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Biodiversity Tools for Marine Resource Exploration

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Project: 101129136 — SustainaBlue — ERASMUS-EDU-2023-CBHE

ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΙΓΑΙΟΥ

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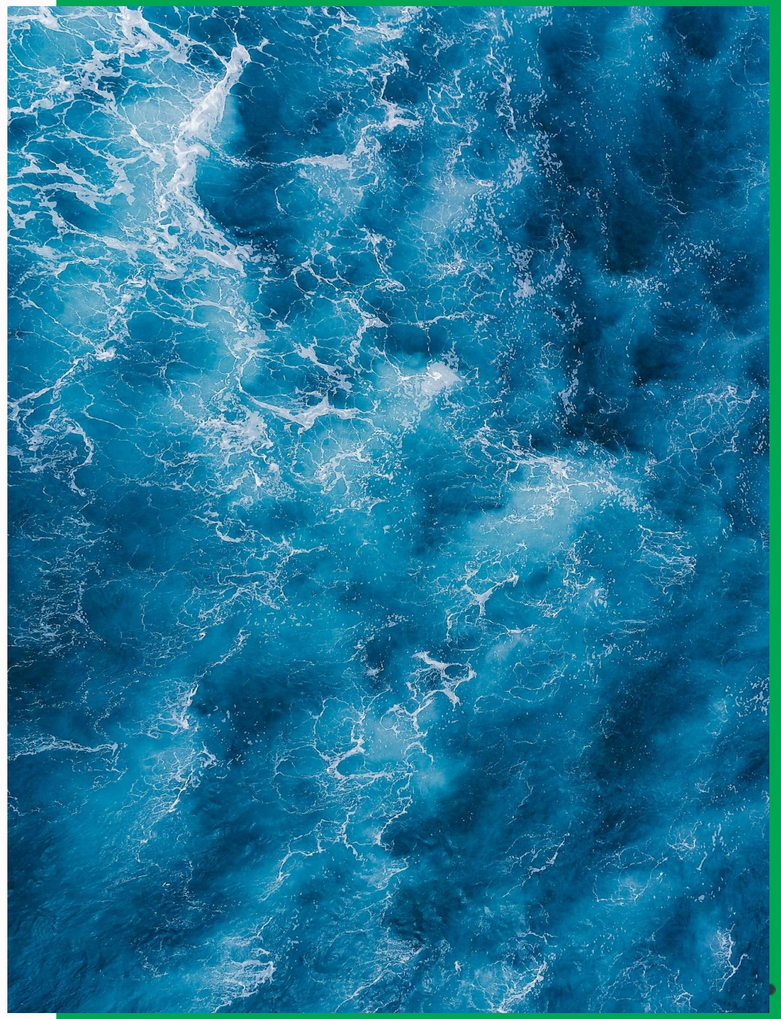
Objectives

01 Understand the fundamental principles of omics science and DNA barcoding in marine biotechnology.

02 Understand the applications of omics and DNA barcoding in conservation, fisheries, and bioprospecting.

03 Understand the principles of biodiversity analysis based on an integrative GIS approach.



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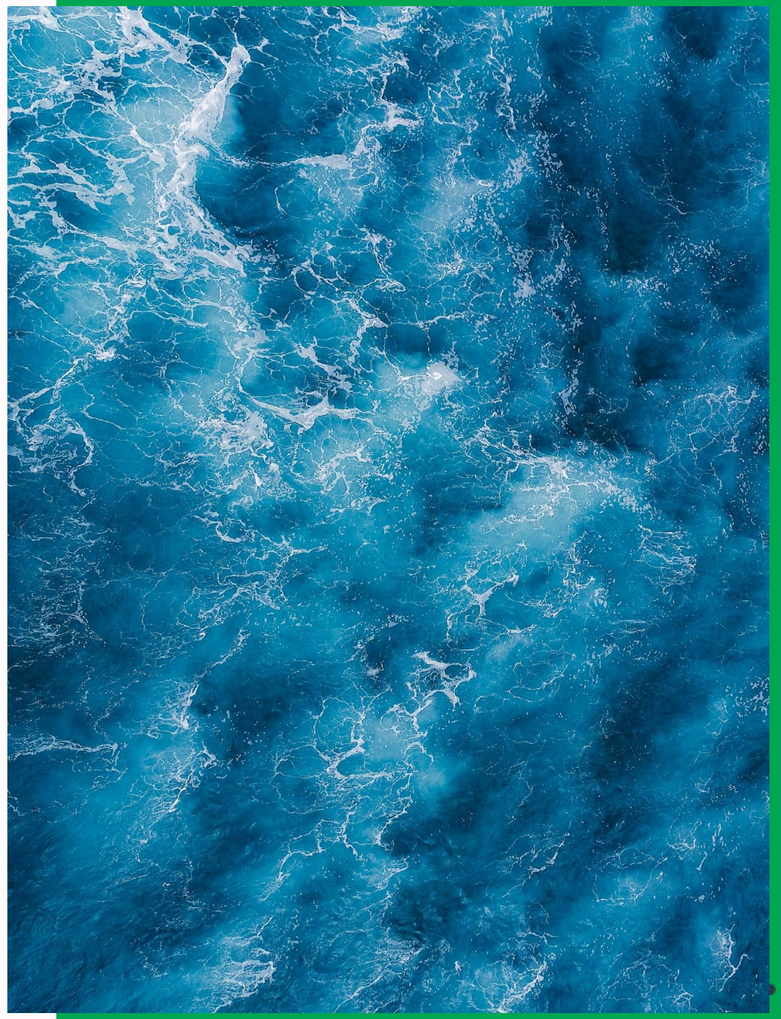
Outcomes

01 Participants are able to describe the fundamental principles of omics science and DNA barcoding within the scope of marine biotechnology.

02 Participants are able to analyze the applications of omics and DNA barcoding in conservation, fisheries, and bioprospecting.

03 Participants are able to integrate GIS approaches and omics science in biodiversity analysis of marine environment.



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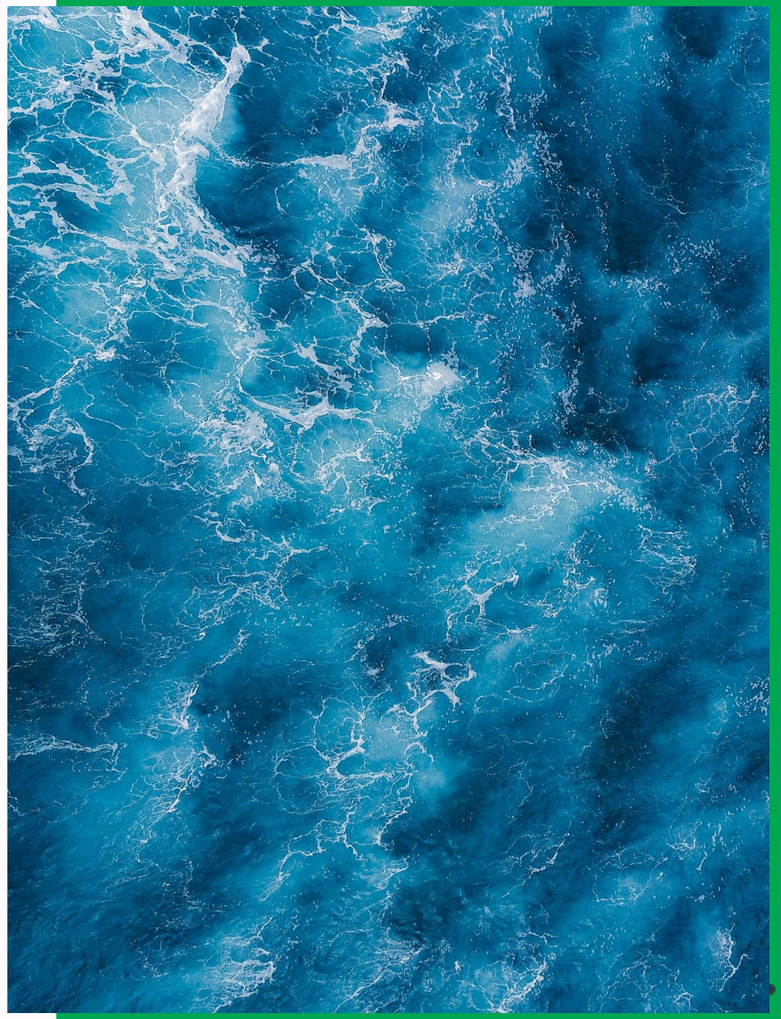

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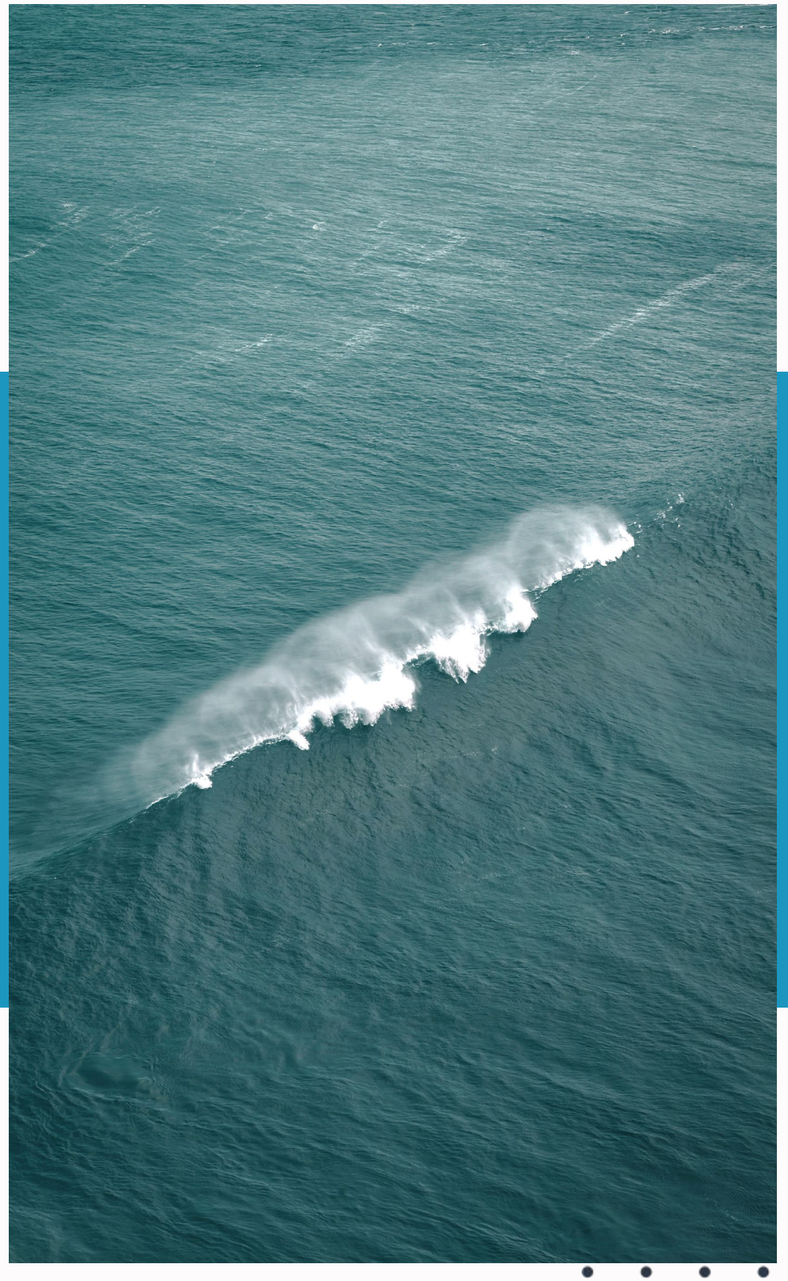
01

Omics Approaches in Marine Biotechnology



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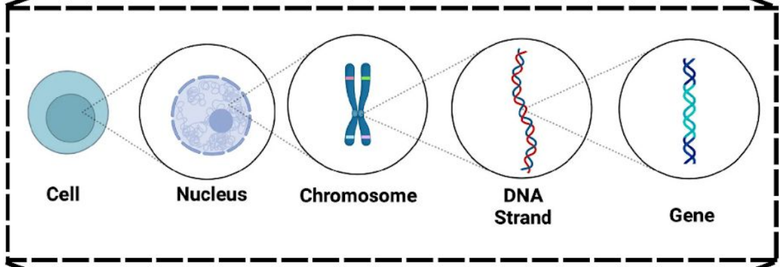
Introduction to Omics Science

