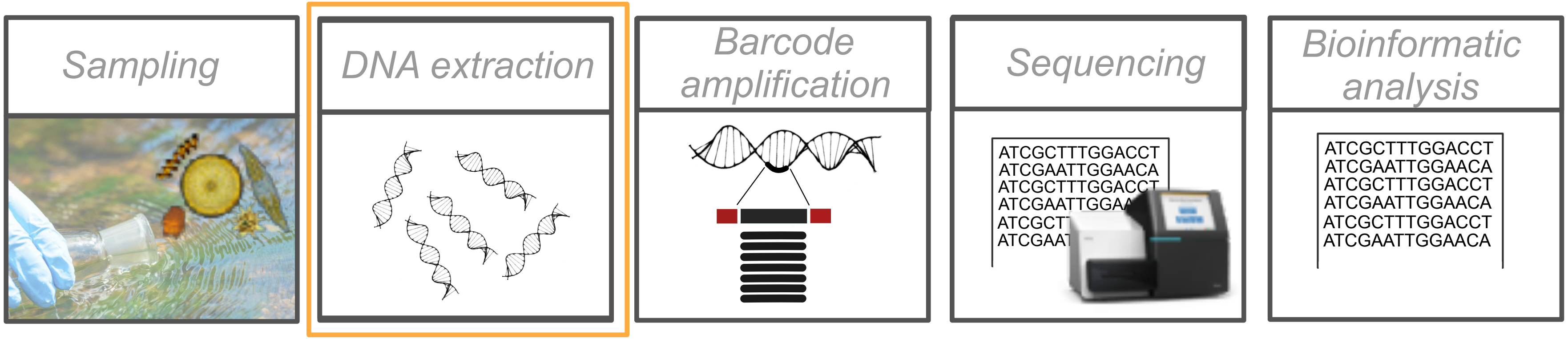
Sampling

Clean dishes.
Use of hydrogen peroxide, HCl, bleach, autoclave, sterile devices …
Add negative controls

Sample preservation : preservation of DNA

-80°C or -20°C that preserve DNA (ideal for long-term preservation)
Other solutions, such as buffers or ethanol

Metabarcoding steps



Sampling

DNA extraction

Barcode amplification

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

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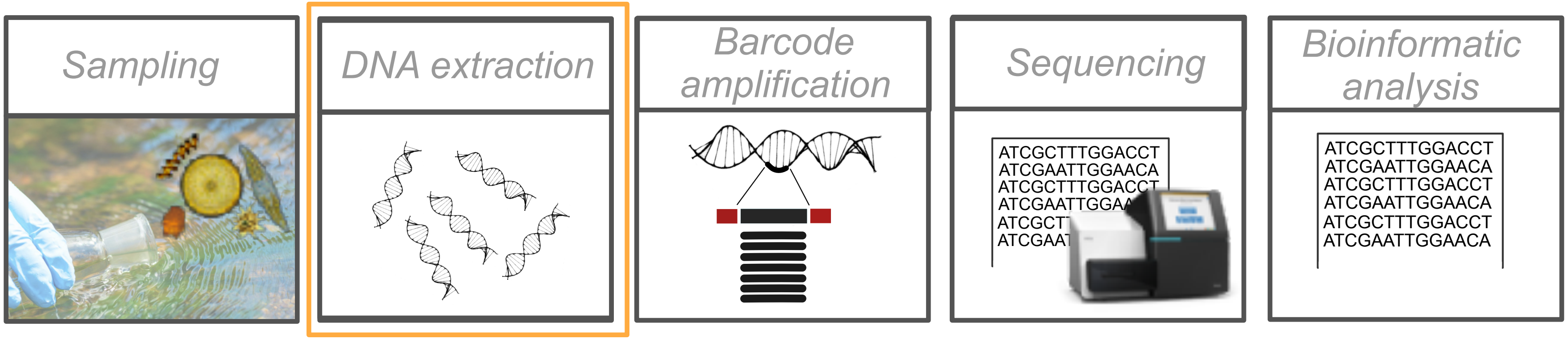
Metabarcoding steps


Sampling

DNA extraction

Barcode amplification

Sequencing

Bioinformatic analysis

Objectives

Extract DNA of good quality and quantity from the targeted organisms

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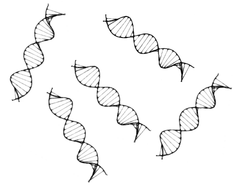
DNA extraction

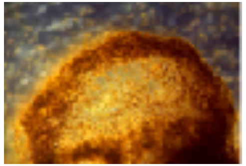
DNA extraction



In a given sample, the DNA molecule is associated with different molecules :

Of the matrix

Mineral and organic molecules



Within the cell

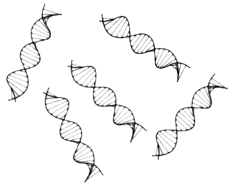
Intracellular organelles

Within DNA molecule

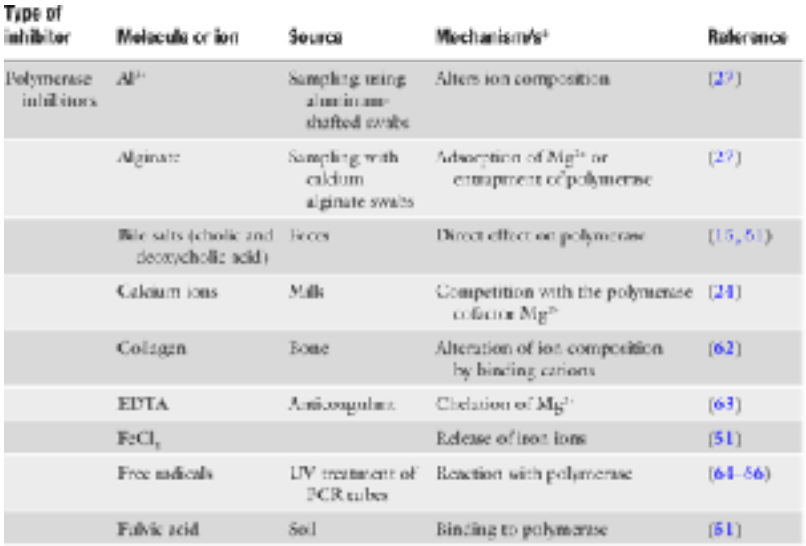
Histones

DNA extraction

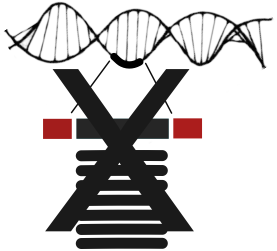
DNA extraction



In a given sample, their can be a lot of inhibitors for PCR



Type of inhibitor	Molecule or ion	Source	Mechanism/s^a	Reference
Polymerase inhibitors	Al^{3+}	Sampling using aluminium-shafted swabs	Alters ion composition	[27]
	Alginate	Sampling with calcium alginate swabs	Adsorption of Mg^{2+} or entrapment of polymerase	[27]
	Bile salts (cholic and deoxycholic acid)	Feces	Direct effect on polymerase	[15, 51]
	Calcium ions	Milk	Competition with the polymerase cofactor Mg^{2+}	[24]
	Collagen	Bone	Alteration of ion composition by binding cations	[62]
	EDTA	Anticoagulant	Chelation of Mg^{2+}	[63]
	$FeCl_3$		Release of iron ions	[51]
	Free radicals	UV treatment of PCR tubes	Reaction with polymerase	[64–66]
	Fulvic acid	Soil	Binding to polymerase	[51]



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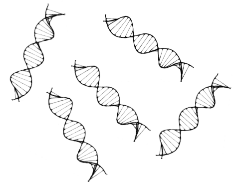
DNA extraction

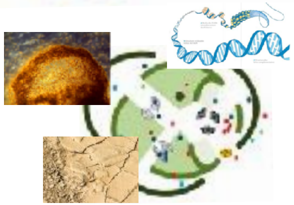
DNA extraction

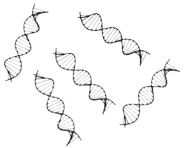


Main steps of DNA extraction

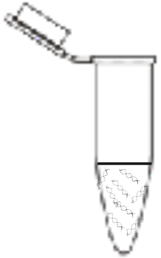
1/ Lysis of cellular membrane

2/ Separation of DNA from cellular debris, proteins and inhibitors





3/ Elution of DNA



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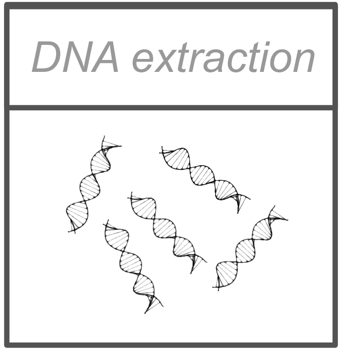
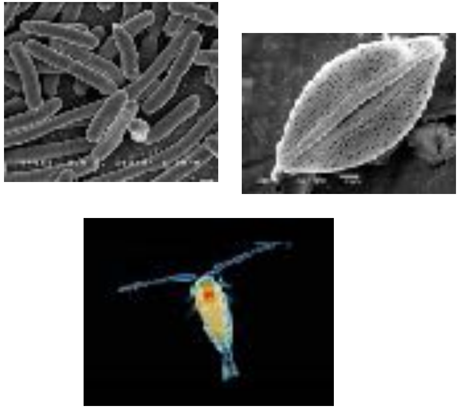
DNA extraction



Needs to be adapted to the matrix

Needs to be adapted to the organisms



Needs to be adapted to the question

DNA extraction

Main steps of DNA extraction

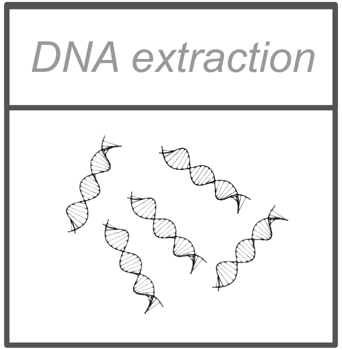
1/ Lysis of cellular membrane

2/ Separation of DNA from cellular debris, proteins and inhibitors

3/ Elution of DNA

Phenol-chloroform protocol

Commercial kits



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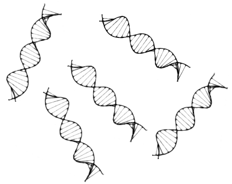
DNA extraction



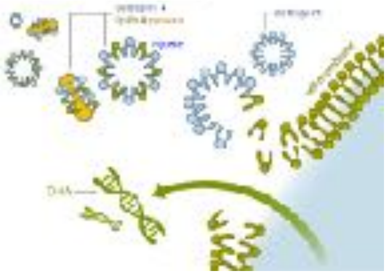
1/ Lysis of cellular membrane

Physical

Break the cells physically
- Bead-beating
- freeze/thaw cycles
- Ultrasonication
- …

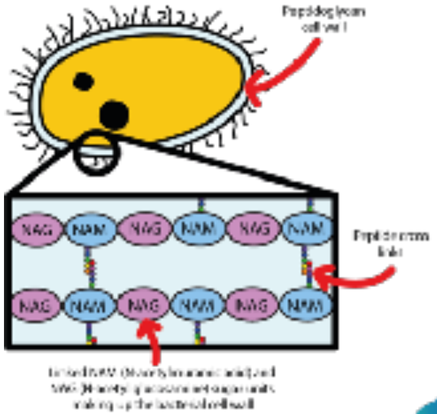
Chemicals

chemicals that will destabilize the lipid membrane :
- Detergents
- Solvents
- Acids
- …



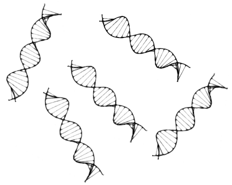
Enzymatic

Use of enzymes that will disrupt the cell membrane:
- Cellulase
- Lysozyme



DNA extraction

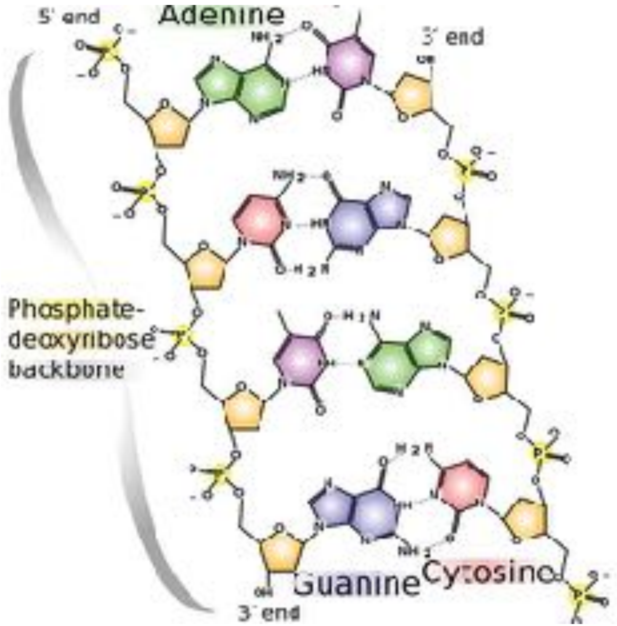
DNA extraction



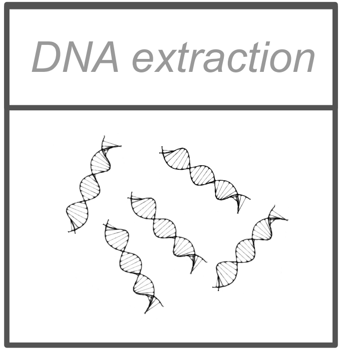
2/ Separation of DNA from membranes debris / proteins

Use the natural properties of DNA :

- hydrosoluble
- Negatively charged

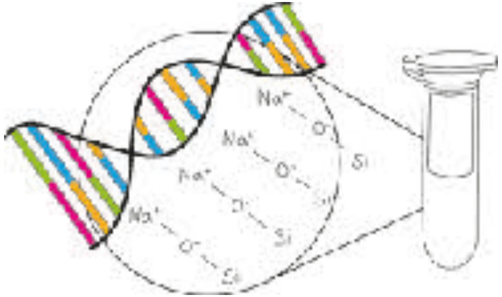


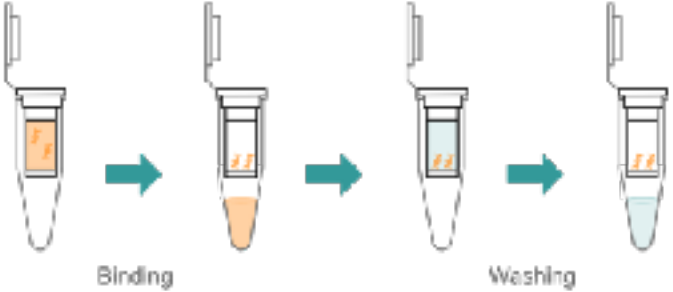
DNA extraction



2/ Separation of DNA from membranes debris / proteins

Use of silice column (kits)





