



Skills evaluation

INRAE

Grab a paper, a pencil



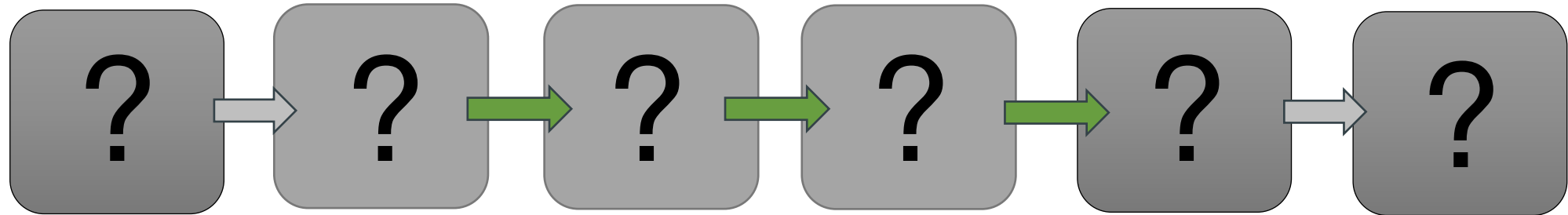
Question 1

Define :

- **barcoding**
- **metabarcoding**

Question 2

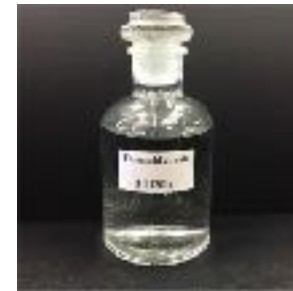
Give the main steps of DNA metabarcoding for diatoms



Question 3

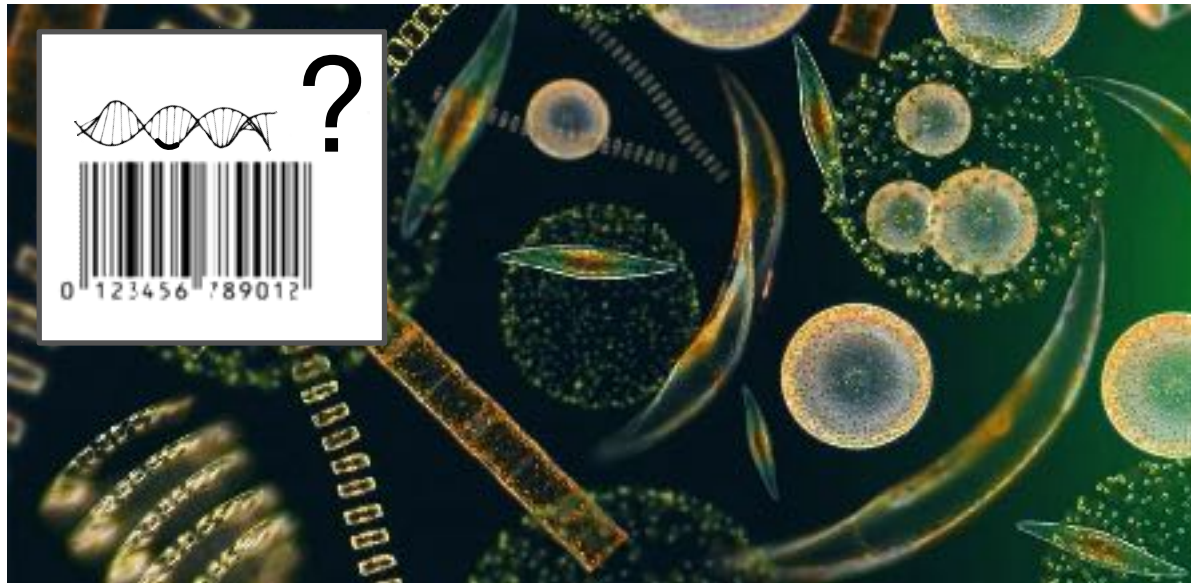
Which strategy are appropriate to store DNA samples?

- A- Ethanol
- B- RNA buffer
- C- Formaldehyde
- D- Preservation -20°C
- E- Rakija



Question 4

What makes a good DNA barcode?



Question 5

After bioinformatic analyses, what can you expect from metabarcoding?

- A- The relative abundance of living and dead organisms in the sample but also traces left from other
- B- The exact abundances of living organisms in the sample
- C- The relative abundances of living organisms in the sample
- D- The presence/absence of living and dead organisms in the sample but also traces left by others

Question 6

What makes a good reference library?