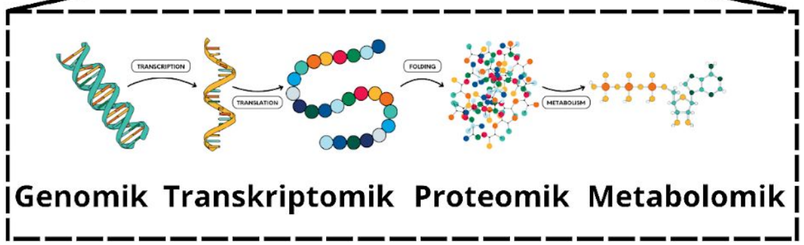
Omics Science in Biology System

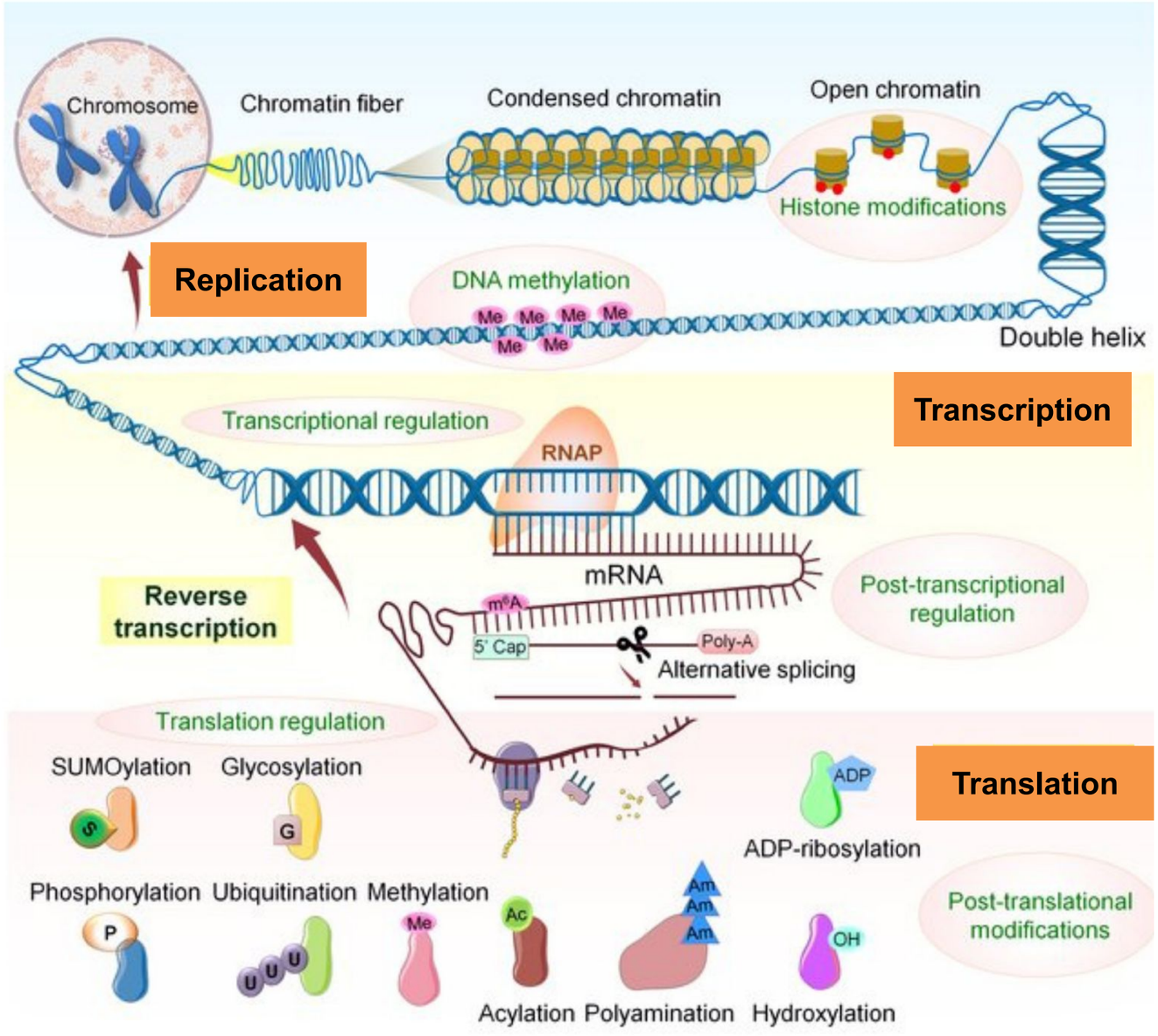
Marine Biodiversity

Biological Systems



Omics Science





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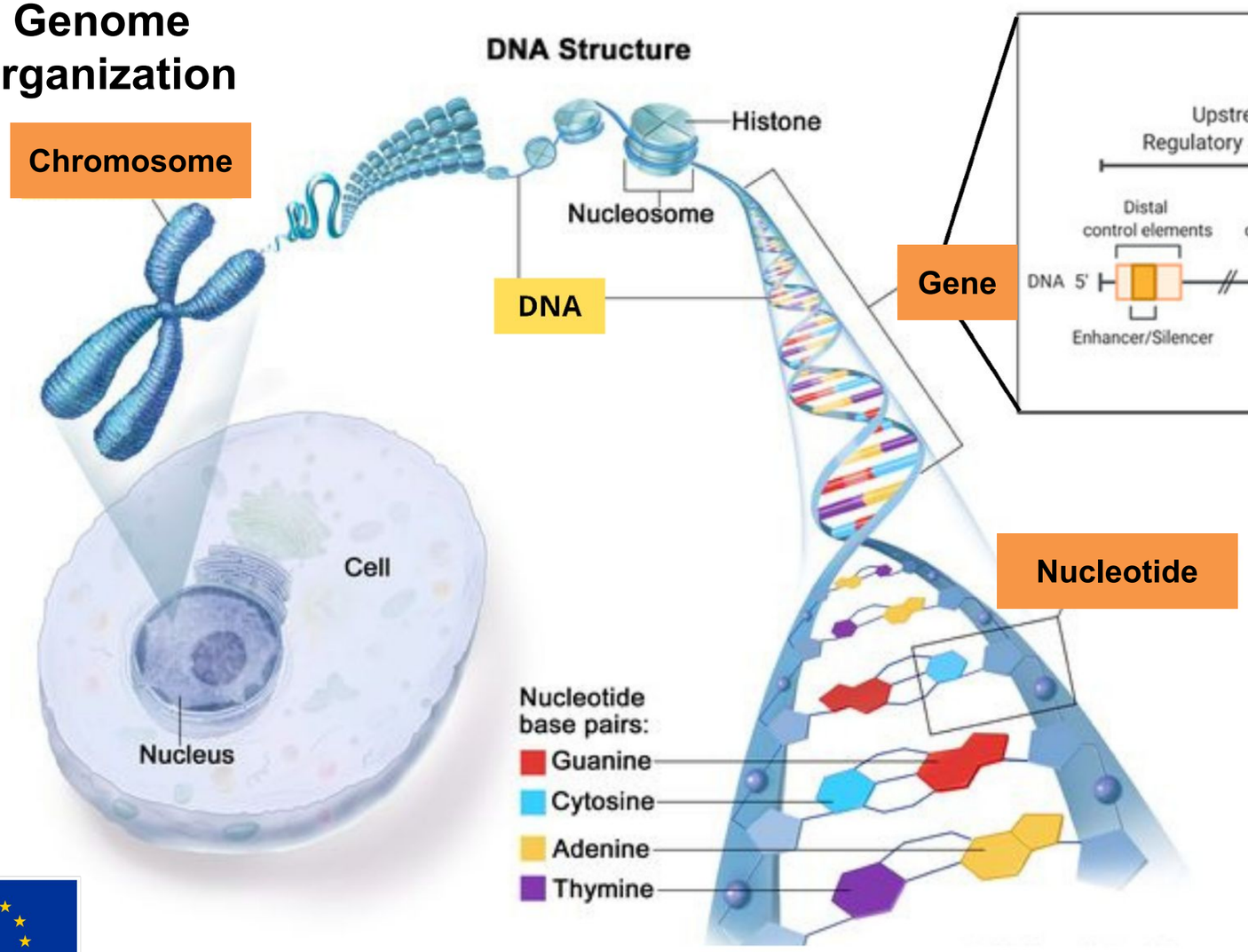
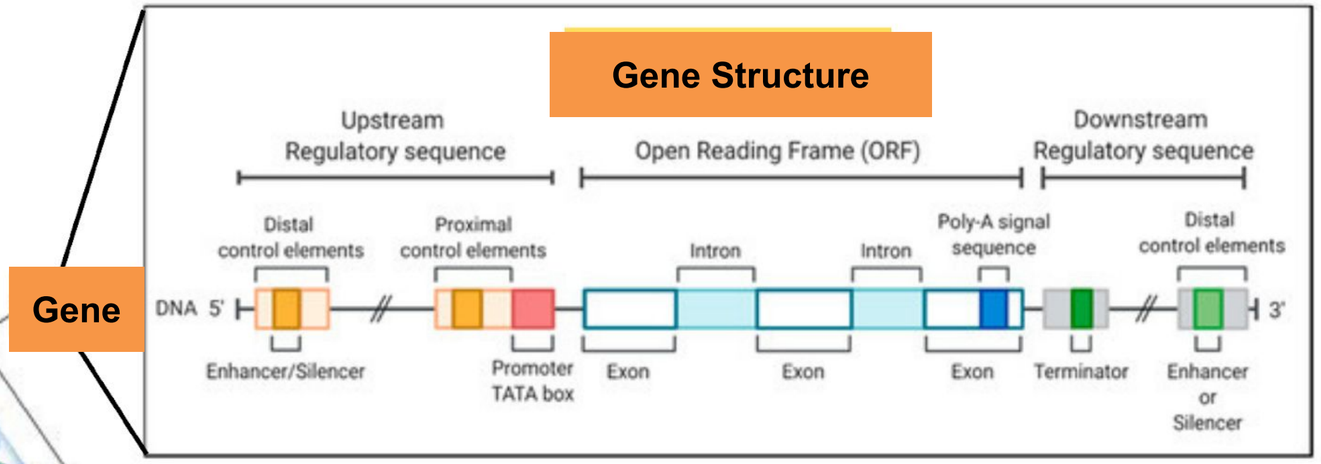
Pramanik et al. 2021; Gen-Fish; CSNK2A1 Foundation

Genomics

Genome Organization





- **Gene** is a segment of DNA that contain information for synthesizing specific protein moleclues, which determine hereditary traits.
- **Genome** refers to the entirety of genetic information in an organism encoded by DNA.