Low pH and high salts
(chaotropic) : DNA binds to the
silicate membrane

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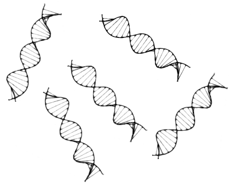
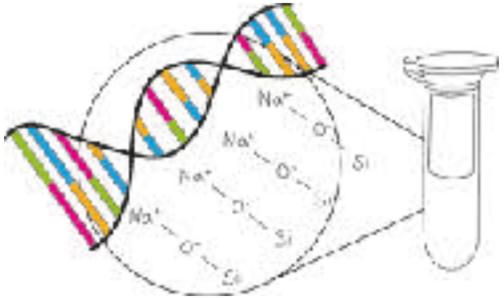
DNA extraction

DNA extraction



2/ Separation of DNA from membranes debris / proteins

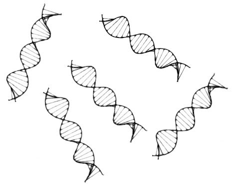
Use of silice column (kits)



Low pH and high salts (chaotropic) : DNA binds to the silicate membrane

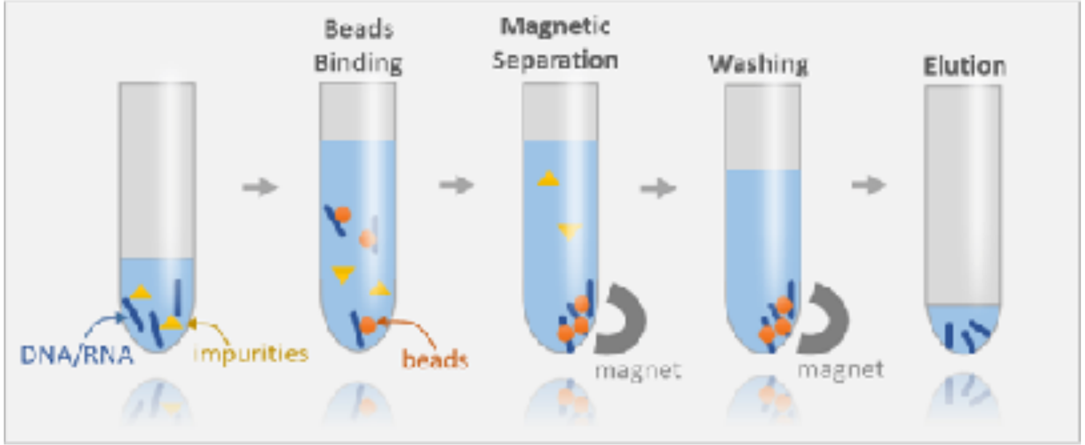
Higher pH - low salt : silicate membrane become negative and DNA is eluted

DNA extraction



2/ Separation of DNA from membranes debris / proteins

Use of magnetic beads (kits)



Magnetic beads are small (20-30nm) iron oxide particles

They are coated with silica to bind dna

Works also with salts and solution with different pH

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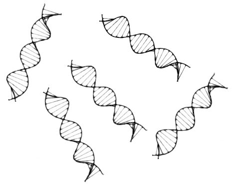
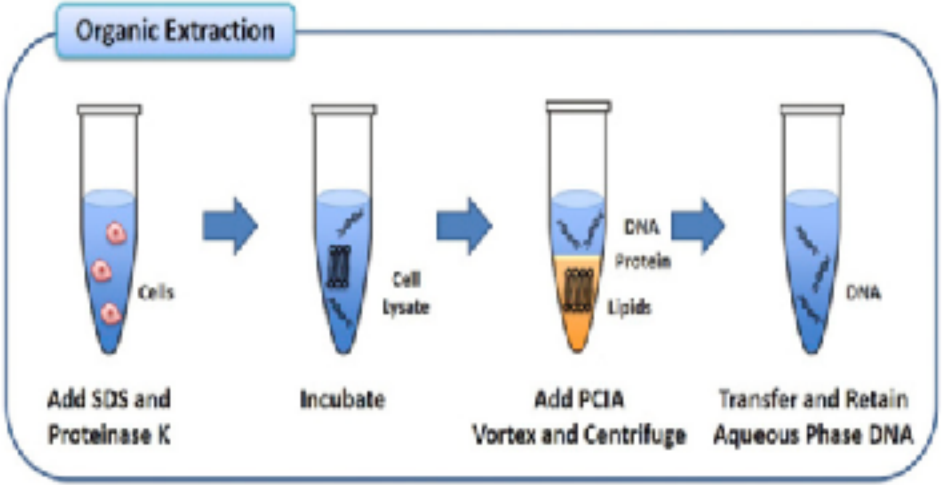
DNA extraction

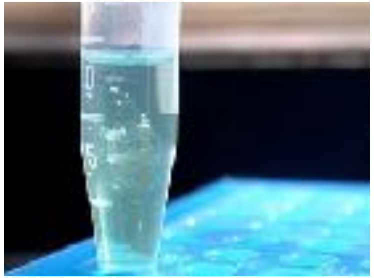
DNA extraction

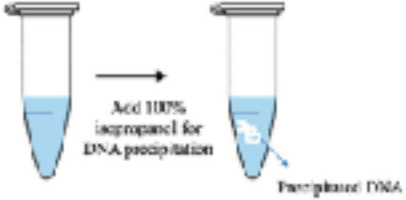


2/ Separation of DNA from membranes debris / proteins

Use of organic solvants (phenol-chloroform extraction)

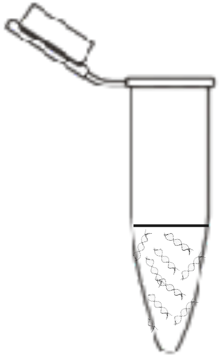


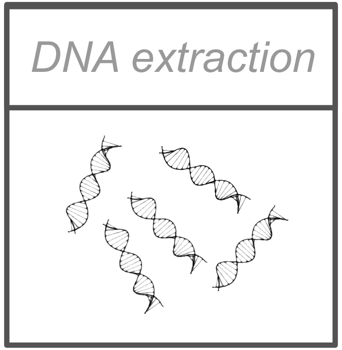


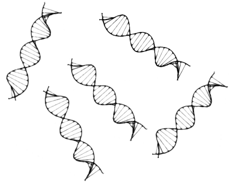


DNA is precipitated with salts and isopropanol

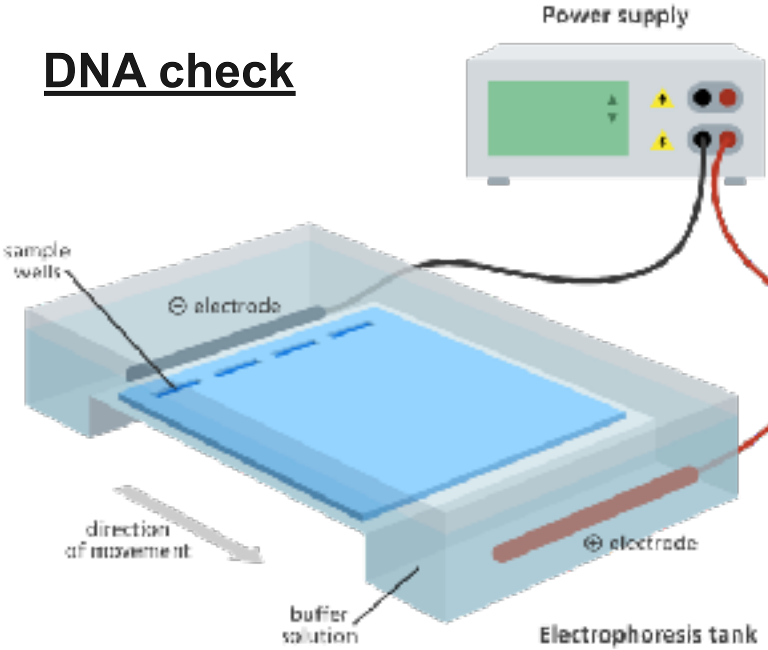
DNA extraction







DNA check



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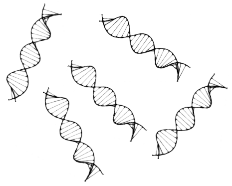
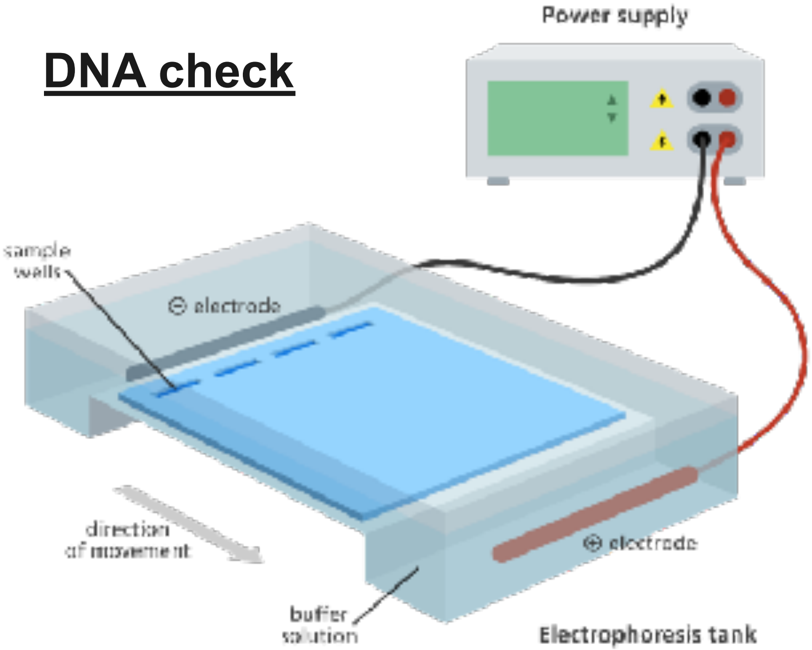
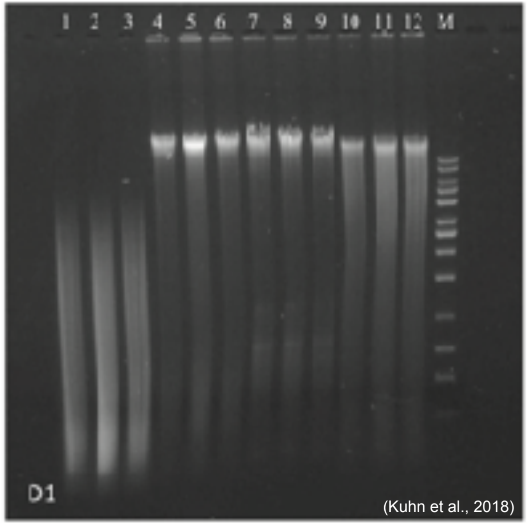


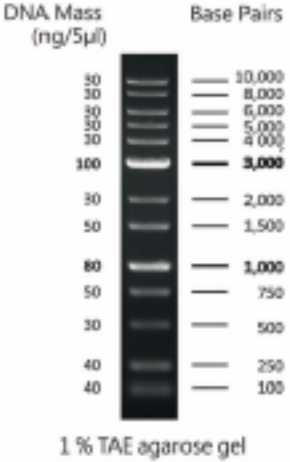
DNA check

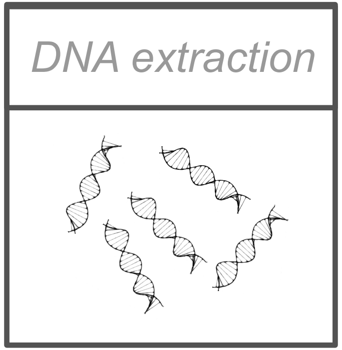




(Kuhn et al., 2018)