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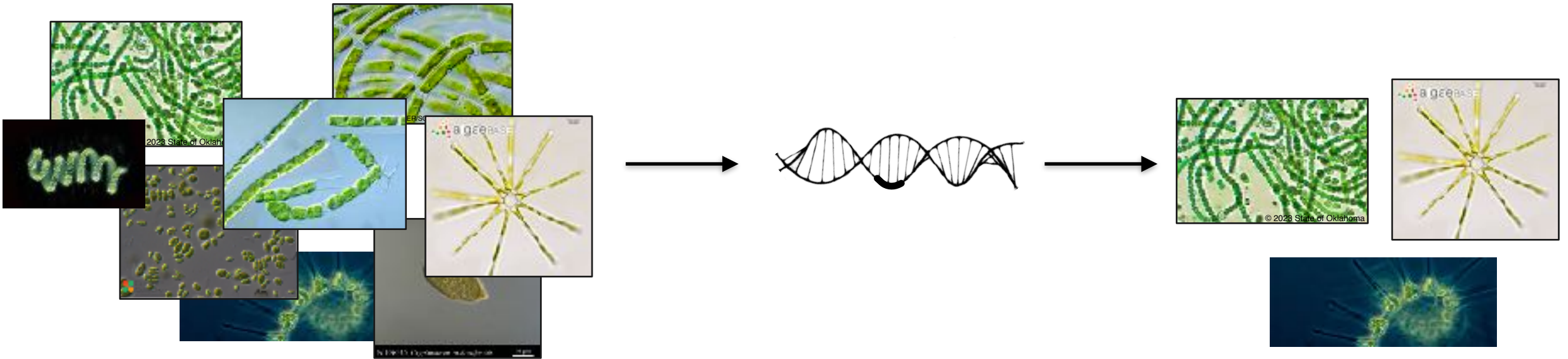
Question 7

**Can you cite several sequencing technologies?
advantages/disadvantages?**



Question 8

You made a mock community with 10 species. After sequencing you recovered only 3 species. Why?



Question 9

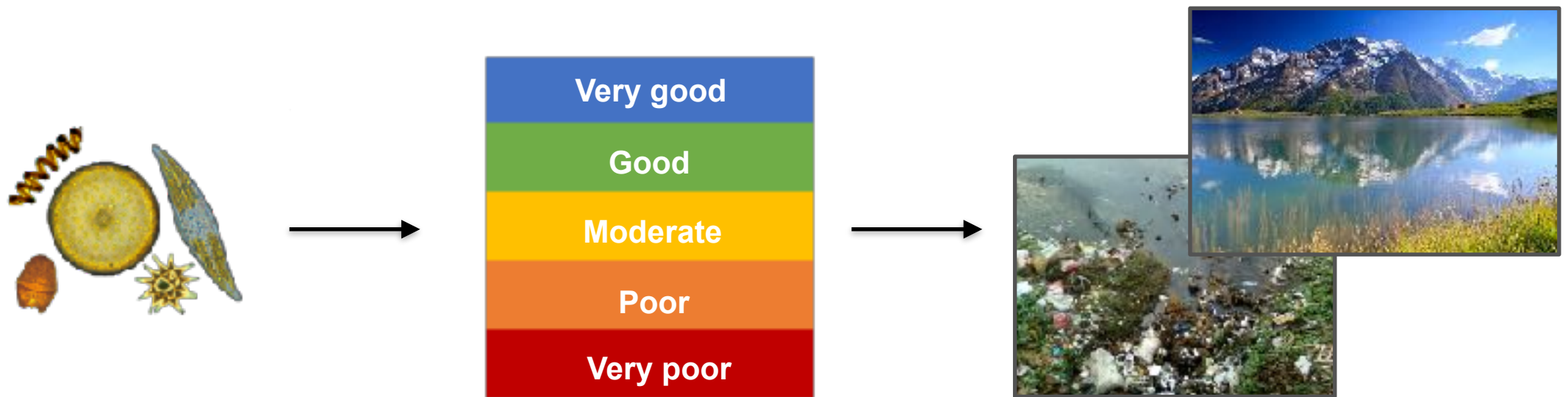
Biolaweb project is finished and a good reference barcoding library for Balkan diatoms is available.

What can explain the differences between diatom lists obtained in microscopy vs metabarcoding in Balkan region?



Question 10

Give 2 different strategies for index development based on metabarcoding data (diatoms or phytoplankton)?