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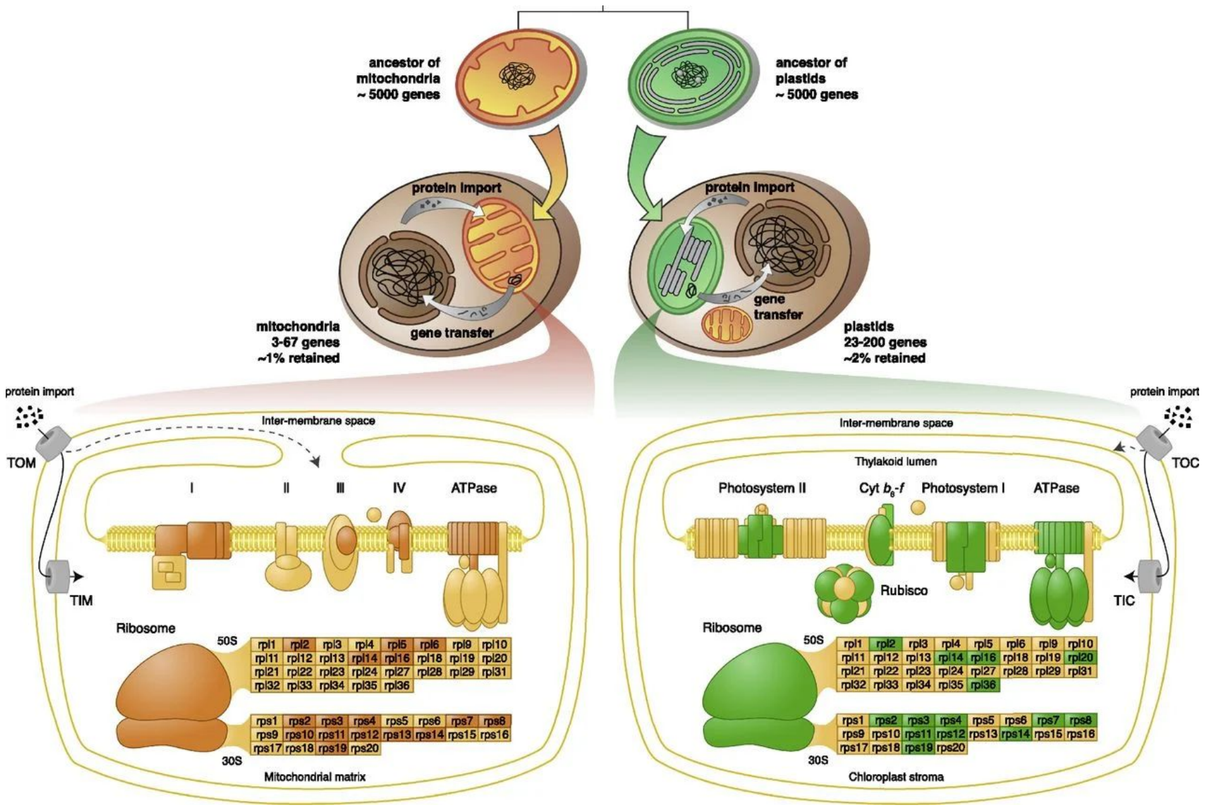
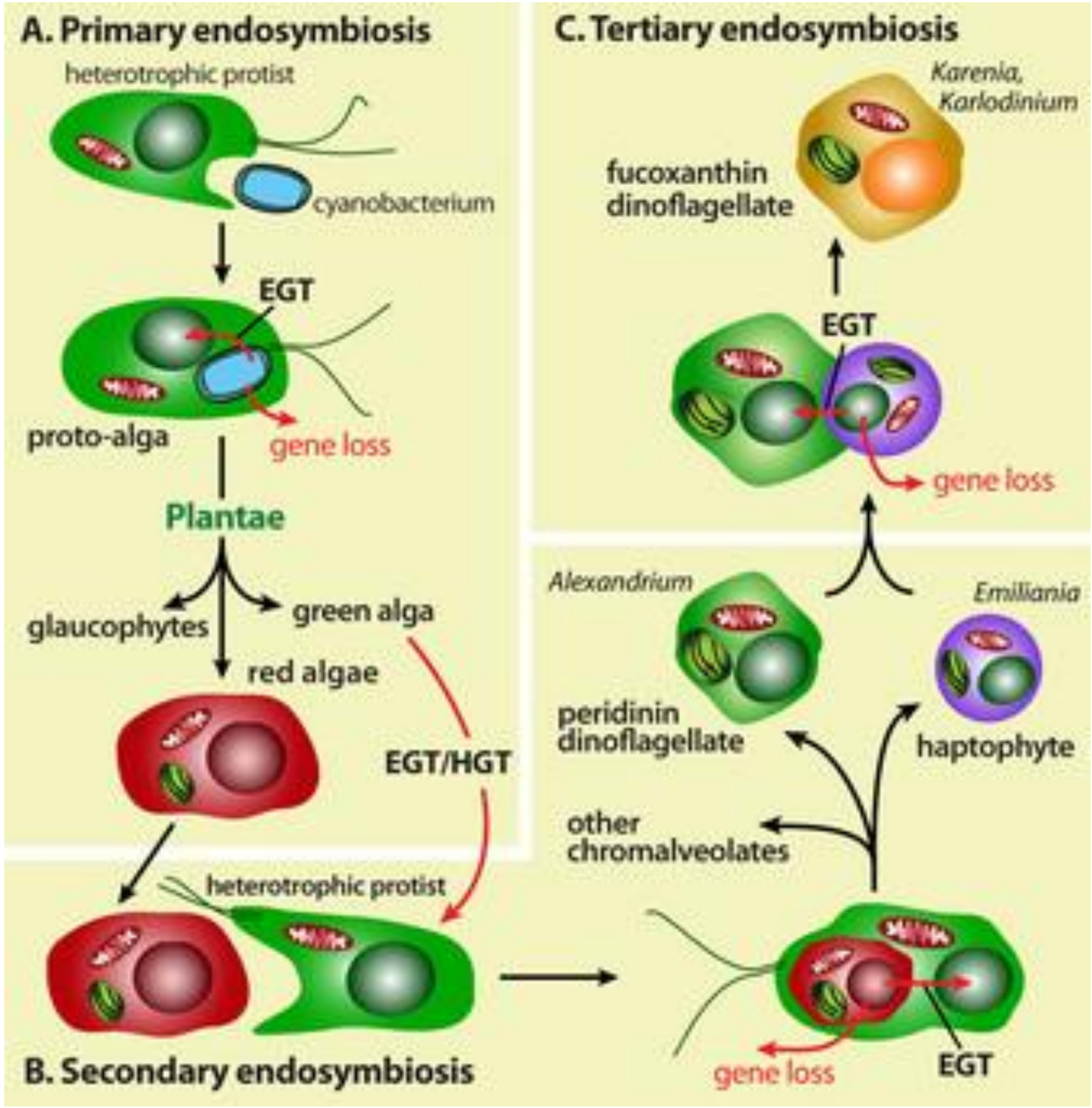
Villao-Uzho et al. 2023

Genomics

Endosymbiosis Theory





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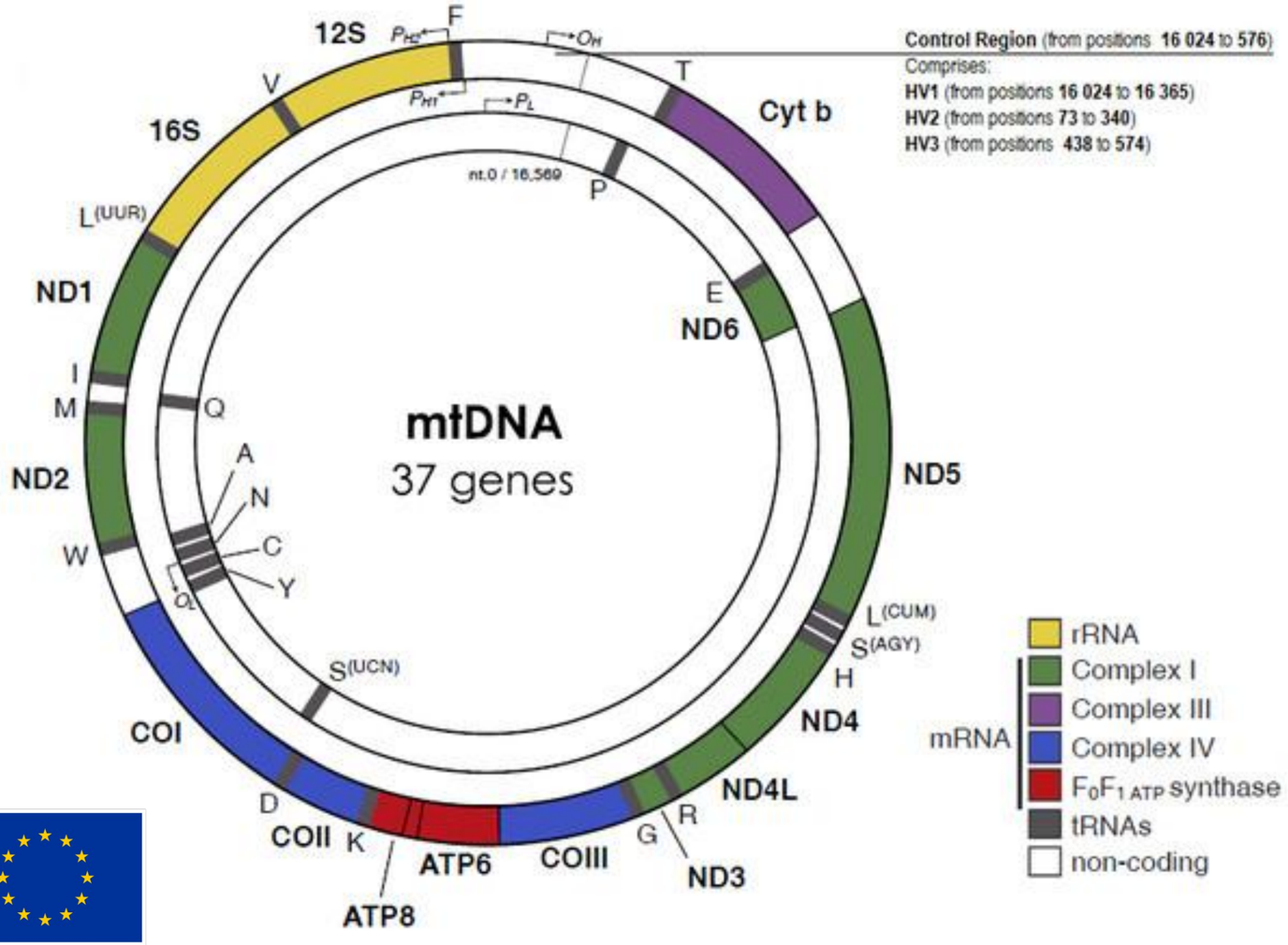
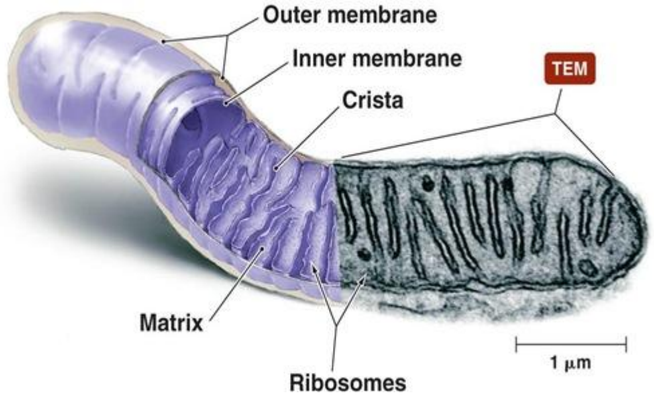
Chan & Bhattacharya 2010; Allen 2015

Genomics

Organel Genome



Nuclear Genome	Mitochondrial Genome
3 billion base pairs (human)	~16,569 base pairs (human)
Linear	Circular
Contains histones	Does not contain histones
Follows Mendelian inheritance	Follows maternal inheritance
~93% non-coding sequences	~3% non-coding sequences
Follows the universal codon usage rules	Some codons do not follow the universal rules
Monocistronic transcription	Polycistronic transcription
Replication dependes on mitosis	Replication does not depend on mitosis
One copy per cell	Multiple copies per cell



Amorim et al. 2019; Jones et al. 2021

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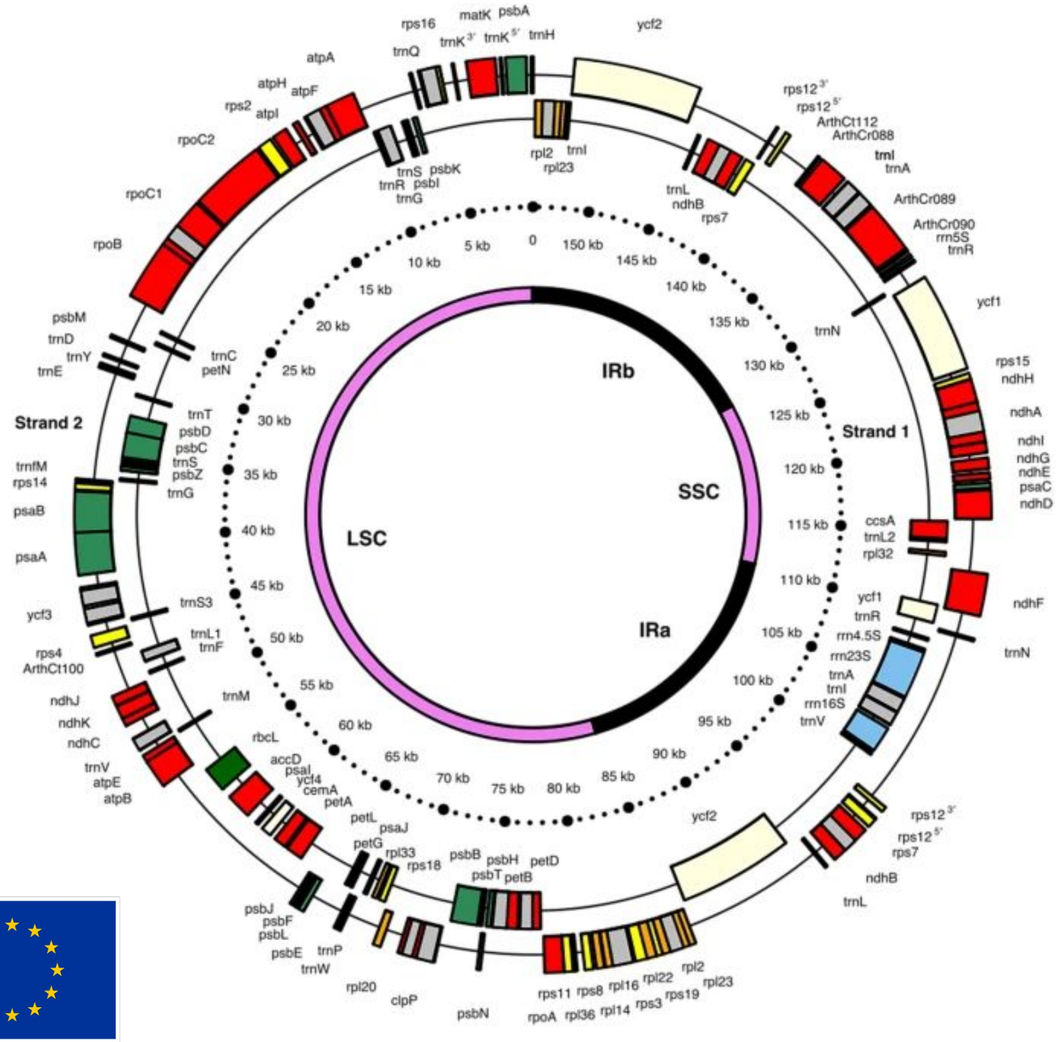
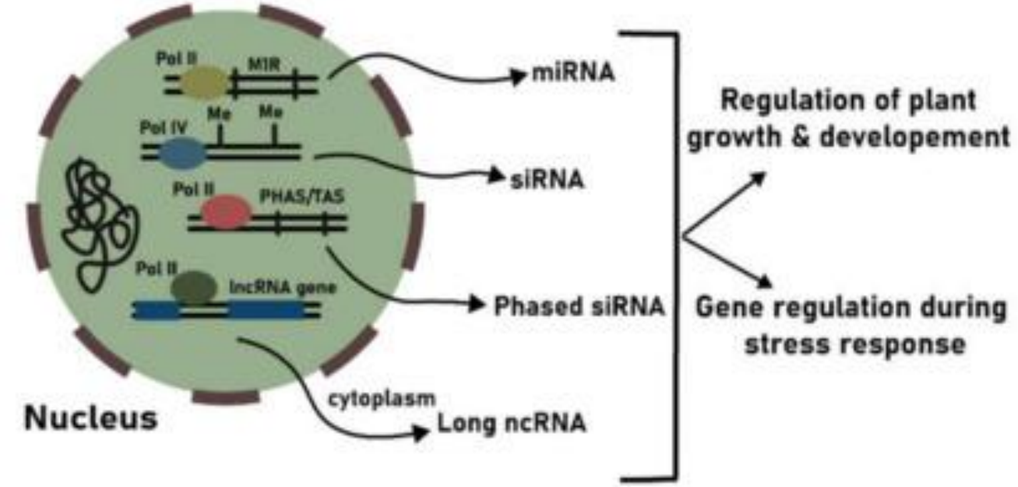
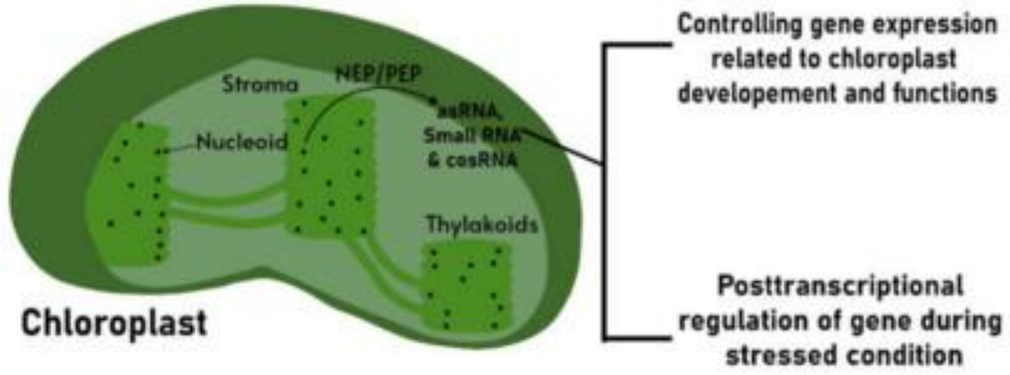
Genomics