DNA intercalant that fluoresce under UV light



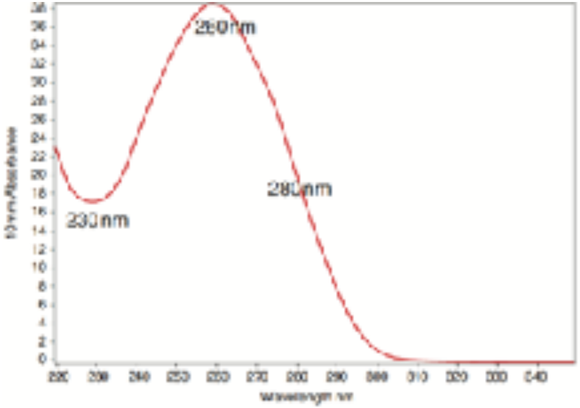


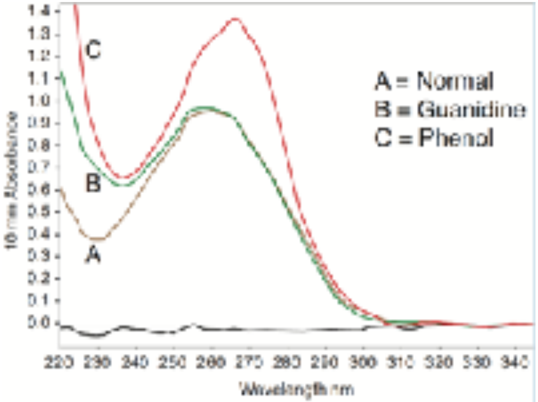
DNA quantity and quality check

Spectrophotometer

Nanodrop

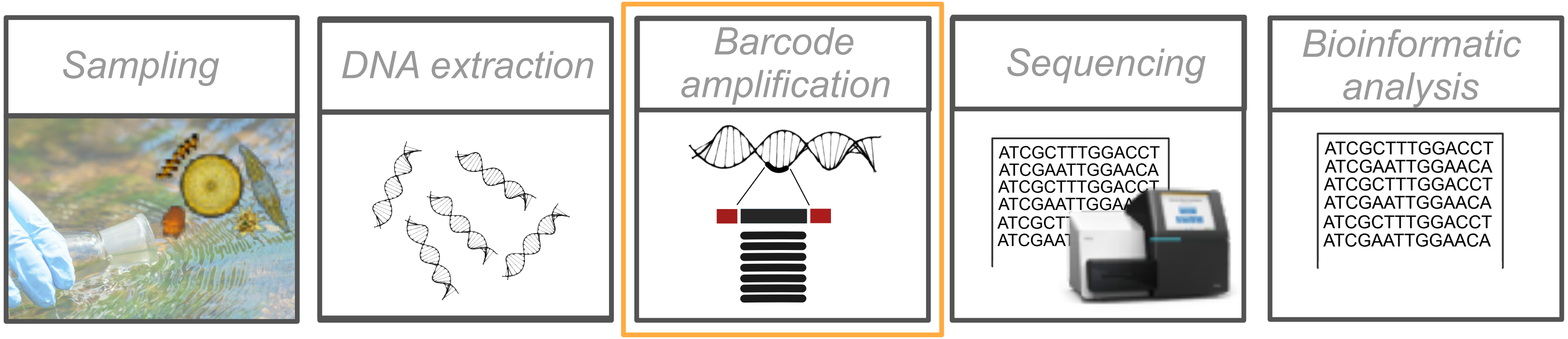
Absorbance at 260nm :DNA quantity





Protein and organic contaminations:
260/230 or 260/280

Metabarcoding steps



Sampling

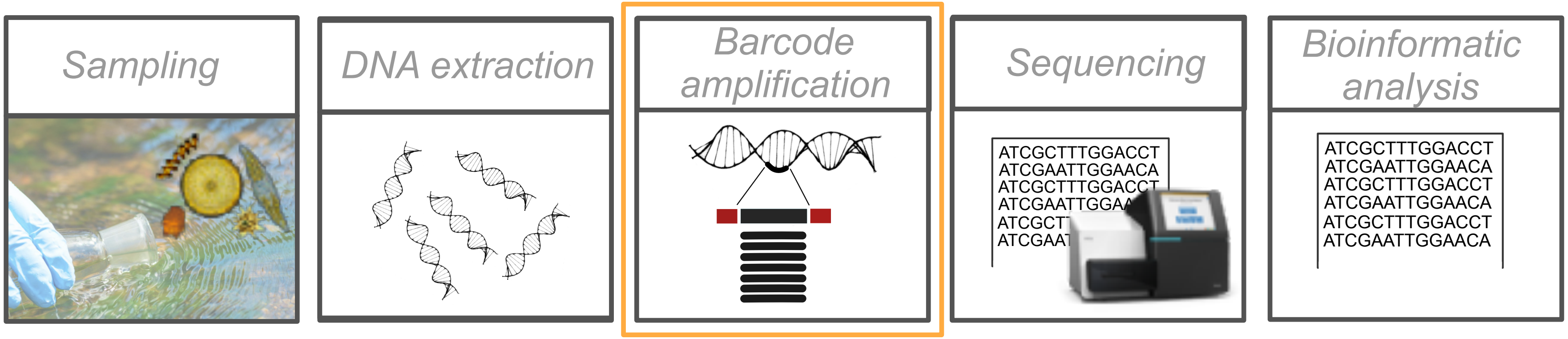
DNA extraction

Barcode amplification

Sequencing

Bioinformatic analysis

Metabarcoding steps



Sampling

DNA extraction

Barcode amplification

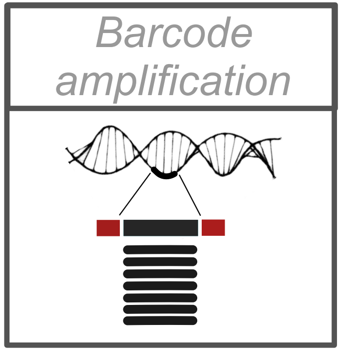
Sequencing

Bioinformatic analysis

Objectives

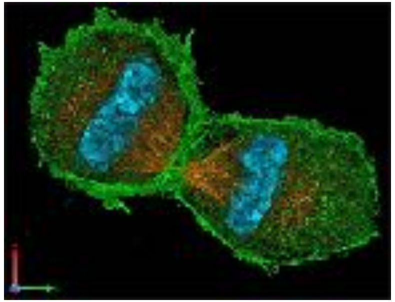
Amplify the barcode in thousands of identical copies

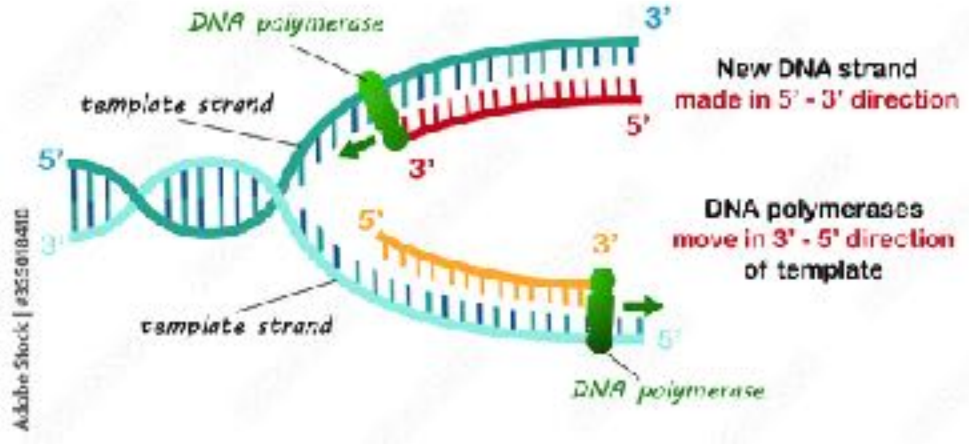
PCR steps



Barcode amplification

In the cell :





The DNA polymerase cannot synthesize a new DNA strand de novo. She can only add new nucleotides to a previous existing strand

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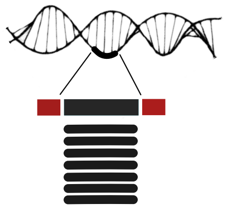
PCR steps

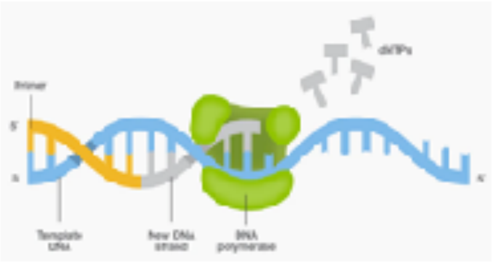
Barcode amplification



In the lab :