Question 1

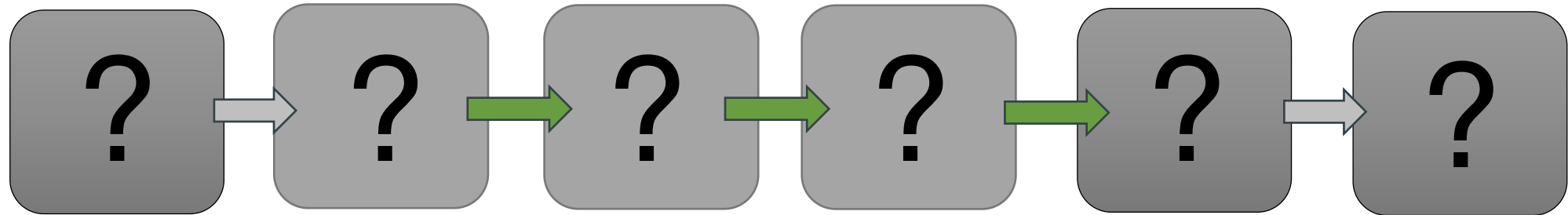
Define

barcoding

metabarcoding

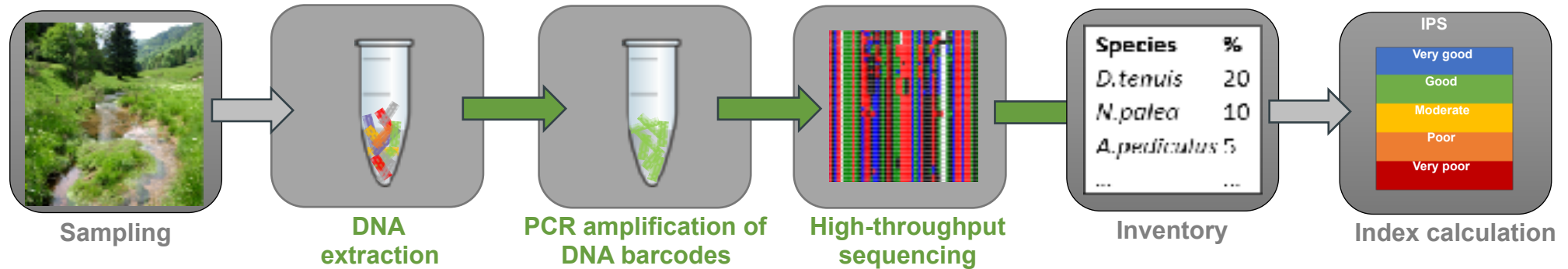
Question 2

Give the main steps of DNA metabarcoding for diatoms



Question 2

Give the main steps of DNA metabarcoding for diatoms



↕
Diat.barcode
Reference library

+ Transformation based of correction factor (biovolume)

Question 3

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- E- Rakija - ???**

Question 4

What makes a good DNA barcode?



Question 4

What makes a good DNA barcode?

- Universality: A single barcode that targets the entire target-group diversity,
- Variability: an efficient barcode able to identify the target-group to species with conserved regions to set primers
- Specificity: a barcode specific of the target-group , not amplifying other groups (e.g. Chrysophytes, etc...)
- Length: the barcode length must fit the sequencing technology (Illumina Miseq)
- References: A barcode with reference barcoding libraries complete enough to analyse the river/lake diversity

Question 5

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Question 5

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B- The exact abundances of living organisms in the sample

C- The relative abundances of living organisms in the sample



D- The presence/absence of living and dead organisms in the sample but also traces left by others

Question 6

What makes a good reference library?



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Question 6

What makes a good reference library?

Completeness

Taxonomic curation

Open access

Data and metadata traceability

When, where, who sampled and how

Sequence production

Voucher: deposited in an open accessible collection

DNA: who extracted, sequenced, following which procedures (primers...), where DNA is stored

If culture: who isolated, date of isolation

Question 7

**Can you cite several sequencing technologies?
advantages/disadvantages?**



Question 7

Can you cite several sequencing technologies? advantages/disadvantages?