Organel Genome



- All known chloroplast genomes are circular DNA molecules, approximately 120-160 Kbp in size. They contain two inverted repeat (IR) regions ranging from 6 to 76 Kbp in length, each encoding duplicate copies of three rRNA genes: 5S, 16S, and 23S rRNA.





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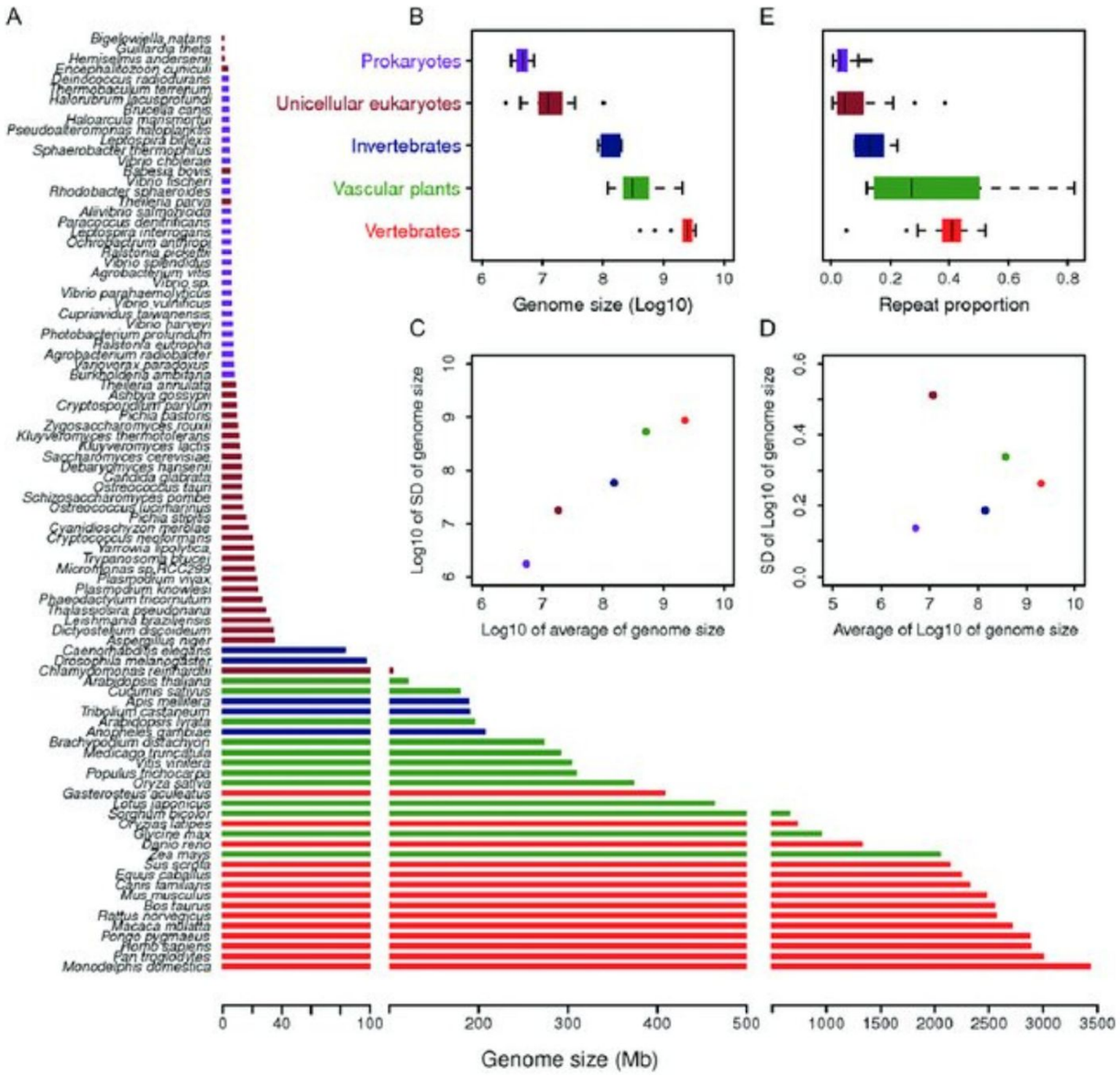
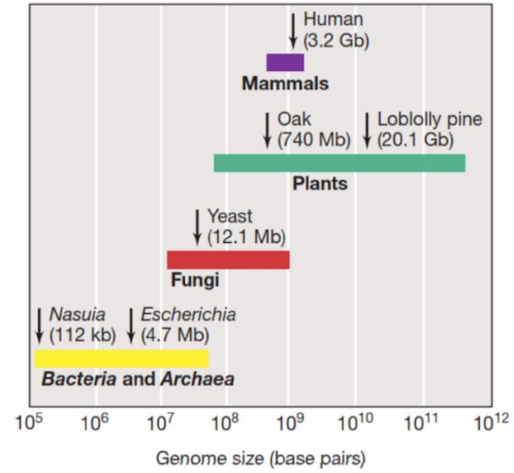
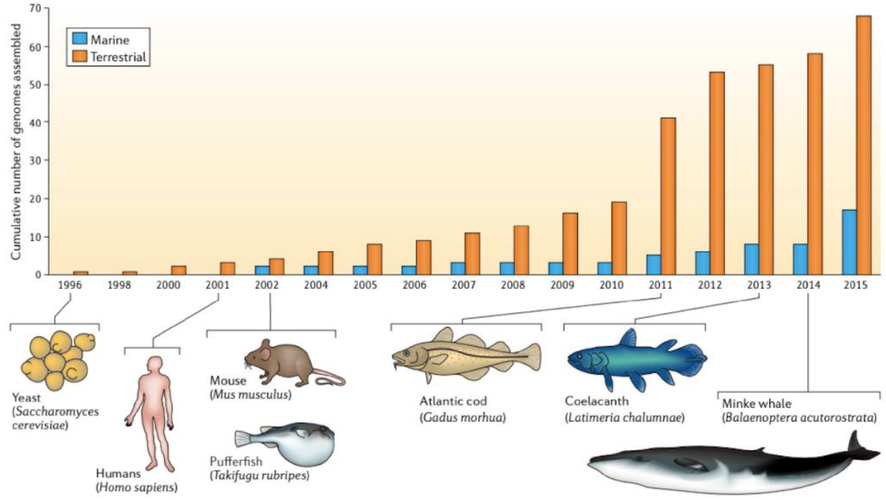
Dobrogojski et al. 2020; Anand & Pandi 2021

Genomics

Genome Size Comparison







Genome assembly data from marine and terrestrial species between 1996 and 2015 show that the number of reference genomes for marine species is significantly lower than that for terrestrial species.

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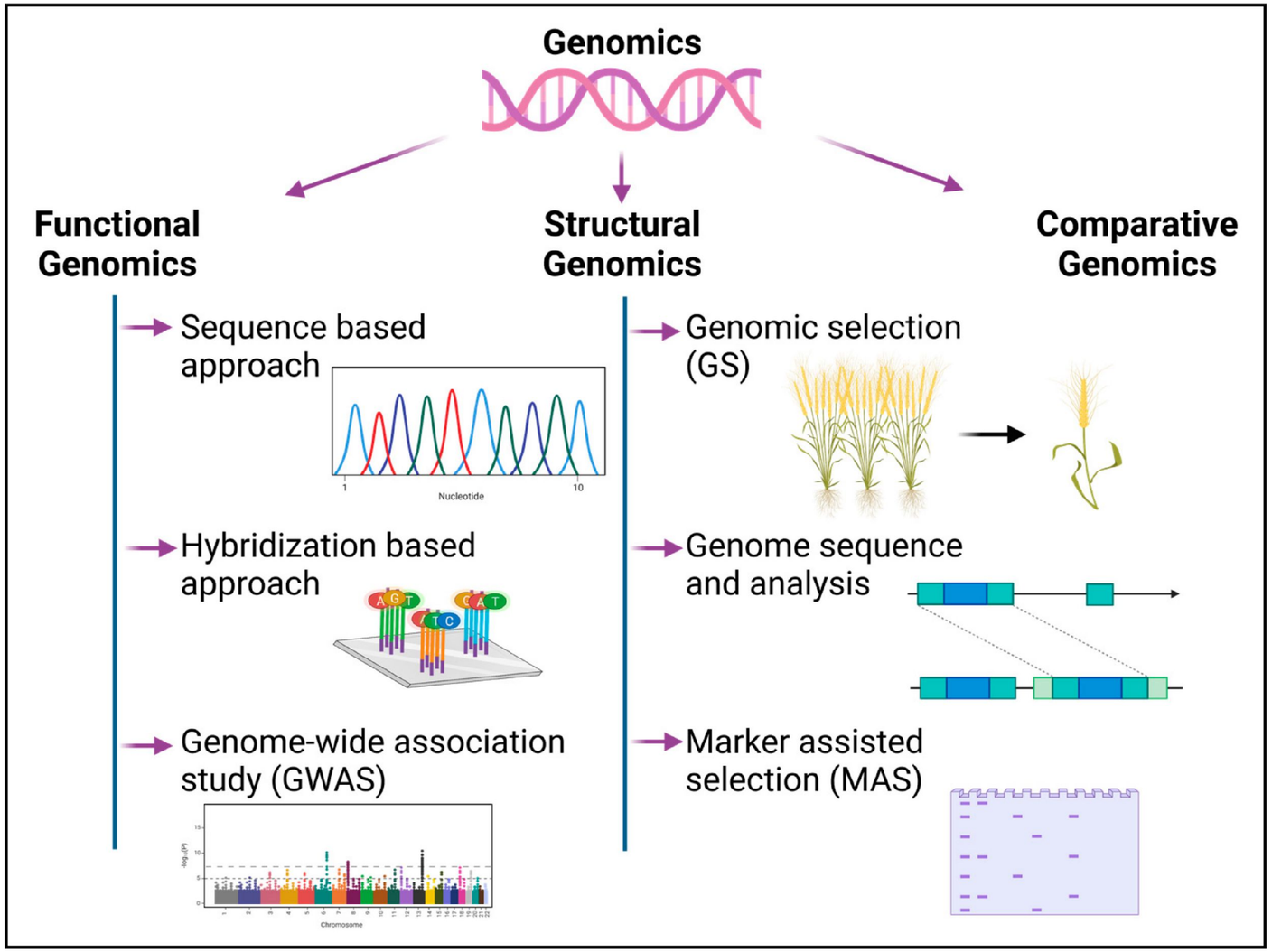
Li et al. 2011; Kelley et al. 2016

Genomics

Genomic Types

- **Structural Genomics** □ complete genome sequencing and annotation aimed at identifying the basic structure of genes and genetic elements.
- **Functional Genomics** □ complete genome sequencing and annotation aimed at determining the function of genes and non-genic sequences; describing gene and protein functions, gene–protein interactions, and the relationship between genotype and phenotype.
- **Comparative Genomics** → Complete genome sequencing and annotation aimed at comparing genome sequences across different species to study evolutionary relationships.