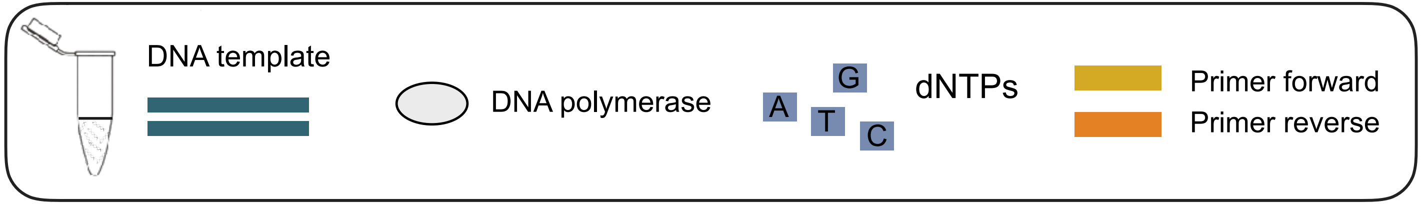
Artificially separate the 2 DNA strands

Use primers for the DNA polymerase to start the synthesis of a new strand

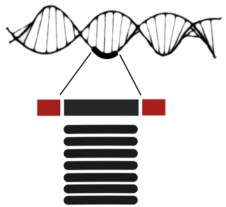


Use of a high-fidelity DNA polymerase

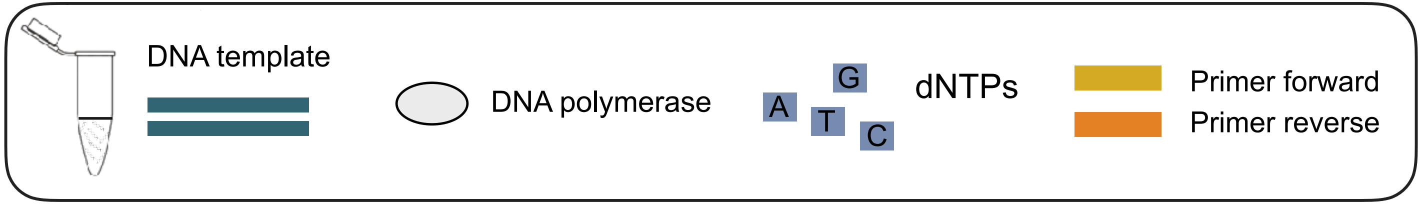
Different steps of PCR



Barcode amplification

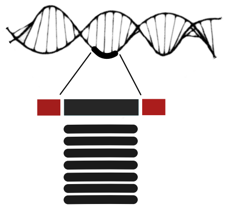


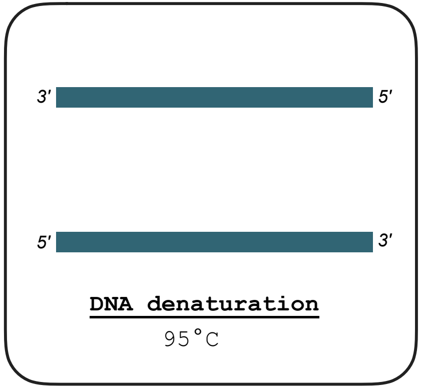
Different steps of PCR


DNA template
DNA polymerase

dNTPs
🟨 Primer forward
🟧 Primer reverse

Barcode amplification

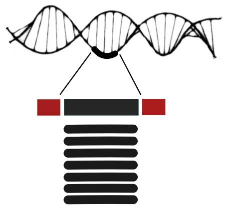


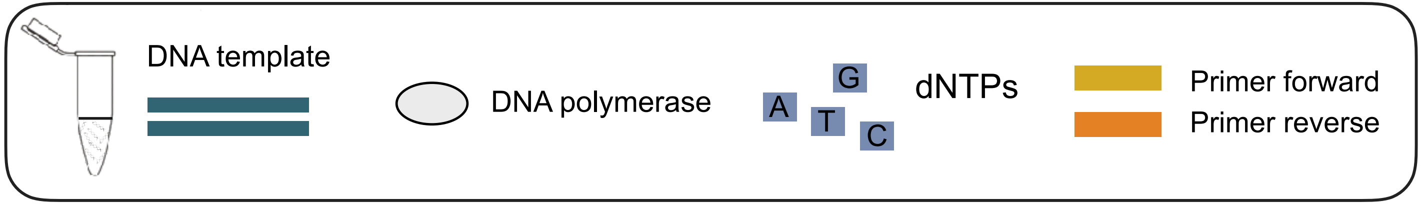


DNA denaturation

95°C

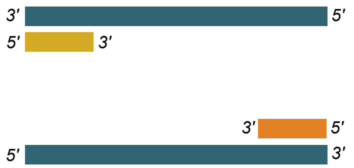
Different steps of PCR





DNA denaturation

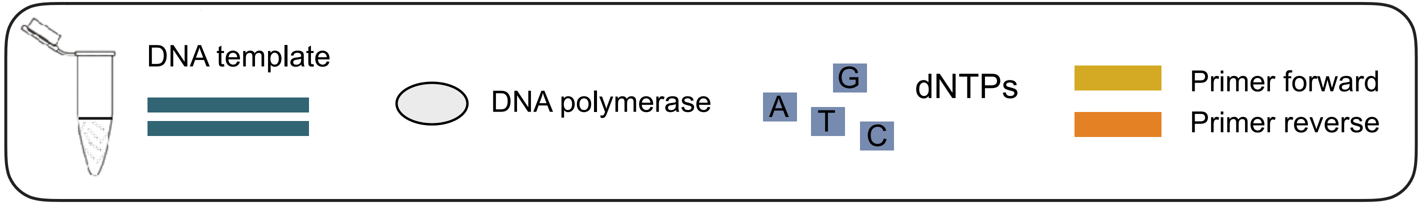
95°C



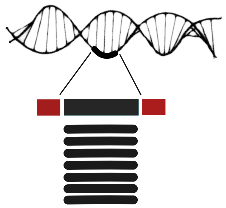
Annealing

50-60°C

Different steps of PCR

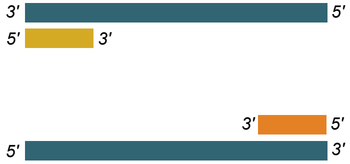


Barcode amplification



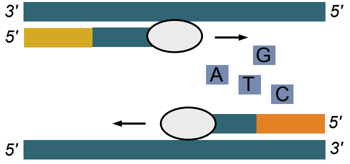
DNA denaturation

95°C



Annealing

50-60°C

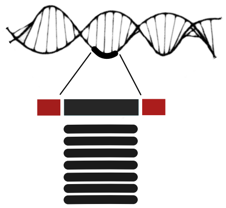


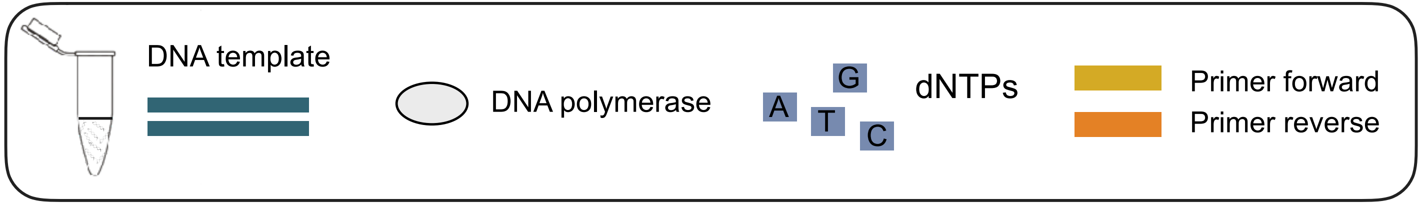
Extension

72°C

Different steps of PCR

Barcode amplification




DNA template

DNA polymerase

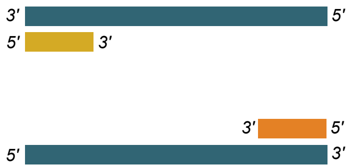
dNTPs

Primer forward

Primer reverse

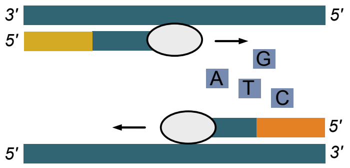
DNA denaturation

95°C



Annealing

50-60°C

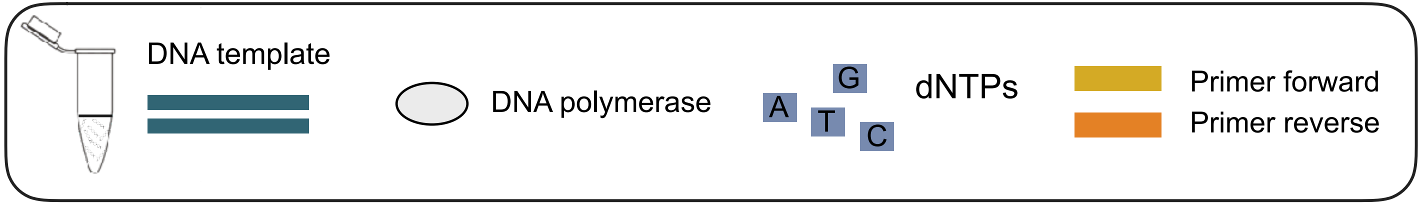


Extension

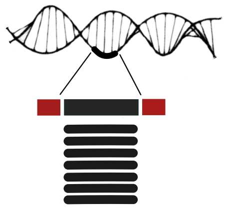
72°C

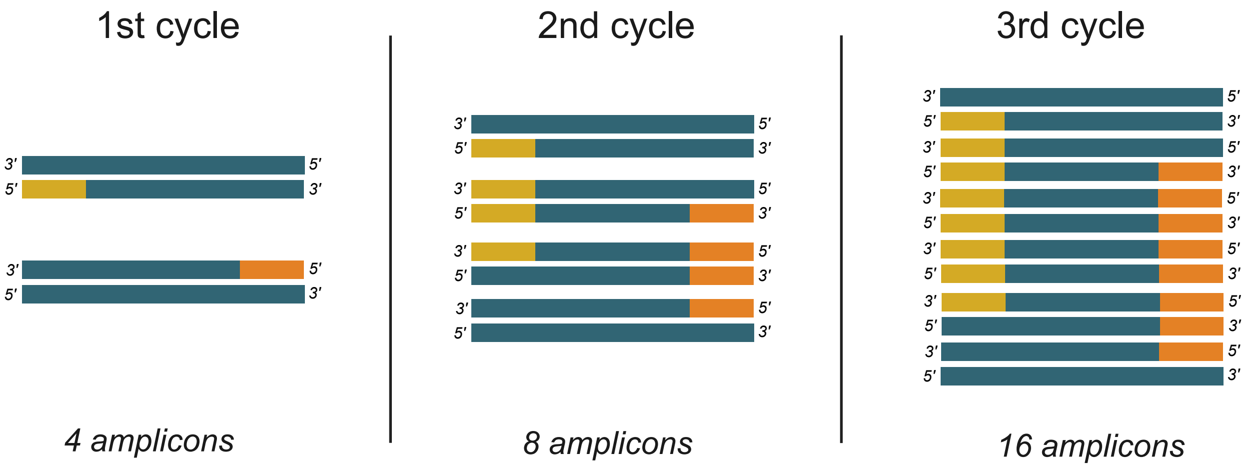
Funded by European Union

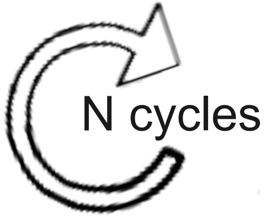
Different steps of PCR



Barcode amplification



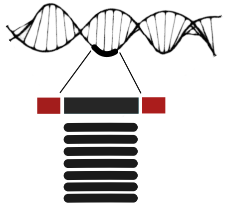




At the end : *in theory* you have 2^n copies of your original molecule

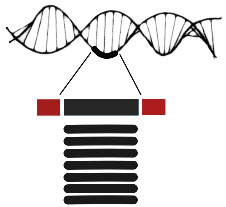
During the PCR…Creation of chimera

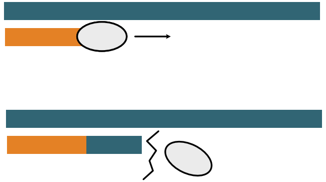
Barcode amplification



During the PCR…Creation of chimera

Barcode amplification



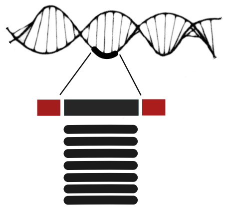


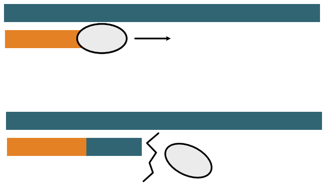
During the amplification, the polymerase stops

Funded by European Union

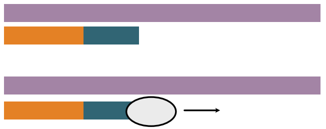
During the PCR…Creation of chimera

Barcode amplification





During the amplification, the polymerase stops

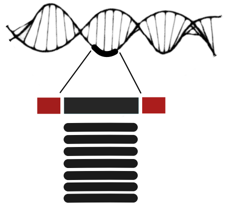


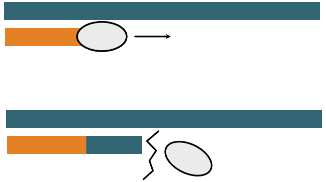
Despite mismatches, the short DNA fragment hybridize in the wrong template

Funded by European Union

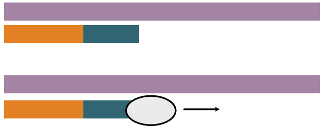
During the PCR…Creation of chimera

Barcode amplification





During the amplification, the polymerase stops

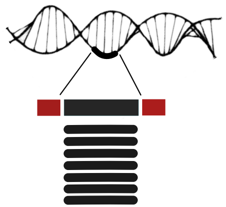


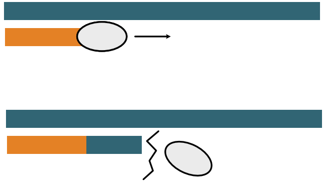
Despite mismatches, the short DNA fragment hybridize in the wrong template

And the PCR continues… the wrong template will be amplified as the others

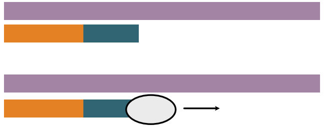
During the PCR…Creation of chimera

Barcode amplification





During the amplification, the polymerase stops

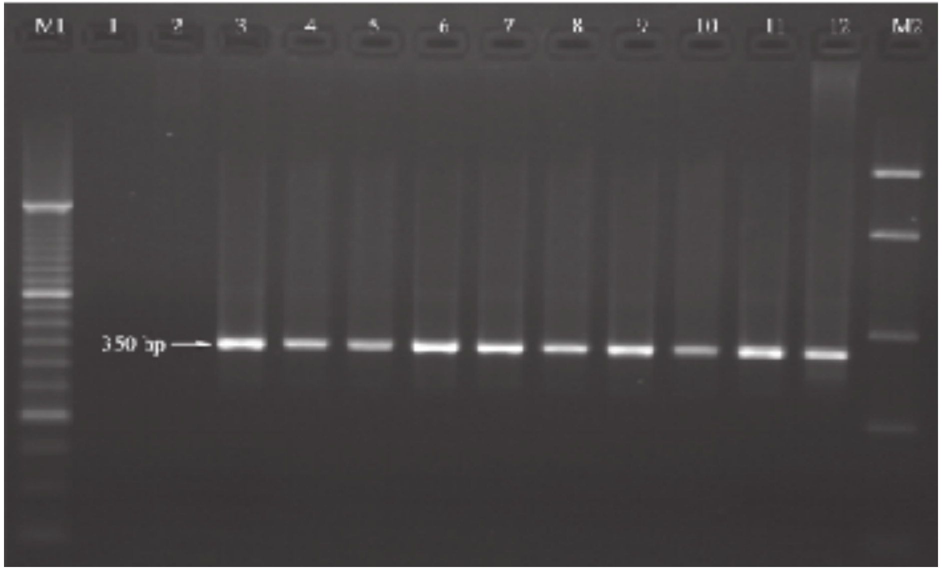


Despite mismatches, the short DNA fragment hybridize in the wrong template

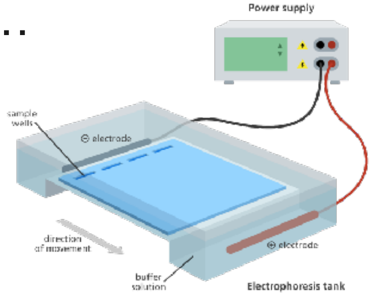
And the PCR continues… the wrong template will be amplified as the others

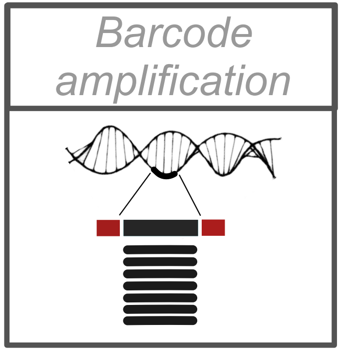
At the end, you have a mix of true amplicons and a chimera

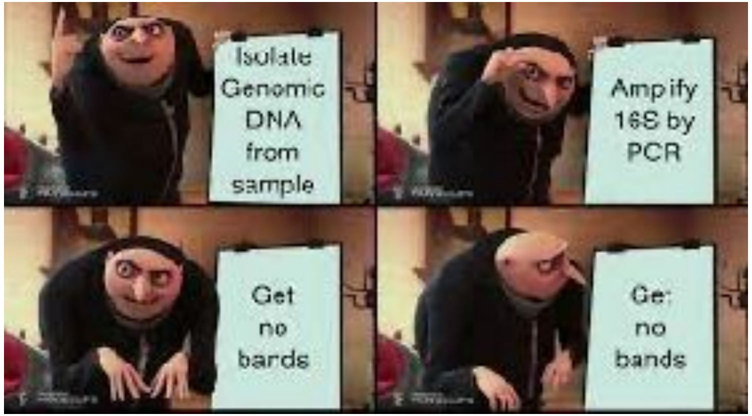
After the PCR…



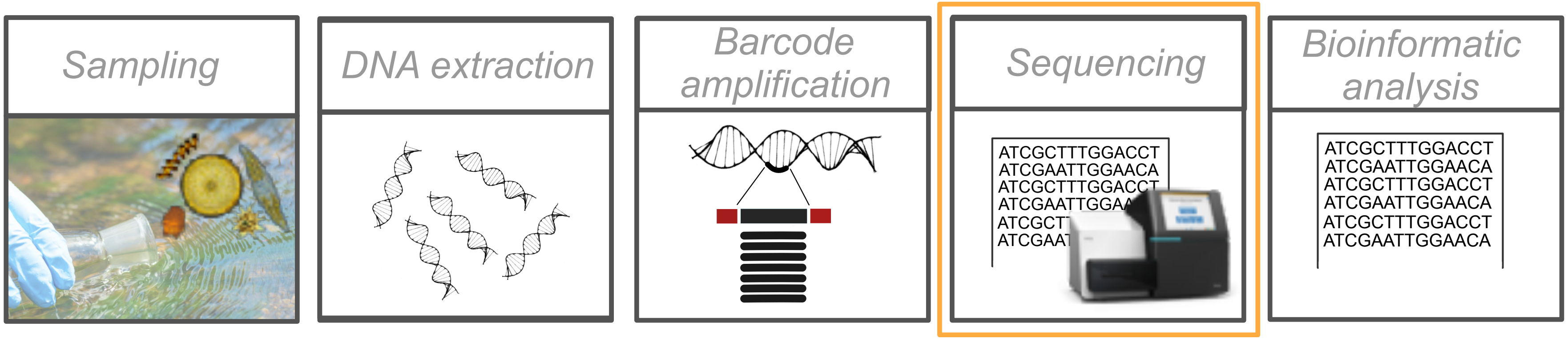
Check if we have well amplified the barcodes with electrophoresis







Metabarcoding steps



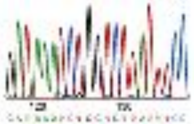
Objectives

Get the DNA sequence of all amplified barcodes

Sequencing technologies

1st generation (1990s)

Sanger



2nd generation (2005 -)

Roche 454, Illumina, Ion torrent

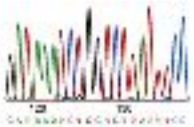
3rd generation (2011 -)

PacBio, Oxford Nanopore

Sequencing technologies

1st generation (1990s)

Sanger



96 seq/run

400-900pb

0.3% error rate

2nd generation (2005 -)

Roche 454, Illumina, Ion torrent

3rd generation (2011 -)

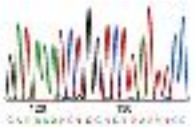
PacBio, Oxford Nanopore

Funded by European Union

Sequencing technologies

1st generation (1990s)

Sanger



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2nd generation (2005 -)

Roche 454, Illumina, Ion torrent

Millions seq/run

150-300 pb

0.1 - 1% error rate

3rd generation (2011 -)

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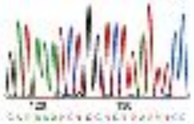
Sequencing technologies

1st generation (1990s)

Sanger



96 seq/run

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2nd generation (2005 -)

Roche 454, Illumina, Ion torrent

Millions seq/run

150-300 pb

0.1 - 1% error rate

3rd generation (2011 -)

PacBio, Oxford Nanopore

~1 millions seq/run

100 000 pb

12% but decreasing

Funded by European Union

Sequencing technologies

1st generation (1990s)

Not adapted to metabarcoding

400-900pb

0.3% error rate

2nd generation (2005 -)

Roche 454, **Illumina**, Ion torrent

Millions seq/run

150-300 pb

0.1 - 1% error rate

3rd generation (2011 -)

PacBio, Oxford Nanopore

Still in development (error rates)

100 000 pb

12% but decreasing

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCTT
ATCGAAT

Illumina MiSeq

Sequencing length of 400-500 bp max

Paired-end sequencing

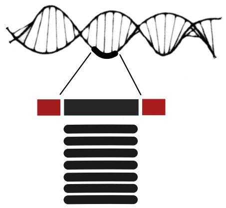
Up to 50 millions paired-end sequences in one run

Run last around 1day

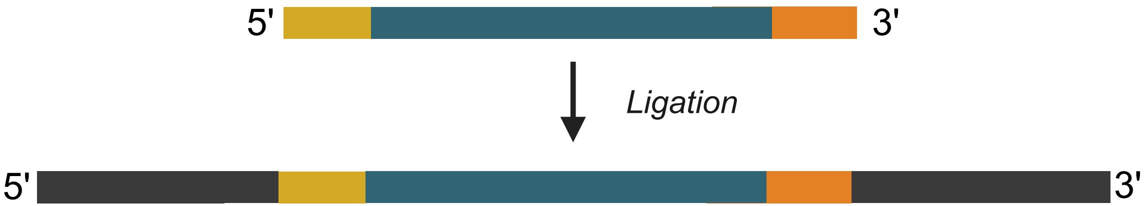
Funded by European Union

Mutliplexed PCR

Barcode amplification

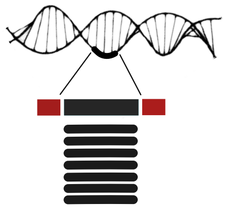


Amplicons needs some additional elements for Illumina technology

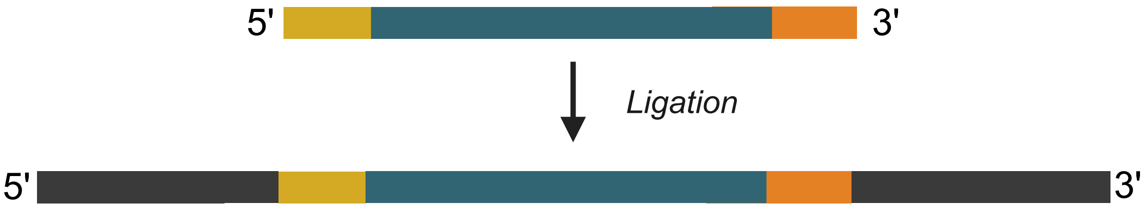


Mutliplexed PCR

Barcode amplification



Amplicons needs some additional elements for Illumina technology

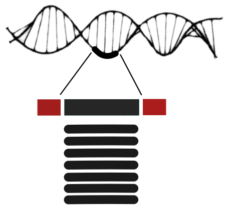


1 run Illumina : 50 000 000 sequences
1 sample : 50 000 -150 000 sequences

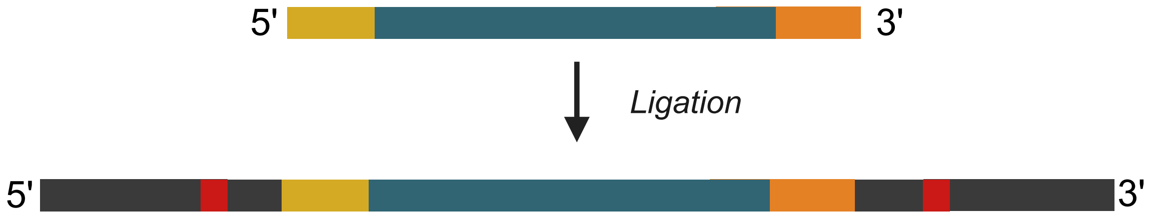
—> Put multiple sample in one run

Mutliplexed PCR

Barcode amplification



Amplicons needs some additional elements for Illumina technology



🟥 Index

1 run Illumina : 50 000 000 sequences
1 sample : 50 000 -150 000 sequences

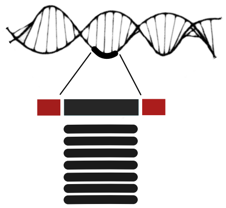
—> Put multiple sample in one run

Add a combinaison of index that will be specific for each sample.