Illumina

Read length: 2 x 150 bp to 2 x 300 bp

0,5% error rate

10.10e6 reads per run

6-7 GB per run

Minion

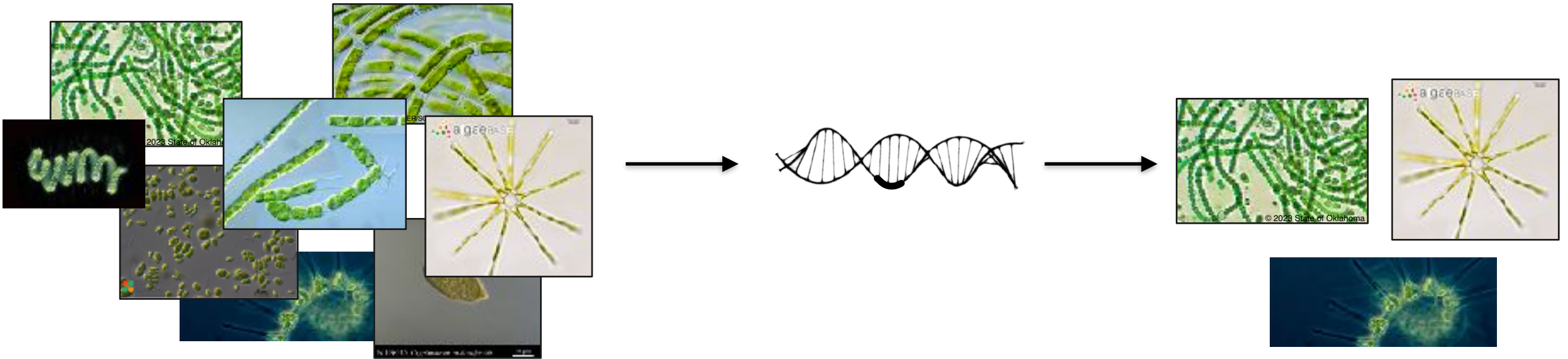
Read length: several 10 kbp

Error rate: 5%

500 MB

Question 8

You made a mock community with 10 species. After sequencing you recovered only 3 species. Why?



Question 8

You made a mock community with 10 species. After sequencing you recovered only 3 species. Why?

- Problem with extraction
- Primers not adapted
- Barcode not adapted to differentiate some species
- Reference library incomplete
- ...

Question 9

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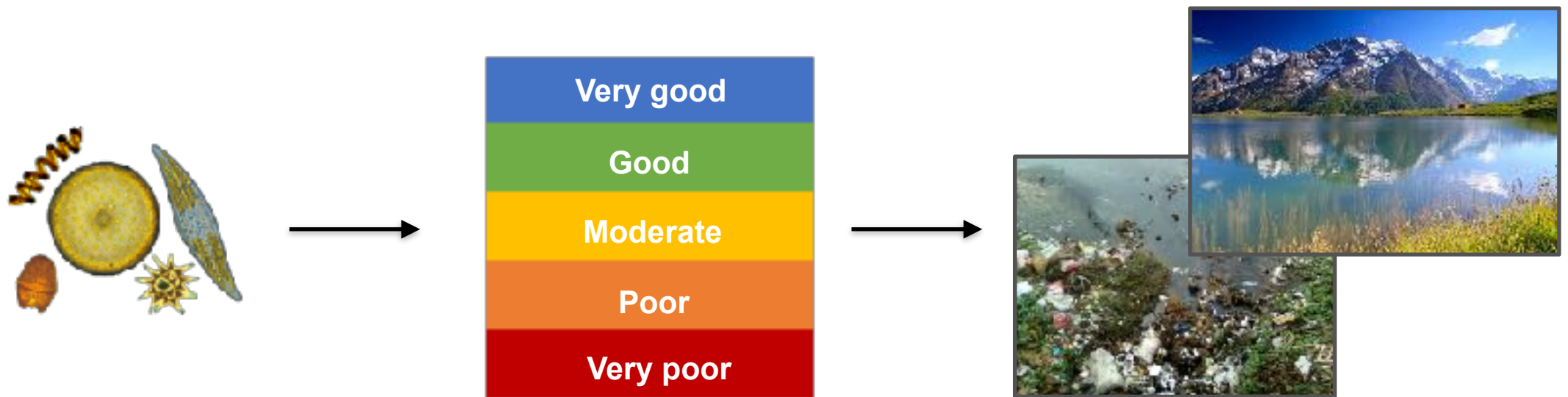
Biolaweb project is finished and a good reference barcoding library for Balkan diatoms is available.

What can explain the differences between diatom lists obtained in microscopy vs metabarcoding in Balkan region?

- Dead frustules
- Barcode length not adapted to differentiate some neighbor species or varieties
- Rare species detection (usually better with DNA)
- Biovolumes
- You put Rakija for sample preservation

Question 10

Give 2 different strategies for index development based on metabarcoding data (diatoms or phytoplankton)?



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Give two different strategies for index development based on metabarcoding data (diatoms or phytoplankton)?

If the barcoding library is complete enough: Mimic existing index

If the reference library is not complete enough: Taxonomy free index

Intermediate situation: use of phylogenetic signal to infer ecology of unassigned sequences and use this information in a existing index

Questions?