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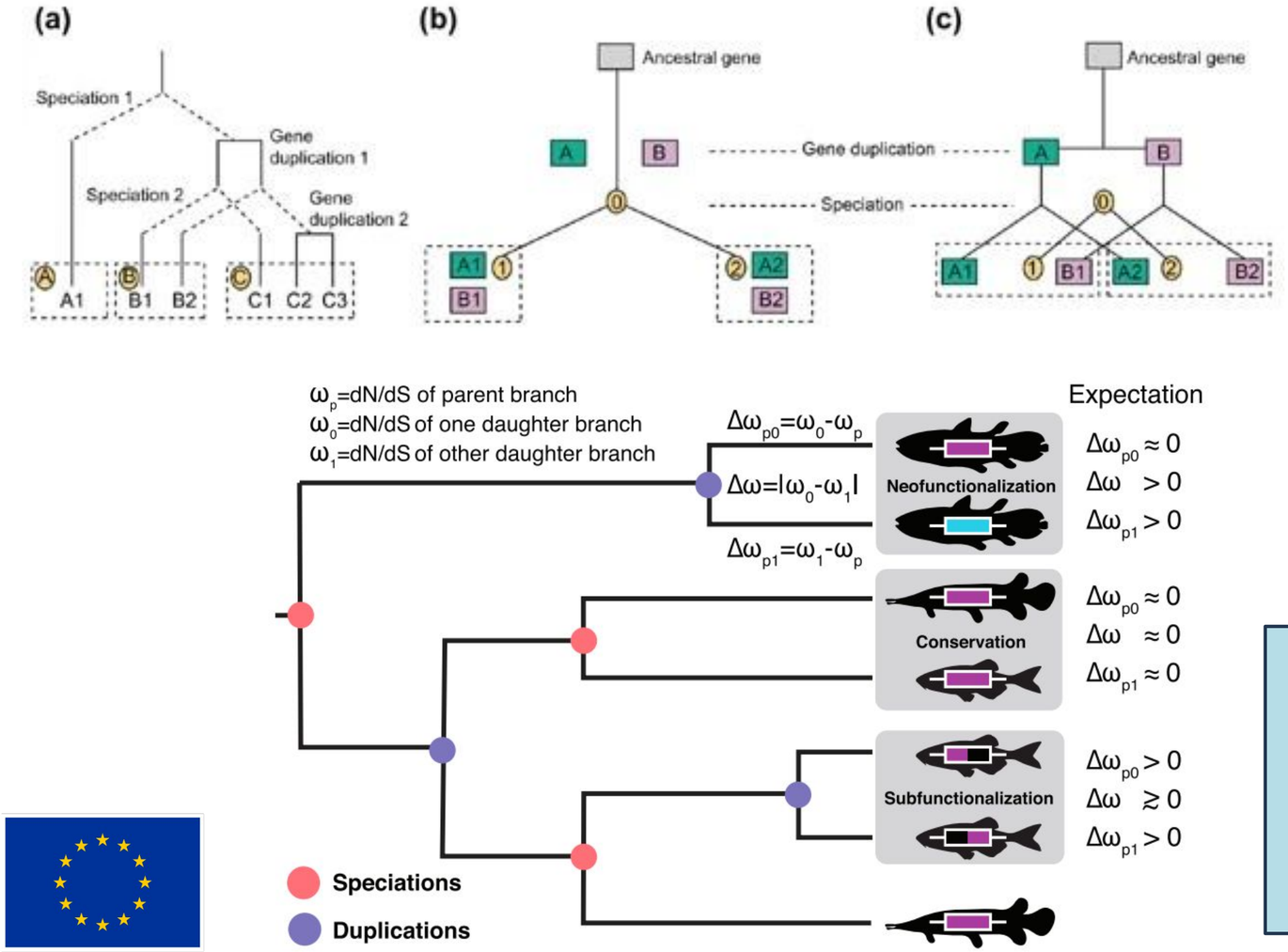
Ebeed & Ceasar 2024; Encyclopedia MDPI

Genomics

Genomics Approach in Biodiversity



Jensen 2001; David et al. 2020

The genome of an organism often contains multiple copies of genes with similar sequences due to a shared evolutionary origin, known as **homologous genes**.

- **Orthologous genes**: Homologous genes that arise from speciation events between different species.
- **Paralogous genes**: Homologous genes that result from gene duplication within the same genome.

Ortholog Conjecture

The difference in selective pressure is generally much smaller between orthologs than between paralogs.

Orthologs are more likely to retain their original function, whereas paralogs are more prone to functional divergence (neofunctionalization).

Evolutionary divergence between paralogs is often **asymmetric**, where one copy may undergo relaxed selection or a functional shift.

Functional genomic annotation → Takes into account the evolutionary origin of genes before assigning their functions.

Whole genome sequencing → Enables the identification of all members of a gene family, including paralogs, which may be highly similar to one another.

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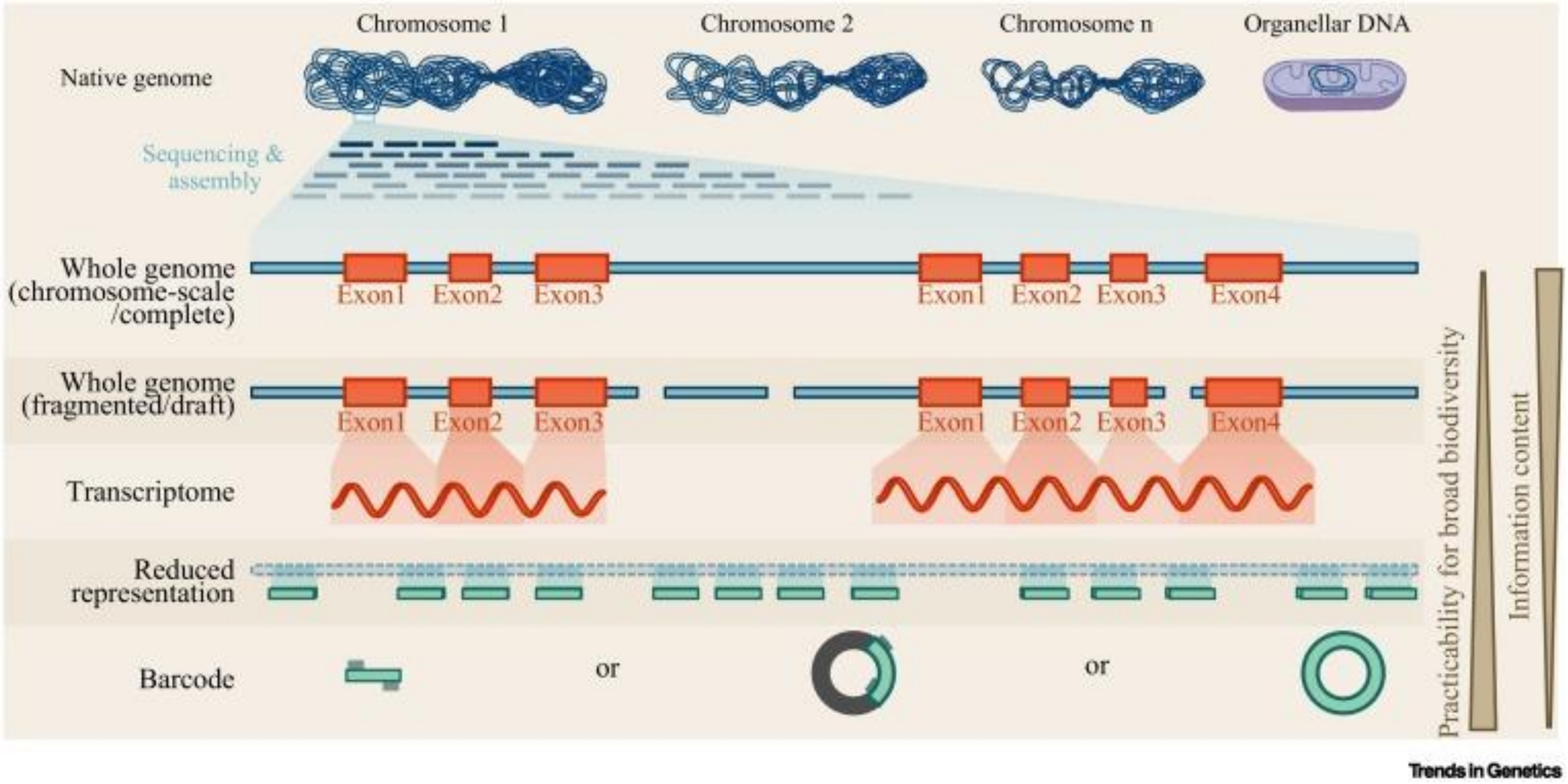
Genomics

Genomics Approach in Biodiversity



Theissinger et al. 2023

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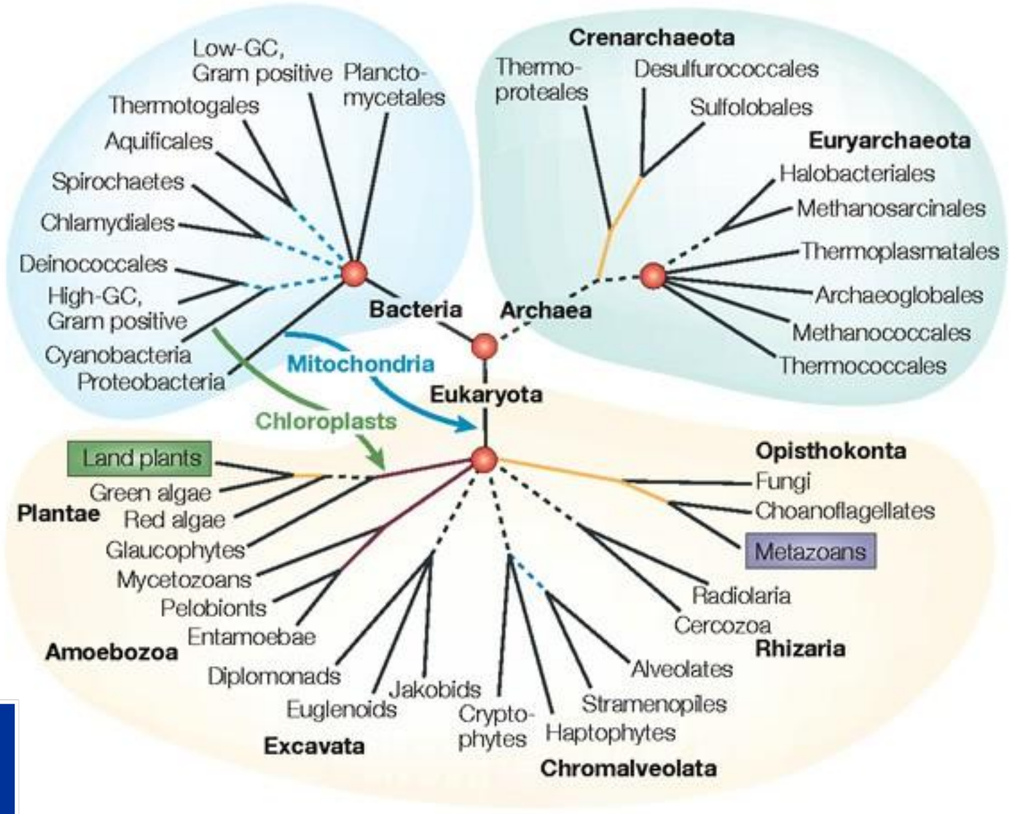
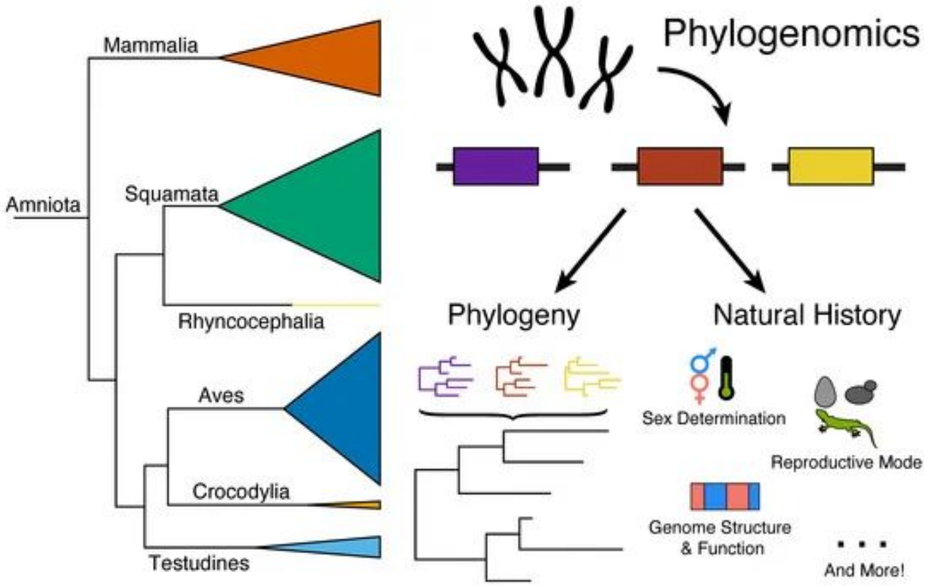
Genomics

Genomics Approach in Biodiversity

- Genomics serves as the foundation for reconstructing phylogenetic trees that reflect evolutionary relationships — a process known as phylogenomics.