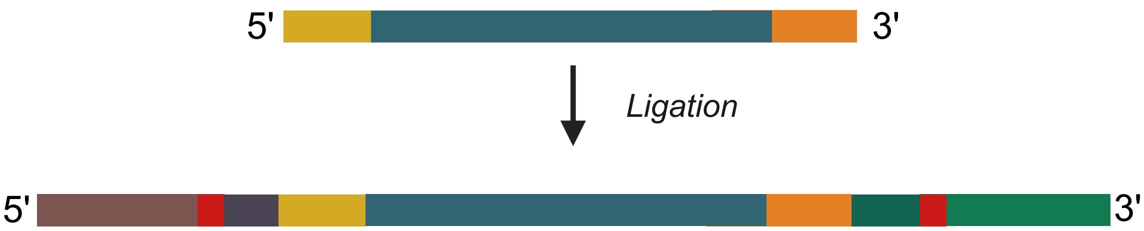
Index : short sequence ~5bp

Mutliplexed PCR

Barcode amplification



Amplicons needs some additional elements for Illumina technology



🟥 Index

🟫 Illumina adapter P5

🟩 Illumina adapter P7

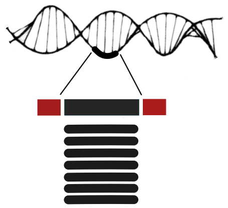
■ ■ sequencing initiation sites

- + Add specific adapters that our amplicon will bind to the Illumina flowcell
- + Sequencing initiation sites

Funded by European Union

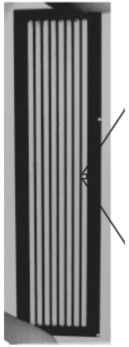
Illumina technology

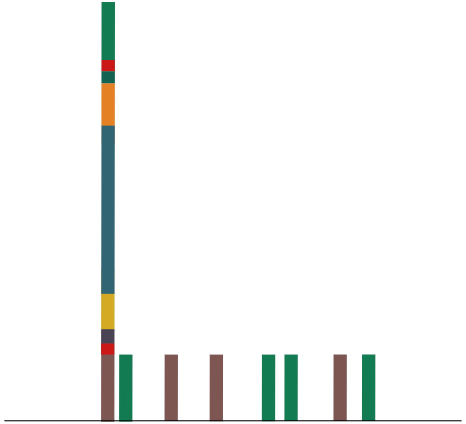
Barcode amplification



Ready for sequencing !

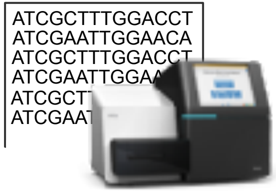
Illumina technology





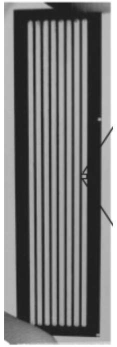
Fixation in the flow cell

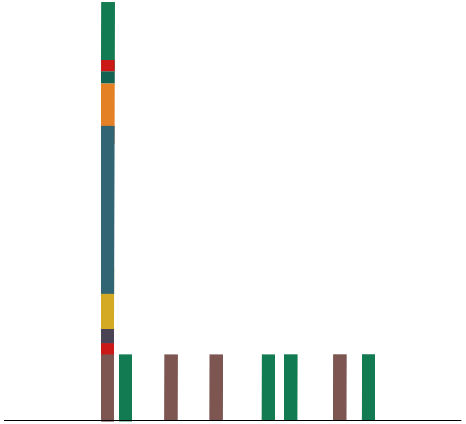
Sequencing



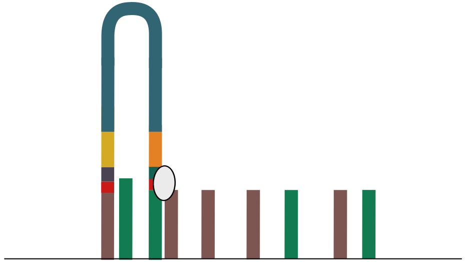
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT

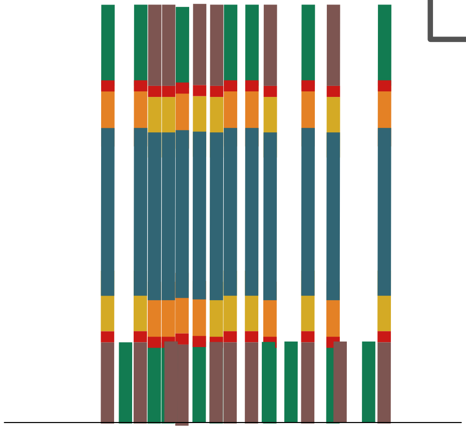
Illumina technology





Fixation in the flow cell

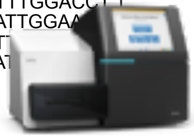




Bridge amplification

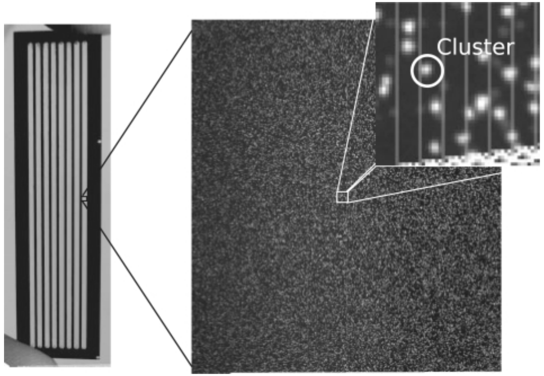
Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCTT
ATCGAAT



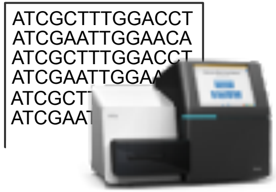
Funded by European Union

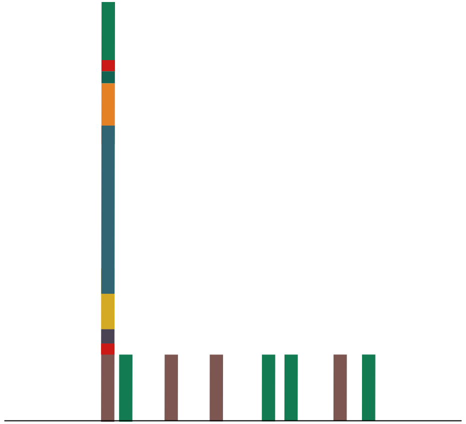
Illumina technology



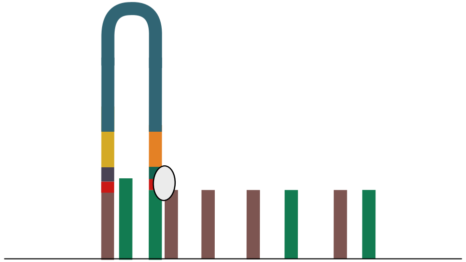
Clusters are visible with a high resolution camera

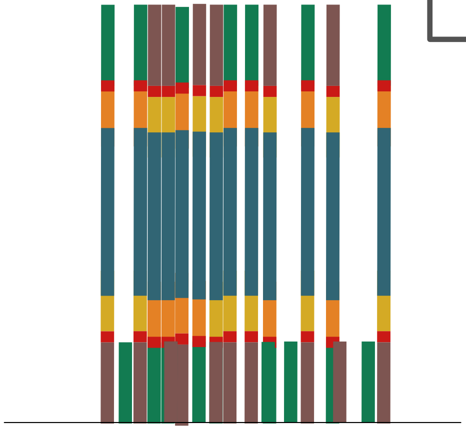
Sequencing


ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT



Fixation in the flow cell

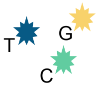




Bridge amplification

Funded by European Union

Illumina technology

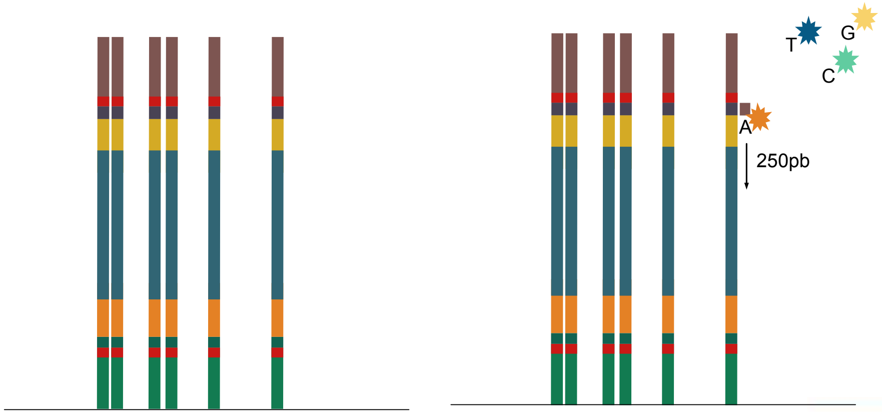


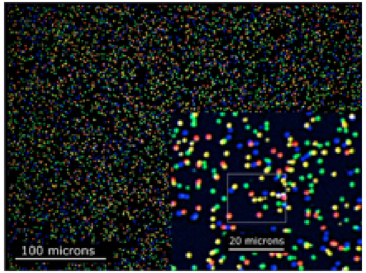
Fluorescent nucleotides with reversible terminator

Sequencing

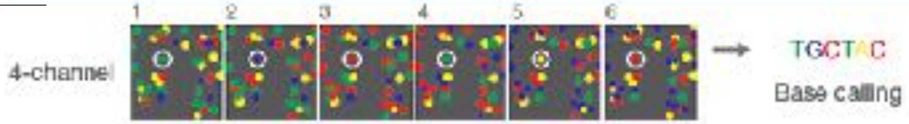
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCTT
ATCGAAT

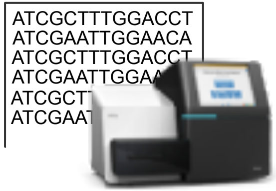
Forward strand



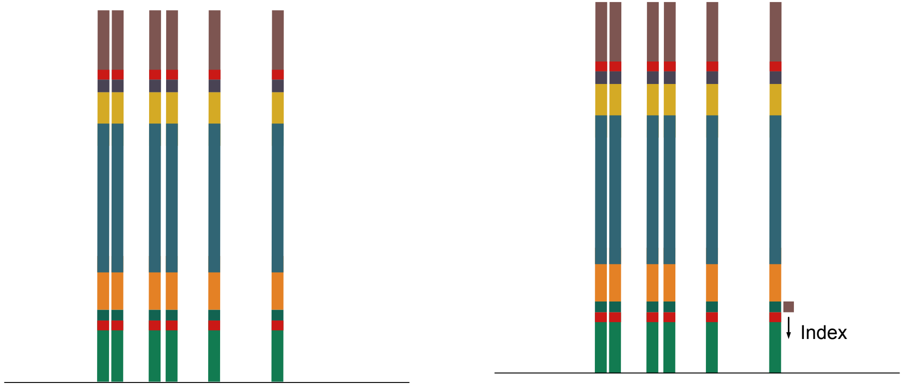


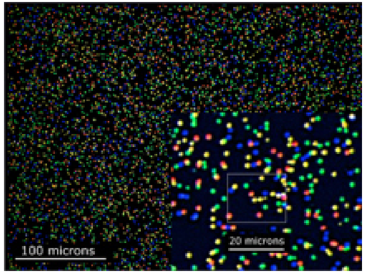
Sequencing of read 1





Illumina technology





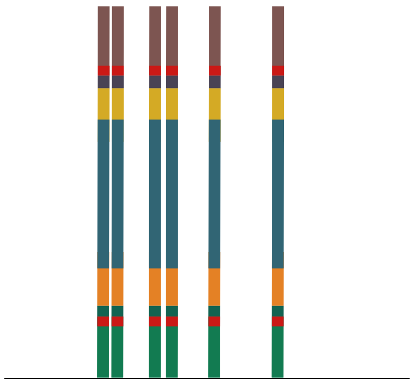
Sequencing of index 1

Sequencing

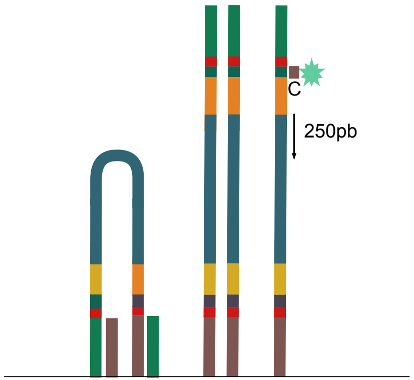
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCTT
ATCGAAT

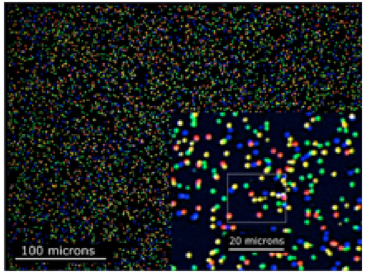
Illumina technology

Forward strand



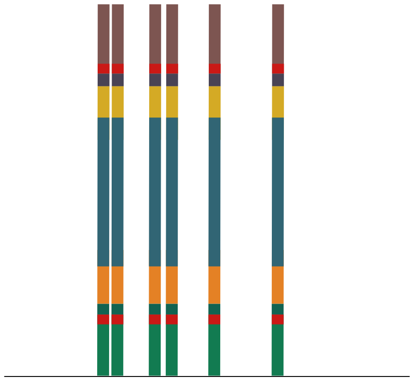
Reverse strand

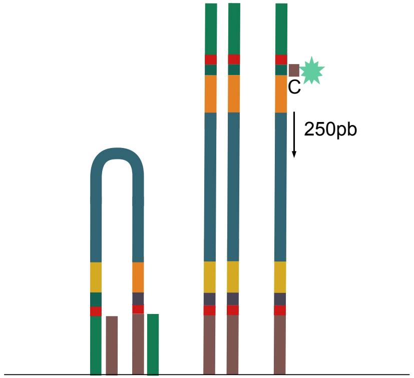


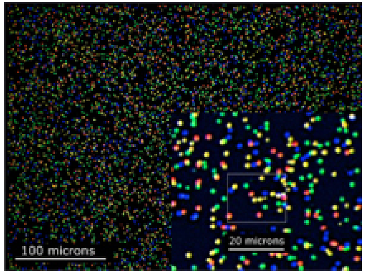


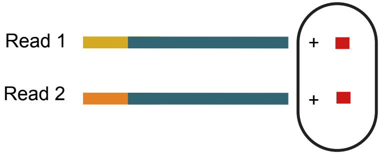
Sequencing of read 2 and index 2

Illumina technology









Demultiplexing

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCTT
ATCGAAT

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCTT
ATCGAAT

Output file

@seq_1_ID
ATCCTGTCGTCTGCT….
+ 1>AAAFAFAFFF
@seq_2_ID
ATCCTGTCGTCCGG…

12 Go

@seq_1_ID
ATCCTGTCGTCTGCT….
+ 1>AAAFAFAFFF
@seq_2_ID
ATCCTGTCGTCCGG…

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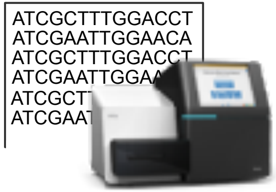
Sequencing