Aspect	Phylogenetics	Phylogenomics
Data scope	Specific genes	Entire genome (full set of genes)
Resolution	Limited by the resolution of biological markers	High-resolution insights
Methods	Sequence analysis of several biological markers	Whole-genome analysis, including RAD-seq and UCEs
Challenges	Limited by marker resolution	Requires high-performance computing and high-quality data





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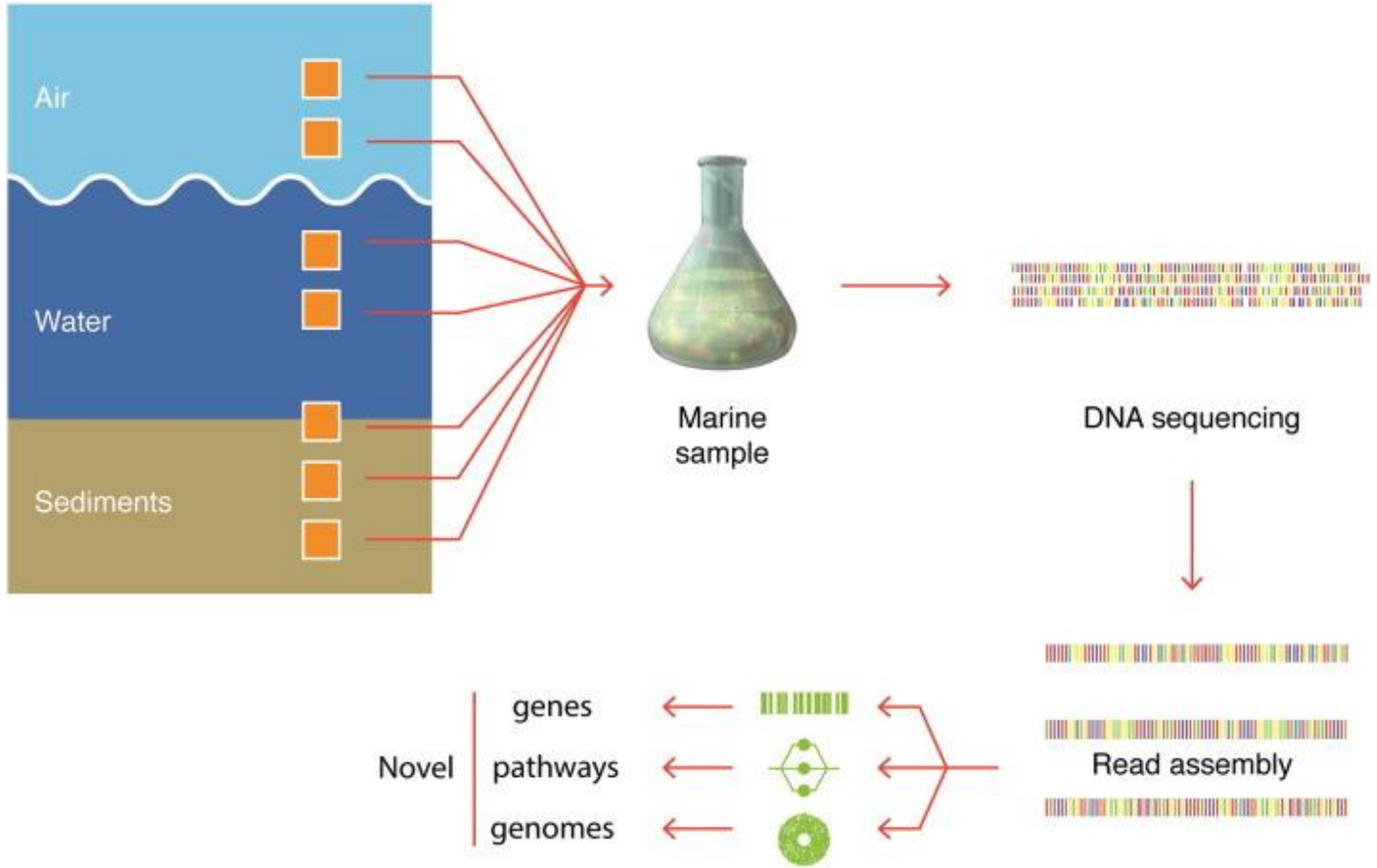
Delsuc et al. 2005; Card et al. 2023

Metagenomics

Metagenomics

- **Metagenomics** (also known as environmental genomics) is an approach used to analyze the combined DNA or RNA from environmental samples that contain organisms which have not been isolated or identified.
- Just as the total genetic content of an organism is referred to as its **genome**, the total genetic content of all organisms inhabiting a particular environment is known as the **metagenome**.
- Metagenomic analysis can be performed through DNA sequencing to explore gene expression patterns within a community of organisms in a given environment.

- **Marine metagenomics** is a promising approach for the development of biotechnological industries (e.g., the discovery of enzymes from marine microbial communities). Covering more than 70% of the Earth's surface, the ocean is an immense reservoir of microbial biodiversity. Marine microorganisms play a crucial role in marine food chains and in the global carbon and energy cycles.



Kodzius & Gojobori 2015

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Metagenomics

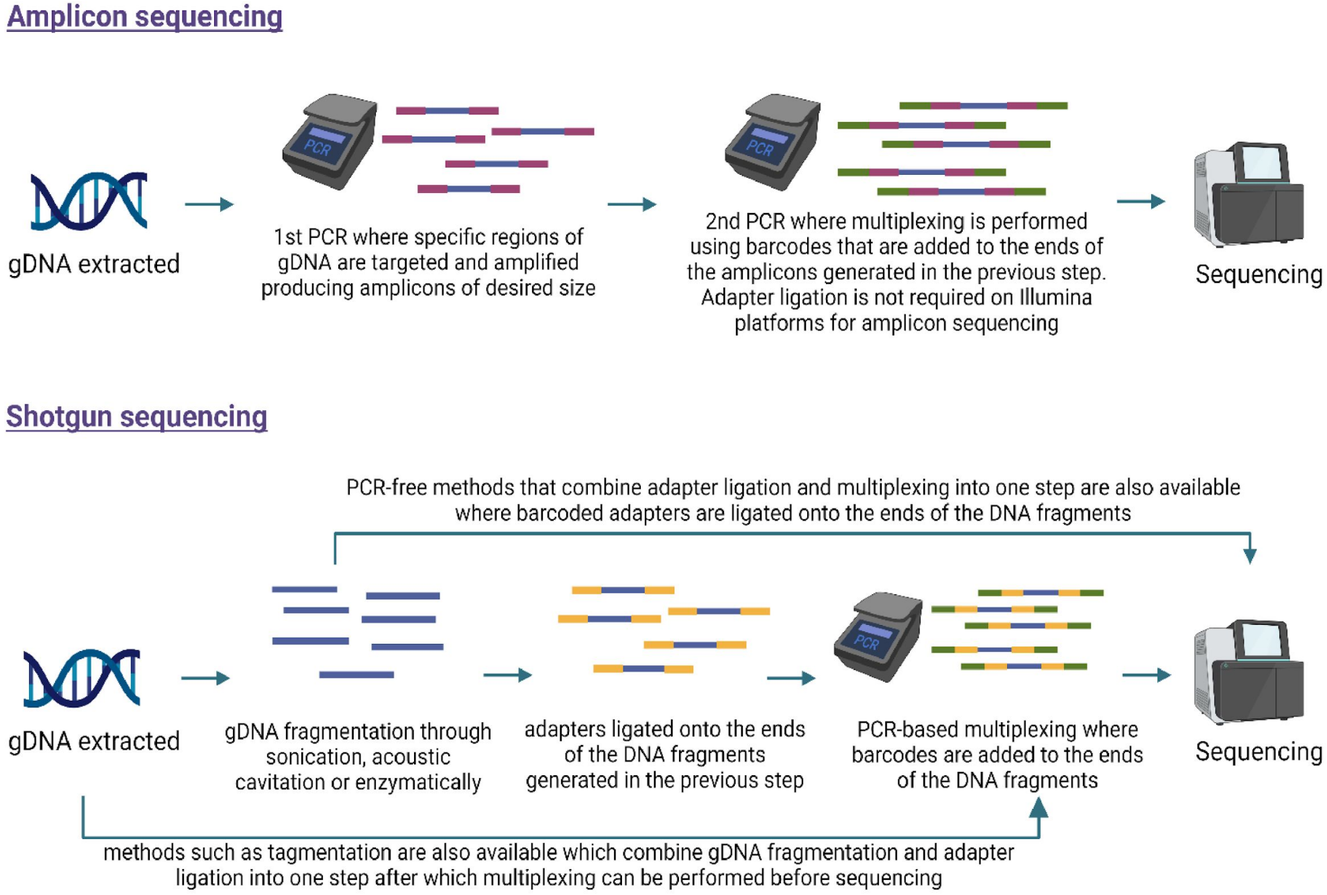
Metagenomic Approaches:

1. Amplicon Sequencing → Relies on sequencing of phylogenetic marker genes following PCR amplification.

- Focuses on a single marker gene (e.g., *16S rRNA* for bacteria, *ITS* for fungi).
- Uses PCR to amplify the marker gene, which is then sequenced.

2. Shotgun Metagenomics Sequencing → Involves sequencing all the DNA from organisms in a sample, rather than targeting a specific marker gene.

- Does not focus on a single gene, but analyzes the entire DNA content of the sample.
- Enables comprehensive analysis of taxonomic composition, genetic functions, and metabolic pathways.



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Srinivas et al. 2022

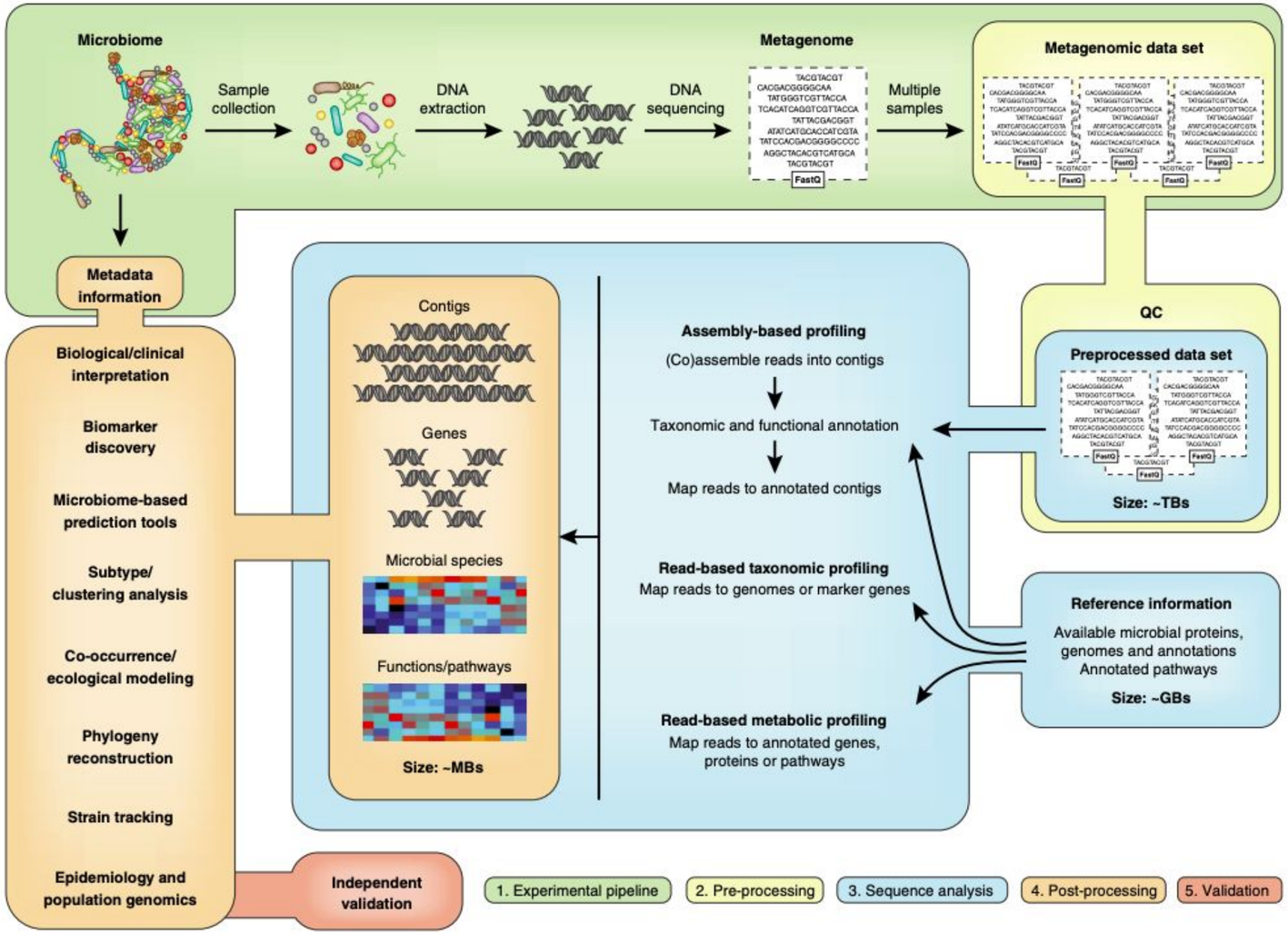
Metagenomics

Metagenomics

Shotgun metagenomics is an untargeted method used to identify taxonomic composition, genetic functions, and to directly reconstruct the genomes of organisms—including microorganisms that cannot be cultured.