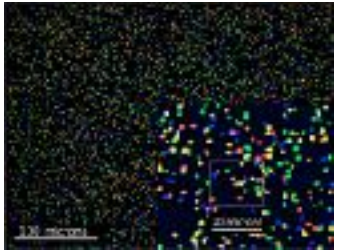
Output file : fastq file

Defline

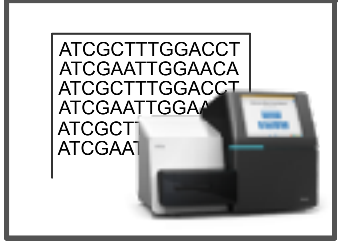
```
@M01028:94:000000000-AHT10:1:1101:15604:1824 1:N:0:10
ATGCTCCAGCAGCTGCGGTAATACGGAGGATGCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTCCGCAGGCGGATCAATAAGT
CAGTGGTGAAAGCCCATAGCTCAACTATGGAACTGCCATTGAAACTGTTGATCTTGAGTCTAGTTGACGTAGGCGGAATGTGACTT
GTAGCGGTGAAATGCATAGATATGTCACAGAACACCAATTGCGAAGGCAGCTTACGTAACTCGACTGACGCTCTTGGAC
+
3AABAFFFFFFFGGGGGGEGGGHHHGEGG2FFB3BDEEGG2F5FECBEEHDFGGFFEFG3AFFG?GCCE0>EE?FG/
>EE3FF3DG44FGHEHGBGE3GGHGF2?GGFF?GFCGFBHHFHFHFBF@2F2
FHGBHHD1GHGGH1CHFF11GFBD1CFCAD1<@CCC0CC0;0<CGF0C-@GA.0;;0C0;;FFCCFBB9090;/;9/
BE..;FB09-9-:....FF;B../;.//9.9..//9.9..9B///
@M01028:94:000000000-AHT10:1:1101:12447:1845 1:N:0:10
```

Sequence

Quality score



Sequencing



Defline

X Y

Sequence

Quality score

```
@M01028:94:000000000-AHT10:1:1101:15604:1824 1:N:0:10
ATGCTCCAGCAGCTGCGGTAATACGGAGGATGCAAGCGTTATCCGGATTTATTGGGTTTAAAGGGTCCGCAGGCGGATCAATAAGT
CAGTGGTGAAAGCCCATAGCTCAACTATGGAACTGCCATTGAAACTGTTGATCTTGAGTCTAGTTGACGTAGGCGGAATGTGACTT
GTAGCGGTGAAATGCATAGATATGTCACAGAACACCAATTGCGAAGGCAGCTTACGTAACTCGACTGACGCTCTTGGAC
+
3AABAFFFFFFFGGGGGGEGGGHHHGEGG2FFB3BDEEGG2F5FECBEEHDFGGFFEFG3AFFG?GCCE0>EE?FG/
>EE3FF3DG44FGHEHGBGE3GGHGF2?GGFF?GFCGFBHHFHFHFBF@2F2
FHGBHHD1GHGGH1CHFF11GFBD1CFCAD1<@CCC0CC0;0<CGF0C-@GA.0;;0C0;;FFCCFBB9090;/;9/
BE..;FB09-9-:....FF;B../;.//9.9..//9.9..9B///
@M01028:94:000000000-AHT10:1:1101:12447:1845 1:N:0:10
```

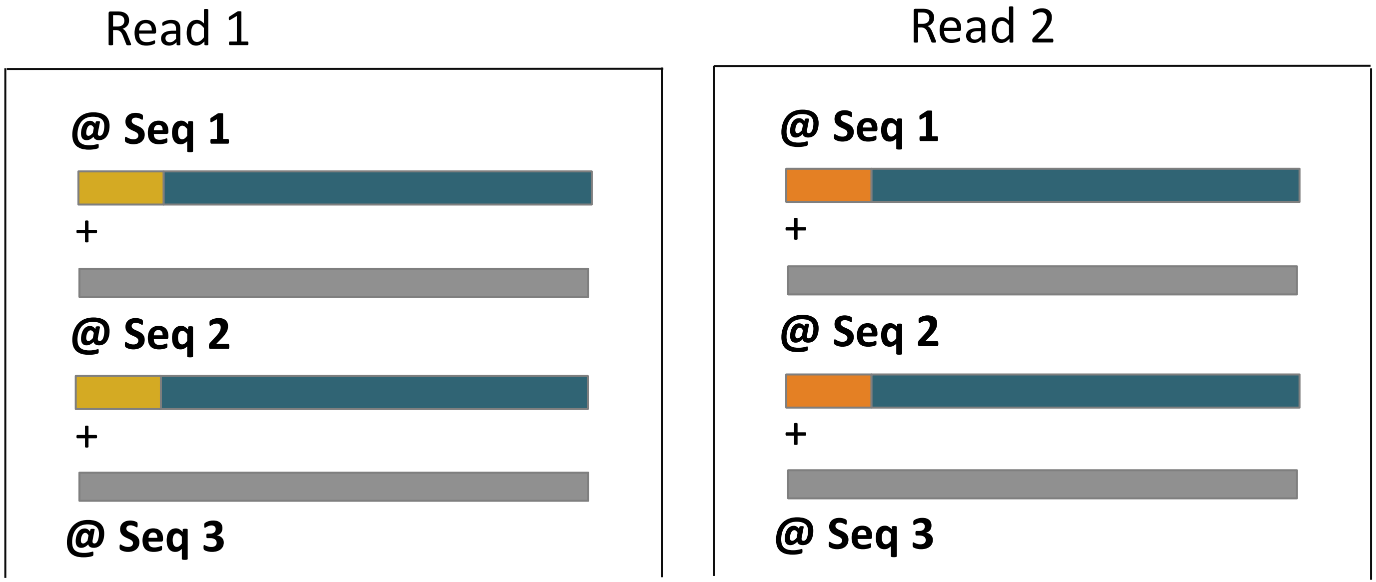
Funded by European Union

Output file : fastq file

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCTT
ATCGAAT

Get a fastq file for each sample



Read 1

@ Seq 1

+

@ Seq 2

+

@ Seq 3

Read 2

@ Seq 1

+

@ Seq 2

+

@ Seq 3

~ 100 000 sequences

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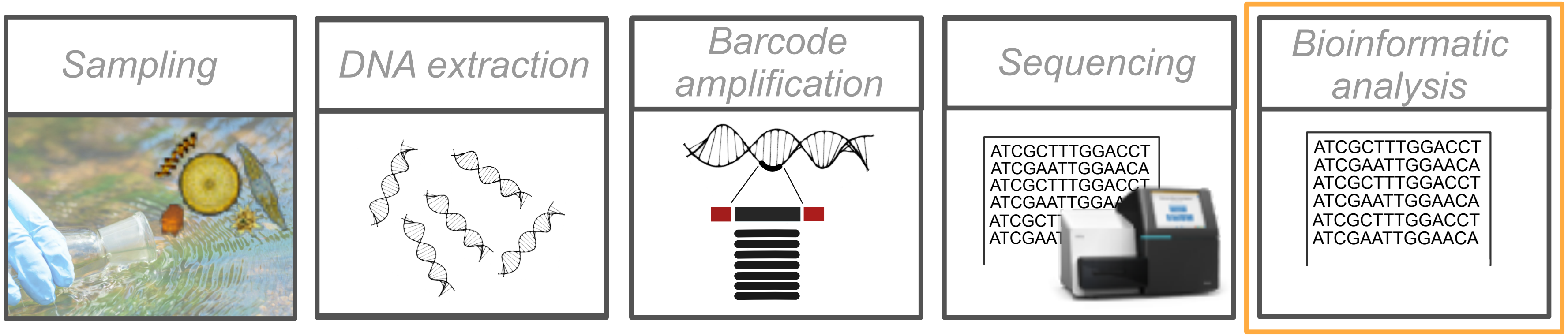
Metabarcoding steps



Sampling

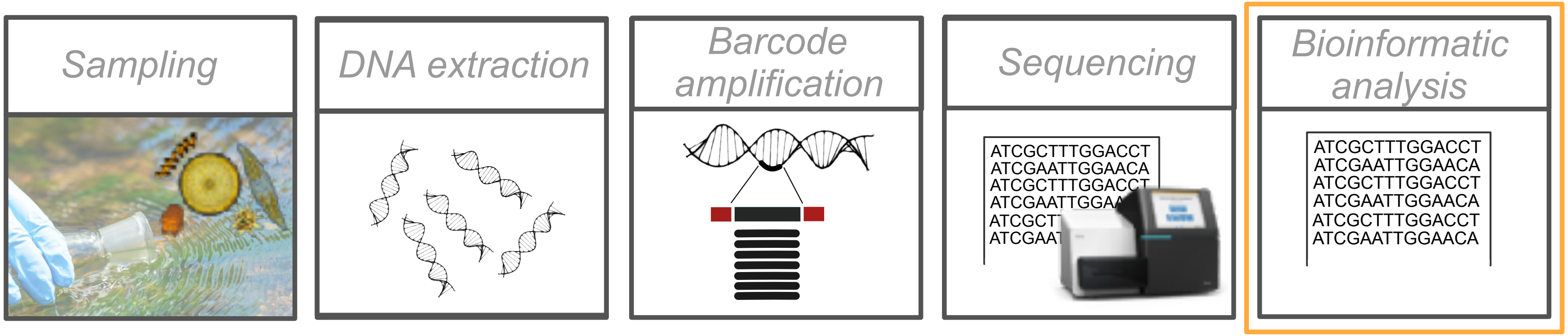
DNA extraction

Barcode amplification

Sequencing

Bioinformatic analysis

Metabarcoding steps



Sampling

DNA extraction

Barcode amplification

Sequencing

Bioinformatic analysis

Objectives

Correct the reads from PCR and sequencing errors, and try to reconstruct meaningful « groups » of sequences that can be equivalent as species

Bioinformatic steps

Bioinformatic analysis

```
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
```

1. **Get complete barcode sequences of high quality**
2. **« Reconstruct » species**
3. **Taxonomic assignation**

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Bioinformatic steps

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

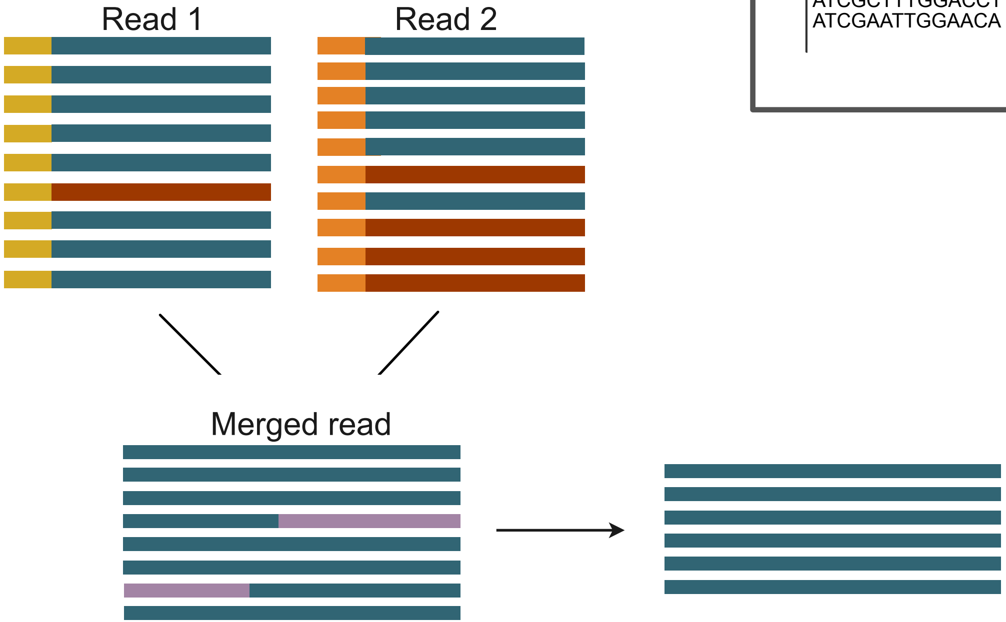
1 Get complete barcode sequences of high quality

Filter reads based on their quality profile

Remove primers

Merge the read 1 and read 2

Remove chimera



Funded by European Union

Bioinformatic analysis

```
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
```

2 « Reconstruct » species

Why? Because we have to deal with 2 things : natural intraspecific variability and sequencing errors.