Shotgun Metagenomics Workflow:

1. Study design
2. Sample collection and DNA extraction
3. Sequencing and DNA library preparation
4. Bioinformatics analysis
5. Post-processing and validation



Quince et al. 2017

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Metagenomics

Metagenomic Approaches

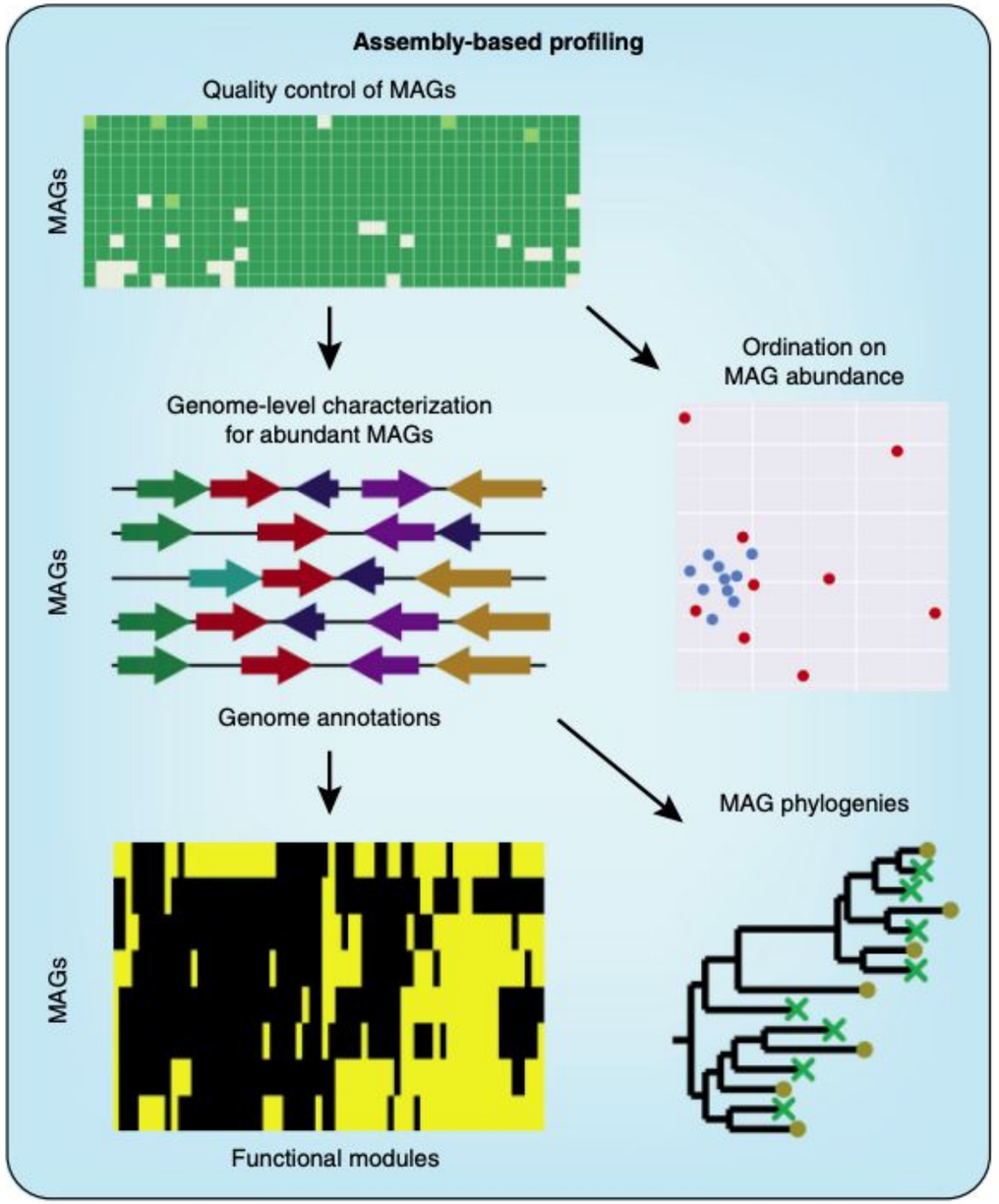
Assembly-Based Profiling
Involves assembling sequencing reads into longer contigs and organizing them into genomes, genes, or metabolic pathways.

Advantages:

- Enables reconstruction of complete genomes, including those of unculturable microorganisms.
- Reveals novel metabolic pathways and unique biological functions.
- Can be used to build phylogenetic trees and conduct evolutionary studies.

Limitations:

- Requires high coverage and large amounts of sequencing data.
- Complexity increases with diverse microbial communities.
- May fail in highly complex communities or with low-quality data.



Quince et al. 2017

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Metagenomics

Metagenomic Approaches

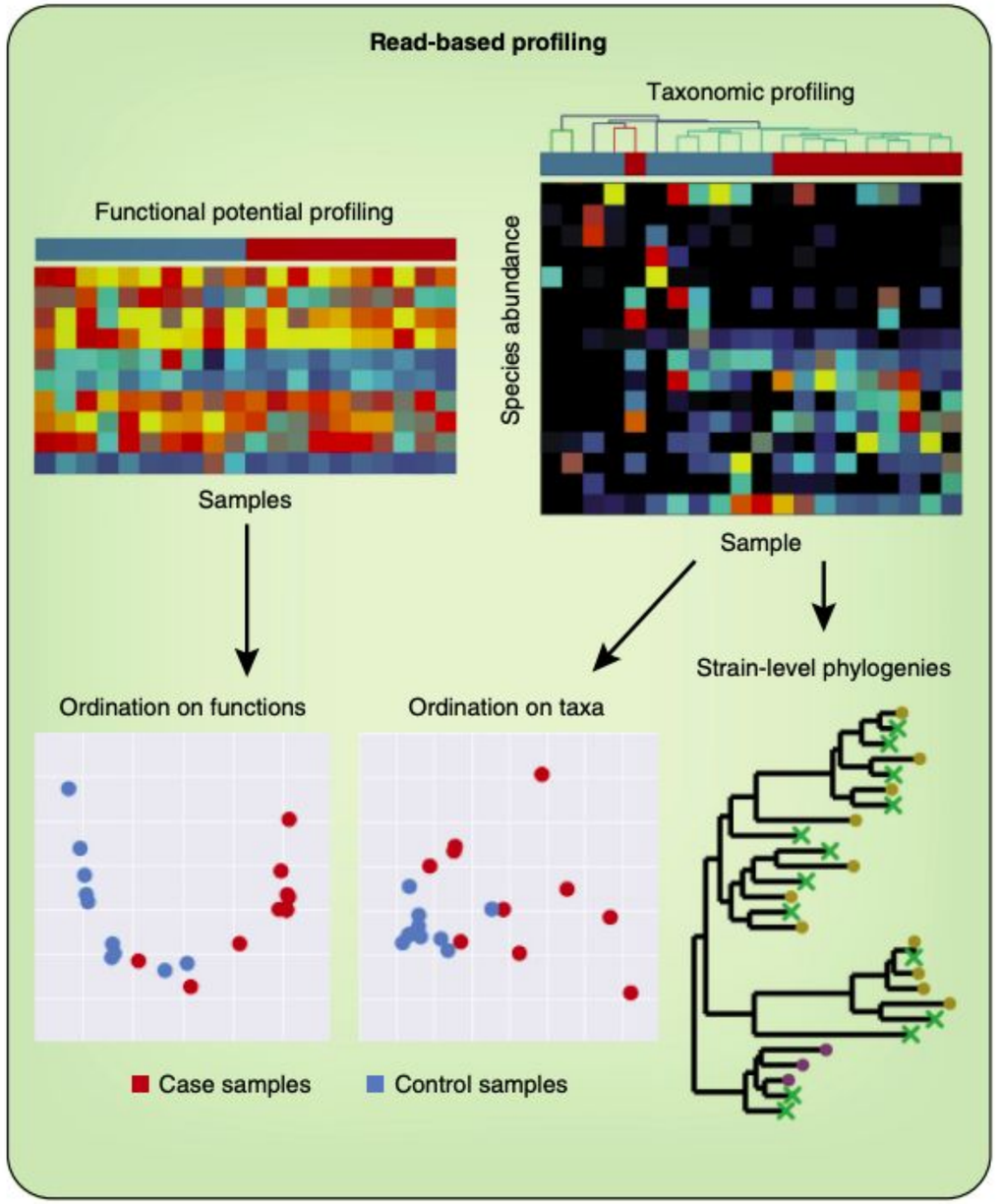
Read-Based Profiling
Involves directly analyzing raw sequencing reads without assembling them into longer contigs. This approach maps reads against a reference database.

Advantages:

- Faster and more efficient, ideal for large-scale analyses or high-throughput sample processing.
- Useful for preliminary screening, taxonomic surveys, or biodiversity studies.

Limitations:

- Relies heavily on reference databases — limited in detecting organisms with uncharacterized genomes.
- Less informative for reconstructing complete metabolic pathways or novel genomes.



Quince et al. 2017

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Metagenomics

Metagenomic Approaches

Aspect	Assembly-based Analysis	Read-based Analysis (Mapping)
Comprehensivenes	Can reconstruct multiple complete genomes, but only for organisms with sufficient sequencing coverage for assembly and binning.	Provides aggregate insights into community structure or function, but only based on the fraction of reads that map to the reference database.
Community Complexity	In complex communities, only partial genomes may be recovered through assembly.	Can handle communities of varying complexity, especially when sequencing depth and reference coverage are sufficient.
Novelty	Can reconstruct genomes of entirely novel organisms without known close relatives.	Cannot identify organisms whose genomes lack close relatives in the reference database.
Computational Load	Requires intensive computation, including assembly, mapping, and binning.	More efficient and scalable, suitable for large-scale meta-analyses.
Genome-based Metabolism	Can link metabolic potential to phylogeny through fully reconstructed genomes, even for novel diversity.	Typically resolves only aggregate metabolic functions of the community; linking to phylogeny is limited to organisms with available reference genomes.
Expert Manual Curation	Requires manual curation for binning, scaffolding, and error detection during assembly.	Generally does not require manual curation, though reference genome selection may require human oversight.
Integration with Microbial Genomics	Assembled genomes can be integrated into microbial genomics pipelines designed for isolate-based genome analysis.	Profiles cannot be directly linked to pure-culture isolate genomes.

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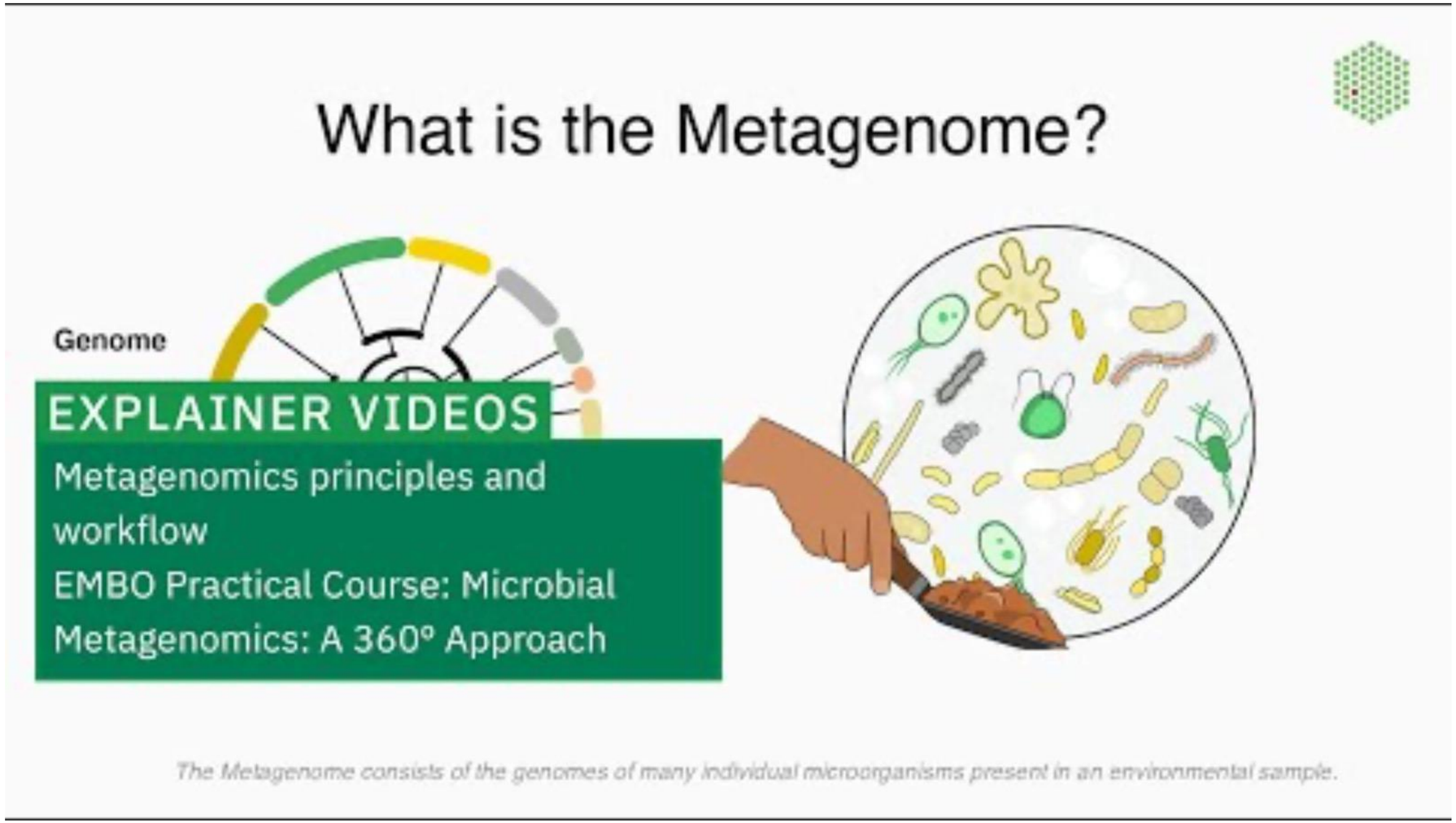
Quince et al. 2017