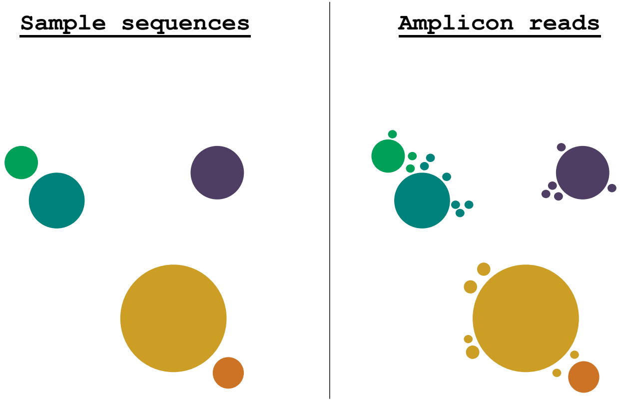
At the end you don’t have a true species as it is artificially reconstructed sequence

Bioinformatic steps

Bioinformatic analysis

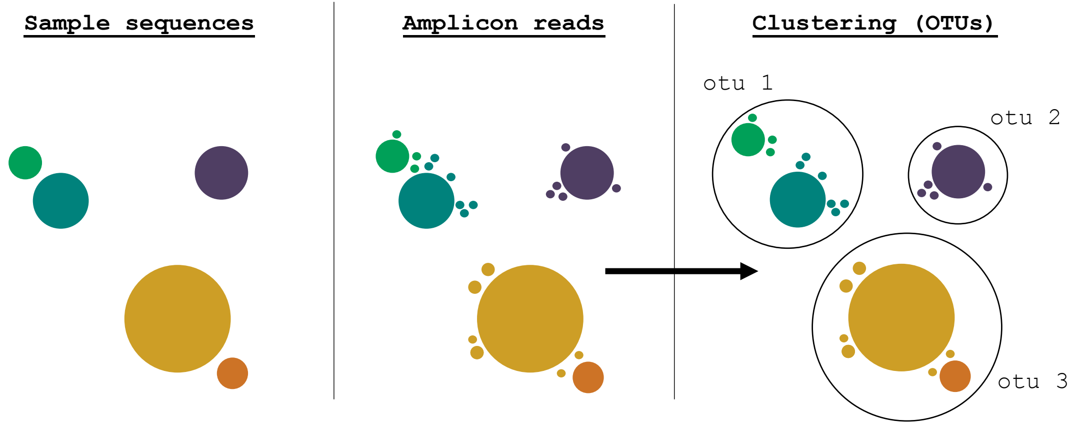
```
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
```

2 « Reconstruct » species



ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

2 « Reconstruct » species

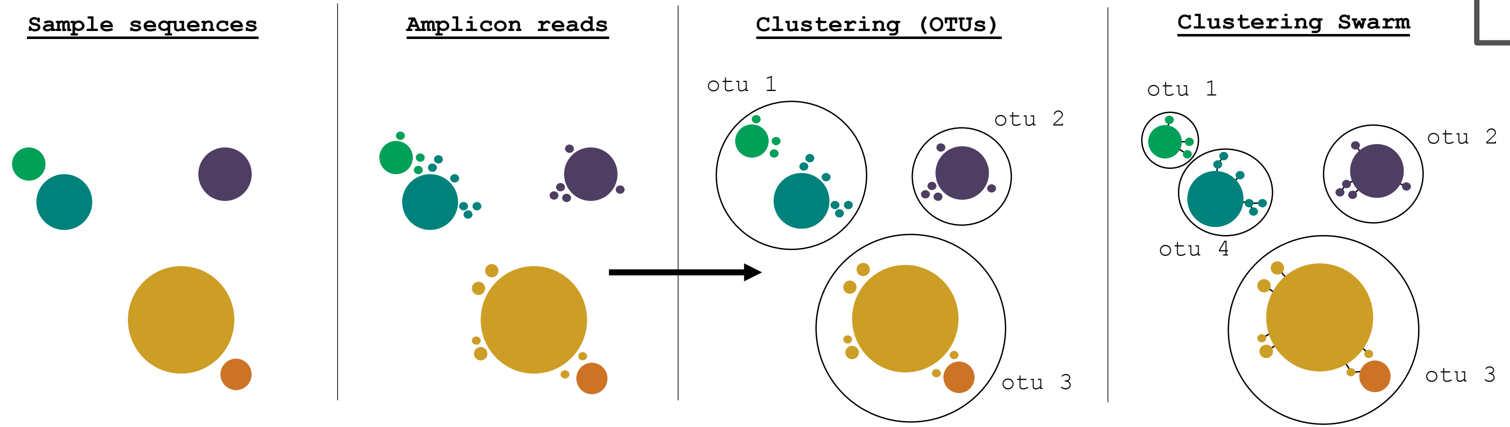


Bioinformatic steps

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

2 « Reconstruct » species



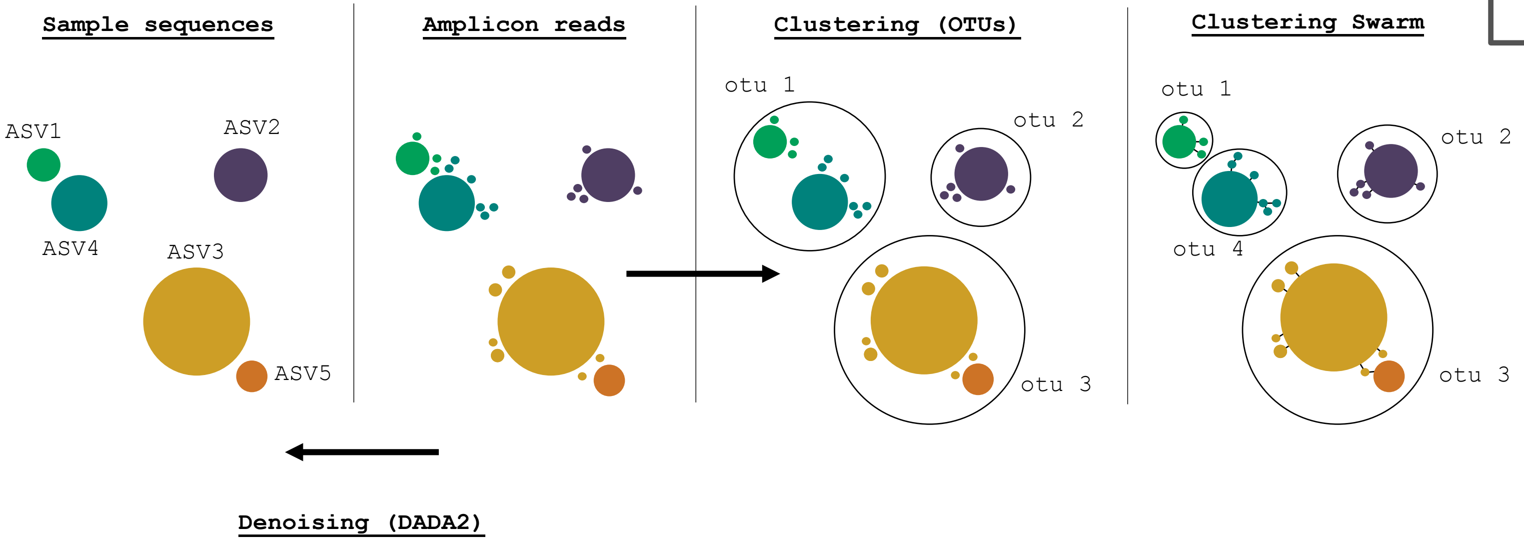
BIOLAWEB

Bioinformatic steps

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

2 « Reconstruct » species



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Bioinformatic steps

Bioinformatic analysis

```
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
```

3 Taxonomic assignation

OTUs/ASV sequences

Sequences are compared to reference databases

Reference database

Sequences are not aligned as it would be too time-consuming and resource expensive

Bioinformatic steps

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

1. **Get complete barcode sequences of high quality**
2. **« Reconstruct » species**
3. **Taxonomic assignation**

Bioinformatic workflow

Different workflows

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

@seq_1_ID
ATCCTGTCGTCTGCT….
+ 1>AAAFAFAFFF
@seq_2_ID
ATCCTGTCGTCCGG…

@seq_1_ID
ATCCTGTCGTCTGCT….
\+ 1>AAAFAFAFFF
@seq_2_ID
ATCCTGTCGTCCGG…

12 Go

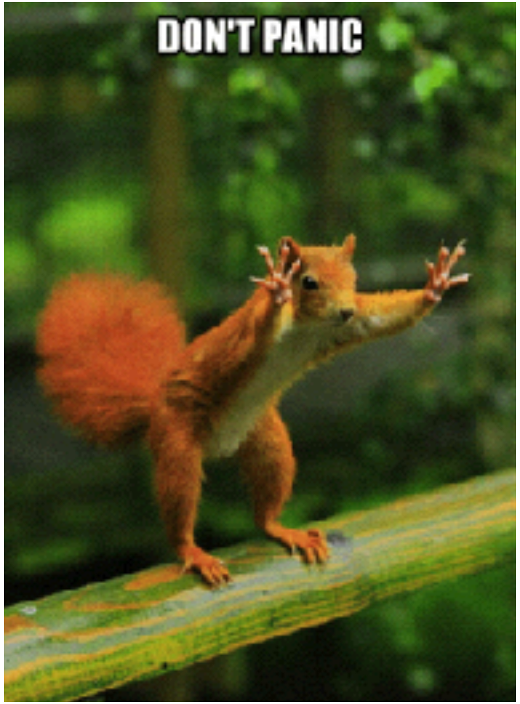
= millions of reads
= thousands of OTUs/ASVs

Use of bioinformatic (it is not an option)

Different workflows

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA



There are different softwares available to do all the bioinformatic analysis :

Mothur : open source software
Own language

QIIME: open source software
Python

DADA2 : open source software
R language

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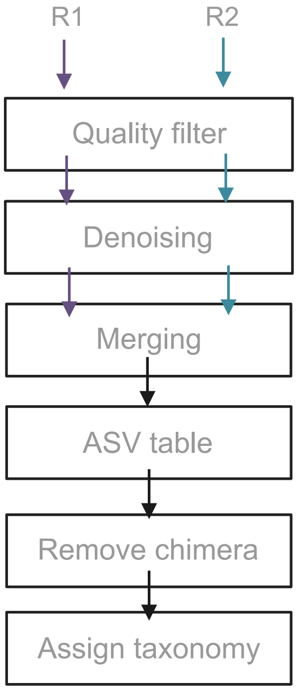

R1
R2
Quality filter
Denoising
Merging
ASV table
Remove chimera
Assign taxonomy

DADA2 pipeline

Online tutorial


DADA2: Fast and accurate sample inference from amplicon data with single-nucleotide resolution

Amplicon Sequencing, Exactly.

Installation

Tutorials

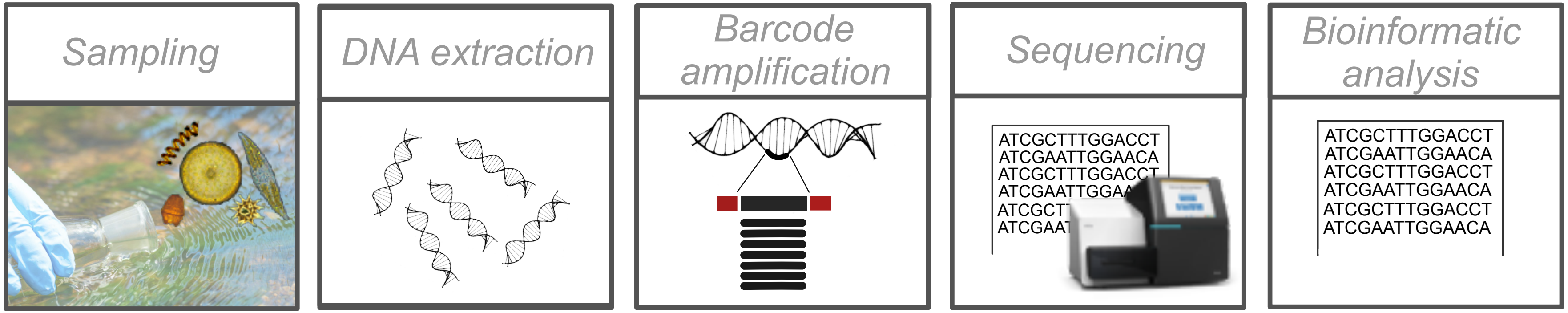
Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

Widely used language within biologists

stackoverflow

Metabarcoding steps



Sampling

DNA extraction

Barcode amplification

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGA
ATCGCT
ATCGAAT

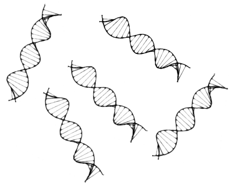
Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

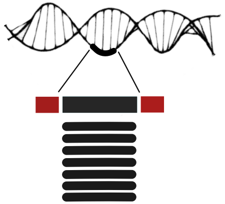
Metabarcoding steps - without optimization

Sampling

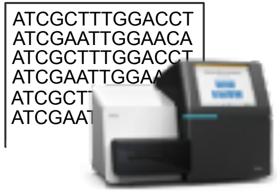
DNA extraction



Barcode amplification



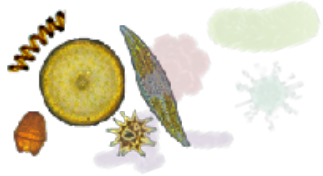
Sequencing



ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGA
ATCGCT
ATCGAAT

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA



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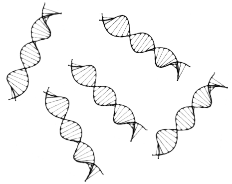
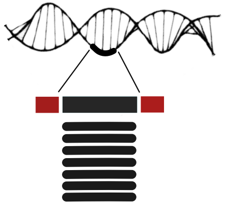
Metabarcoding steps - without optimization

Sampling

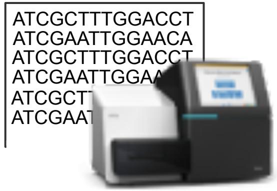
DNA extraction



Barcode amplification



Sequencing



ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

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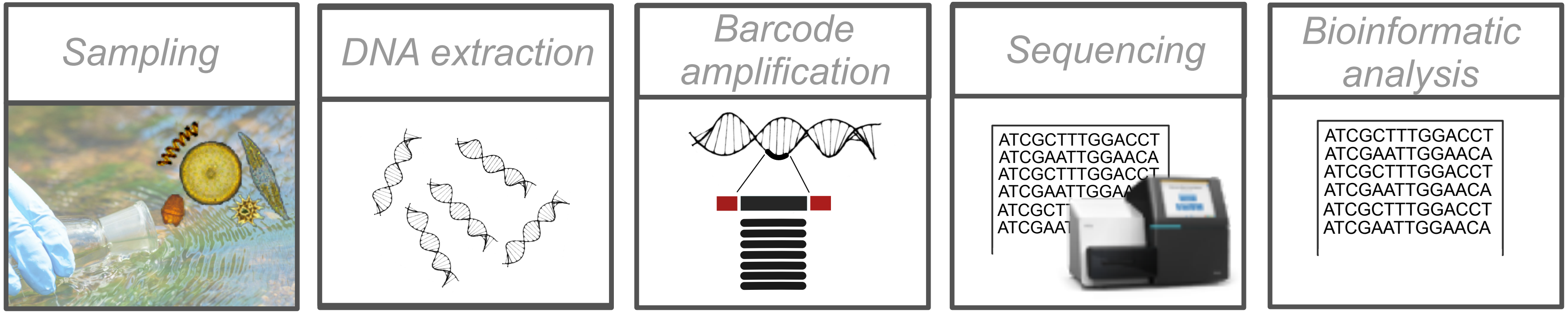
Metabarcoding steps - without optimization



Sampling

DNA extraction

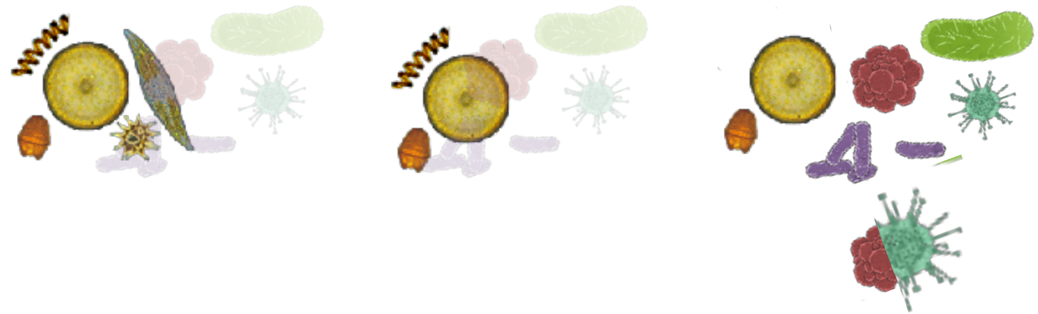
Barcode amplification

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCTT
ATCGAAT

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA



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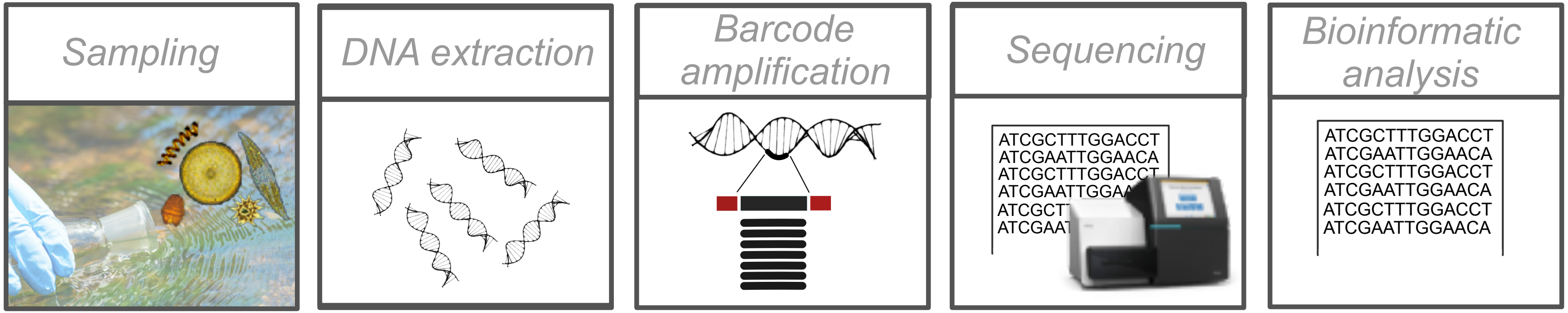
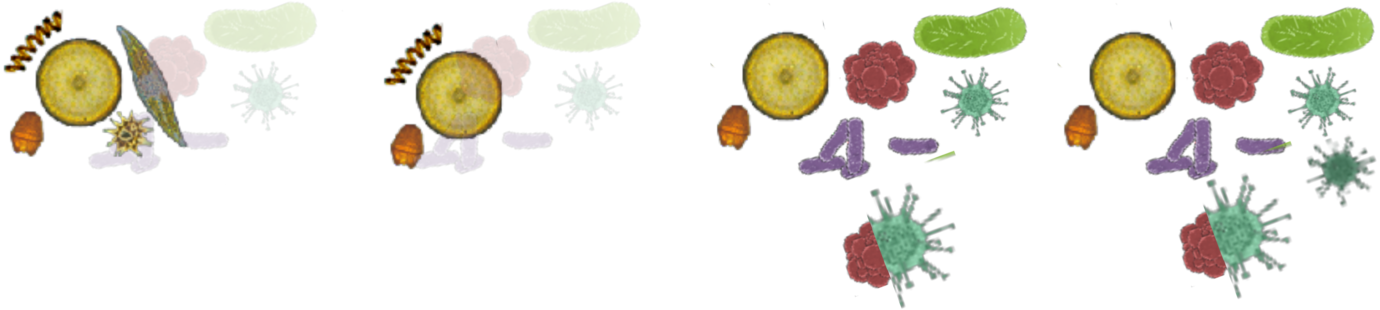
Metabarcoding steps - without optimization





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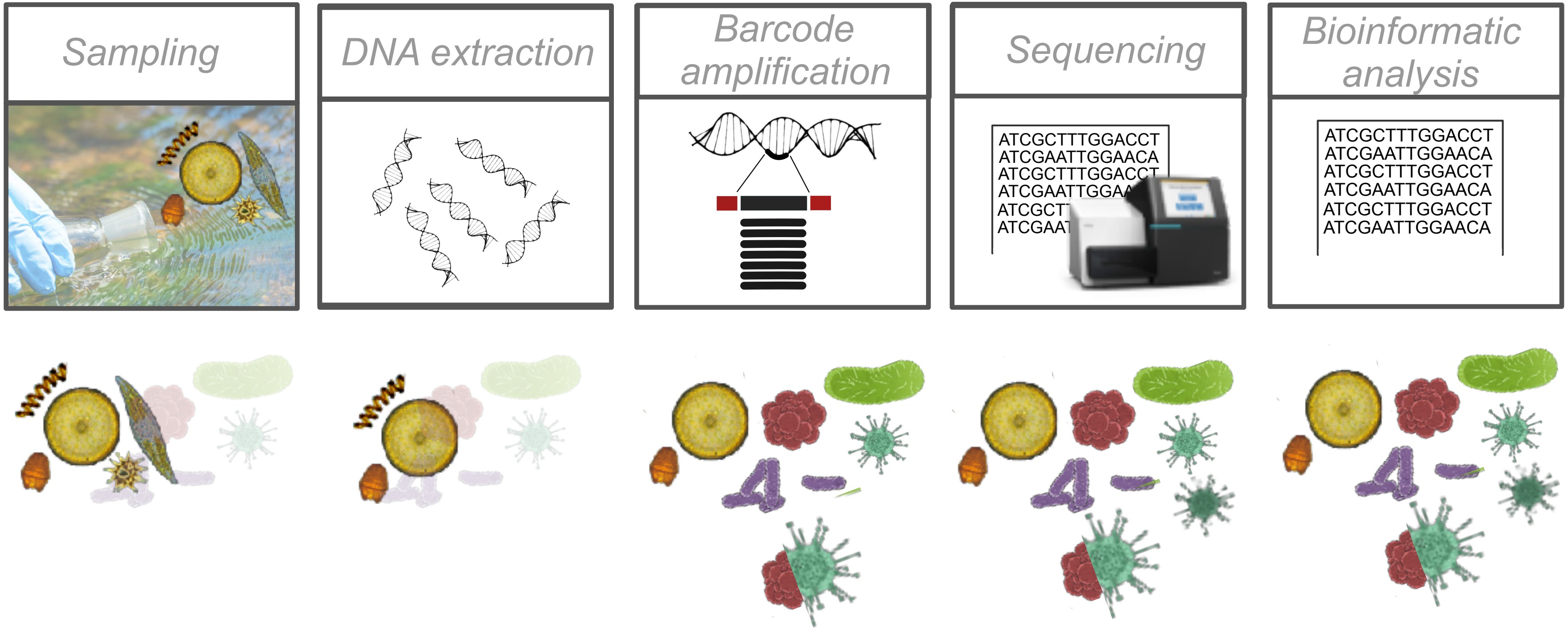
Metabarcoding steps - without optimization



Sampling

DNA extraction

Barcode amplification

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

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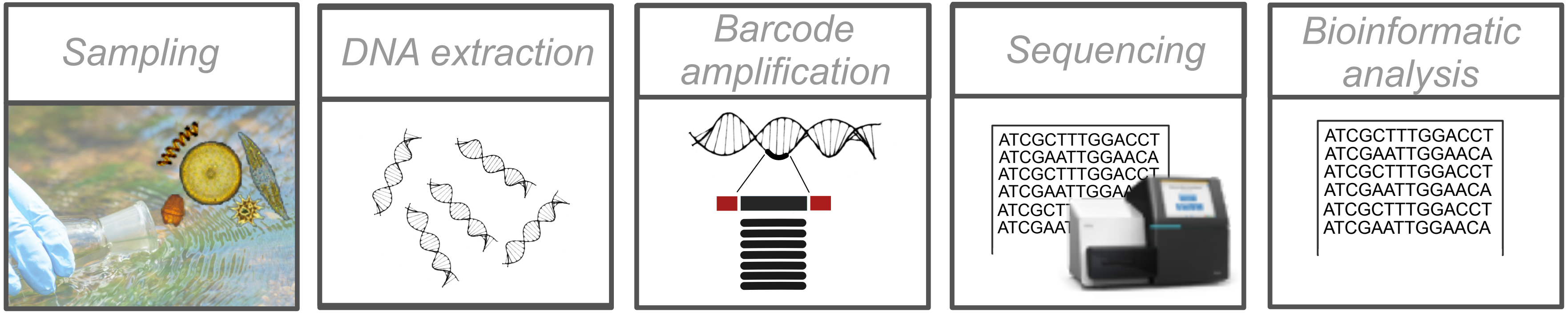
Metabarcoding steps - without optimization



Sampling

DNA extraction

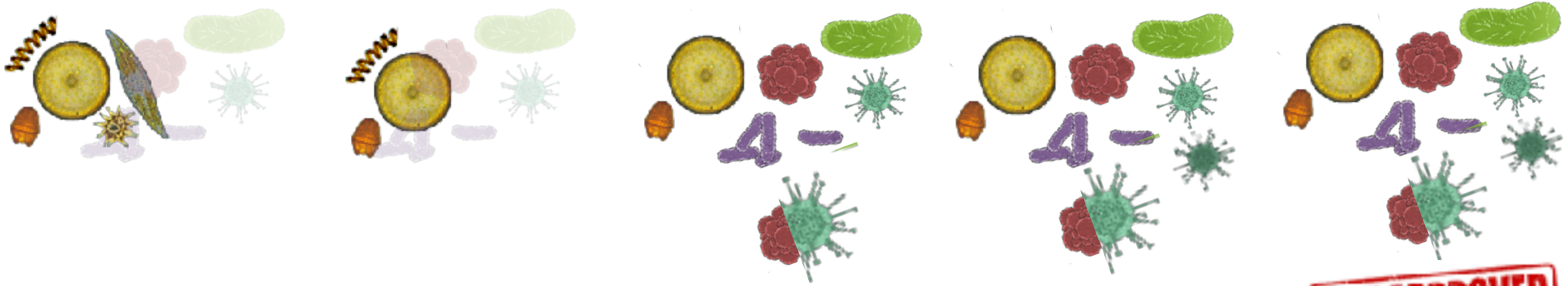
Barcode amplification

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT

Bioinformatic analysis

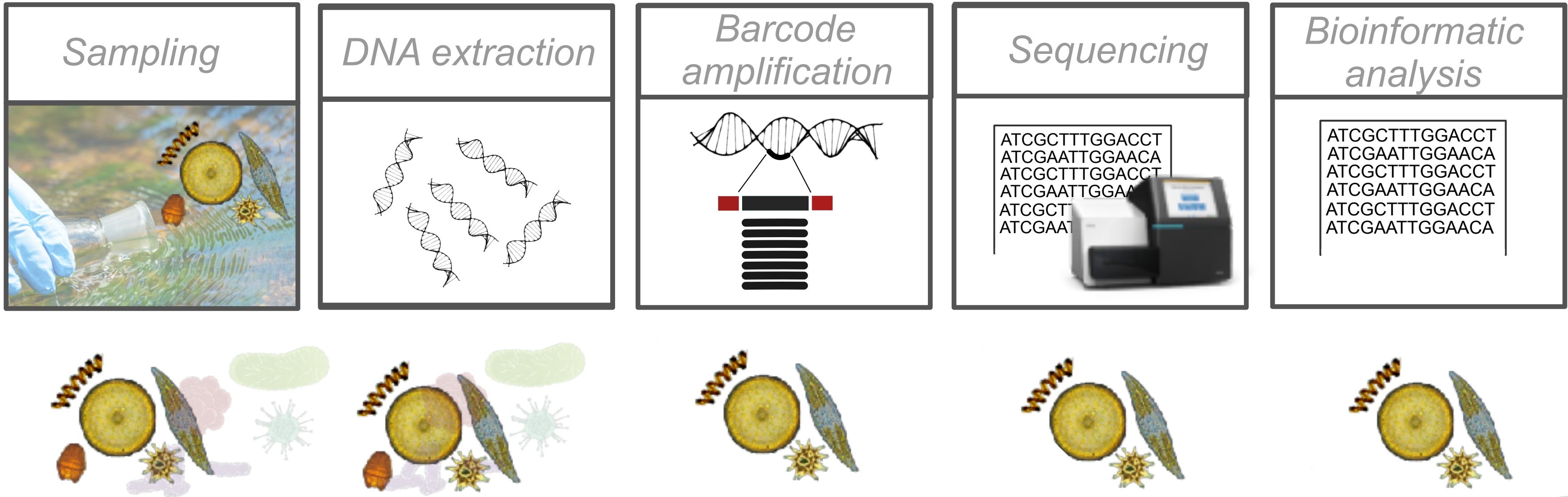
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA



NOT APPROVED

Funded by European Union

Metabarcoding steps - with optimization



Sampling

DNA extraction

Barcode amplification

Sequencing

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAA
ATCGCT
ATCGAAT

Bioinformatic analysis

ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA
ATCGCTTTGGACCT
ATCGAATTGGAACA

✓APPROVED

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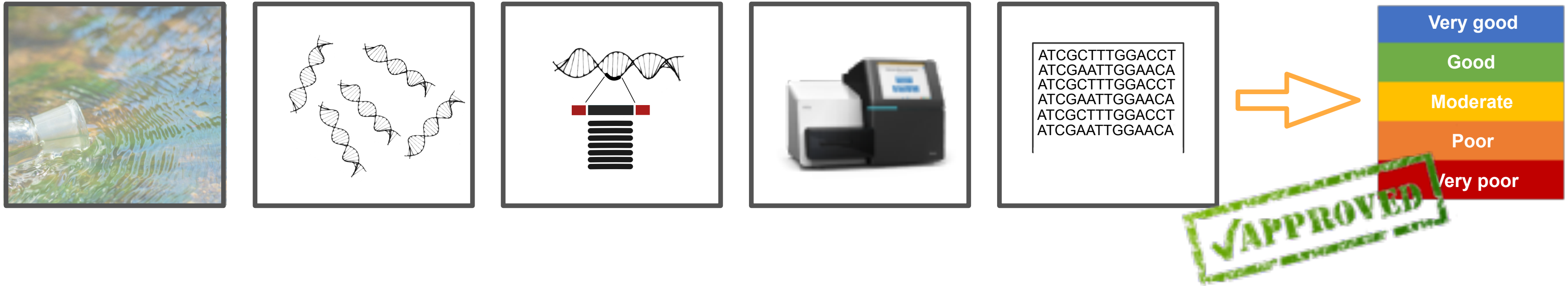
BIOLAWEB

You’ll see in the next presentations how this workflow was designed, optimized, tested and verified to do biomonitoring of diatoms



ATCGCTTTGGACCT
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Very good
Good
Moderate
Poor
Very poor

How we are currently doing this for the biomonitoring of phytoplankton…

Funded by European Union


Any questions?