Metagenomics

Metagenomic Approaches



https://youtu.be/RcYXTpNS_XU?si=3y5yoQxFpqePXLqV

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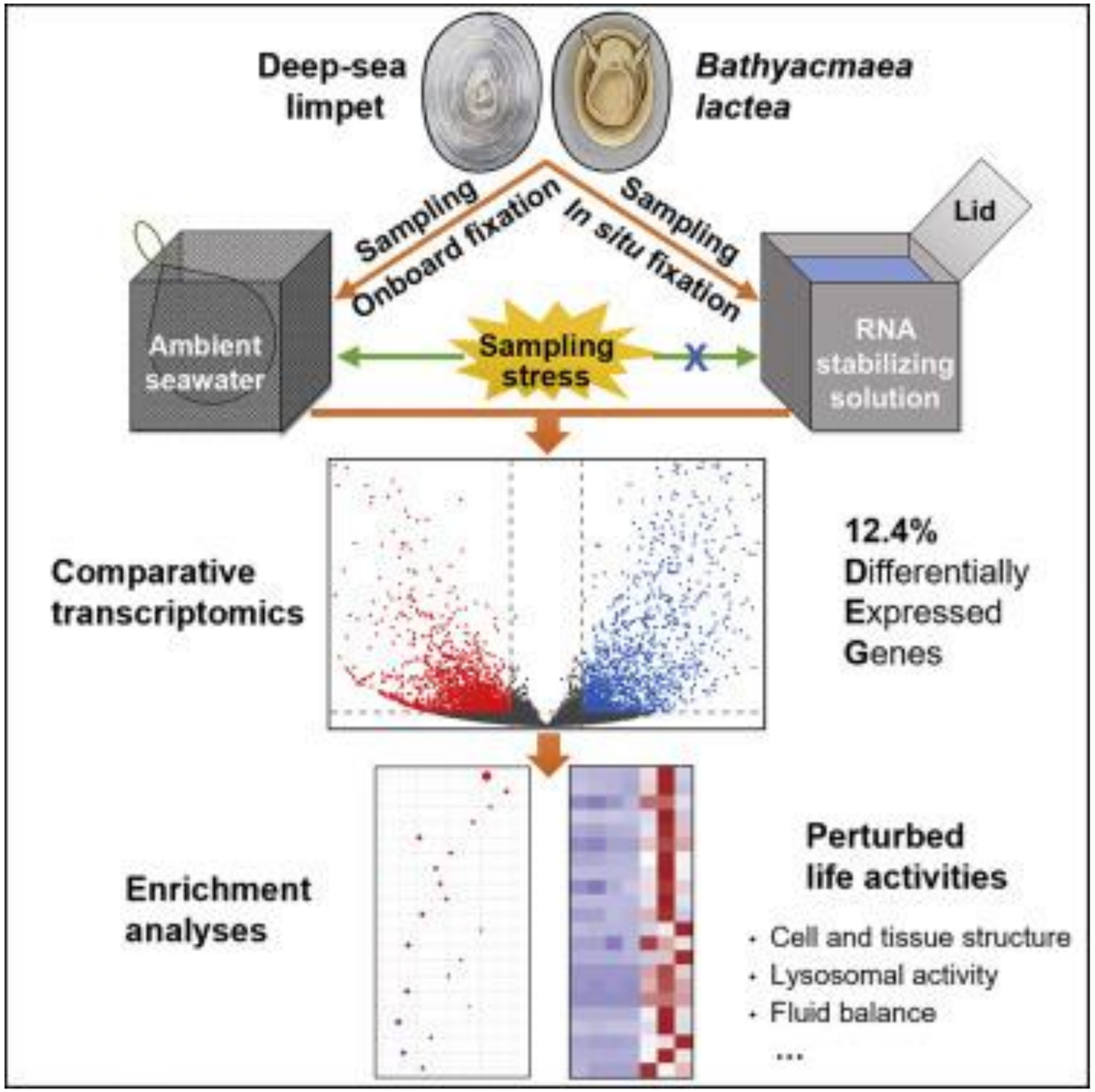
Transcriptomics

Transcriptomics

Transcriptomics is an approach used to study the **transcriptome**, which refers to the complete set of RNA molecules synthesized by a cell or tissue under specific conditions.
RNA reflects the genes that are actively being expressed, providing insights into gene activity at particular times and under specific circumstances.

Transcriptomics can be applied to investigate stress responses in marine organisms (e.g., during the sampling process from the deep sea to the surface).
A study on the species *Bathyacmaea lactea* demonstrated that sample collection without **in situ fixation** can lead to significant biases in gene expression.



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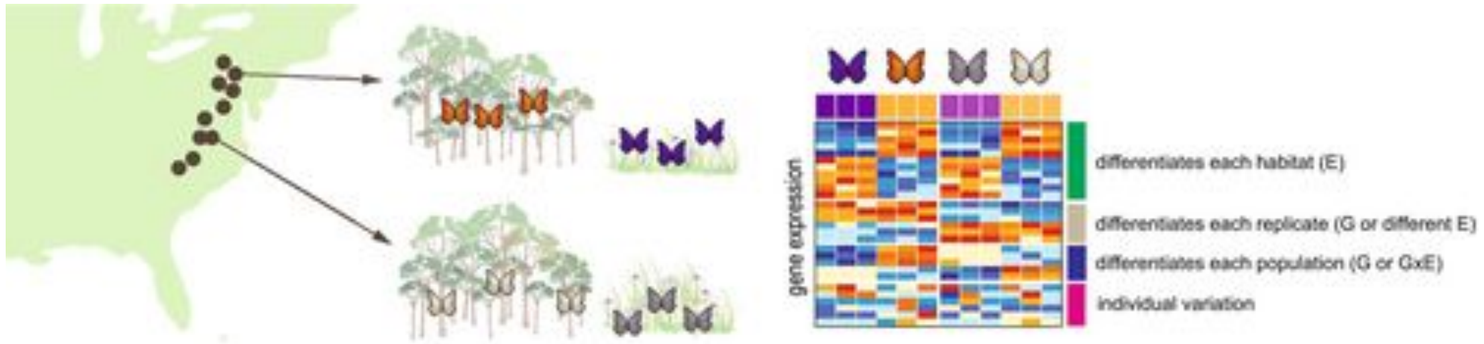
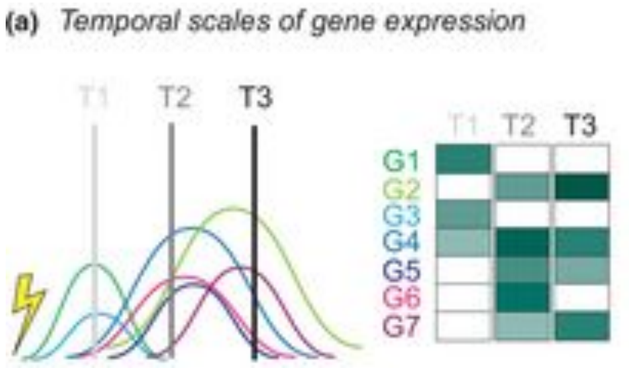
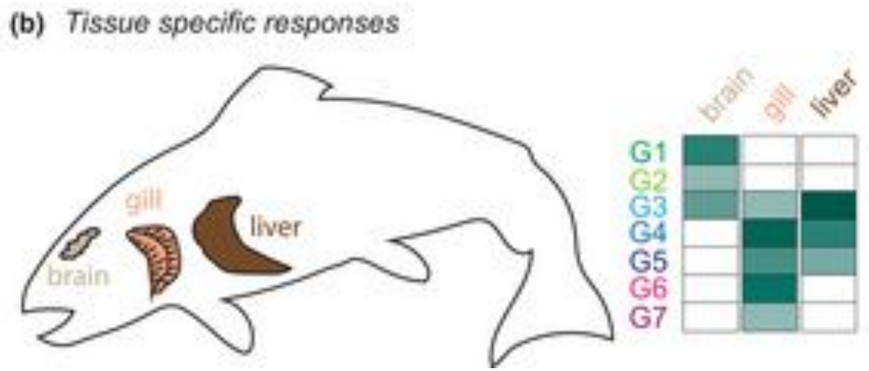
Yan et al. 2022

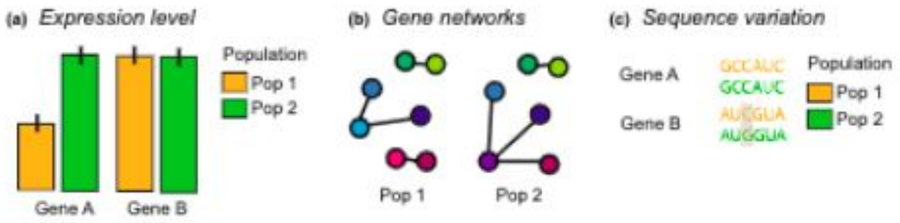
Transcriptomics

Landscape Transcriptomics









Keagy et al. 2022

Landscape Transcriptomics

Landscape transcriptomics is an emerging approach that integrates:

1. **Gene expression data** (transcriptomics)
2. **Large-scale environmental data** (ecological landscape)

The goal of landscape transcriptomics is to understand how organisms respond to environmental changes—such as climate shifts, pollution, habitat fragmentation, and other stressors.

Examples:

- **Salmon**: Transcriptomes are used to detect responses to temperature, salinity, or disease.
- **Coral reefs**: Baseline gene expression is analyzed to identify genes associated with heat tolerance.

Challenges in Landscape Transcriptomics

- **Temporal specificity**: Gene expression can change rapidly; precise time of sampling is critical.
- **Tissue specificity**: Different tissues express different sets of genes, requiring careful sample selection and interpretation.

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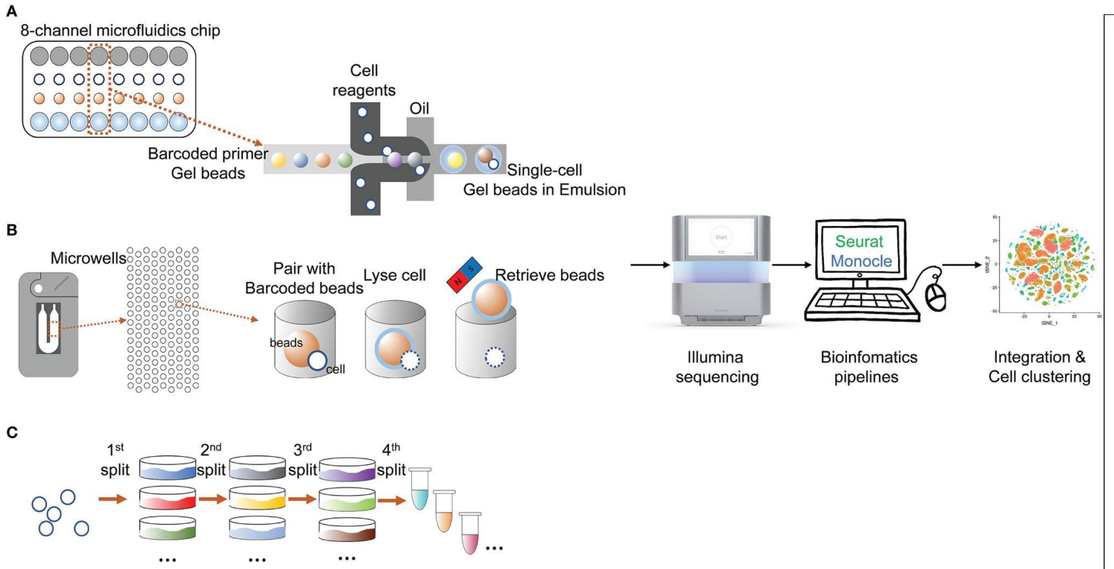
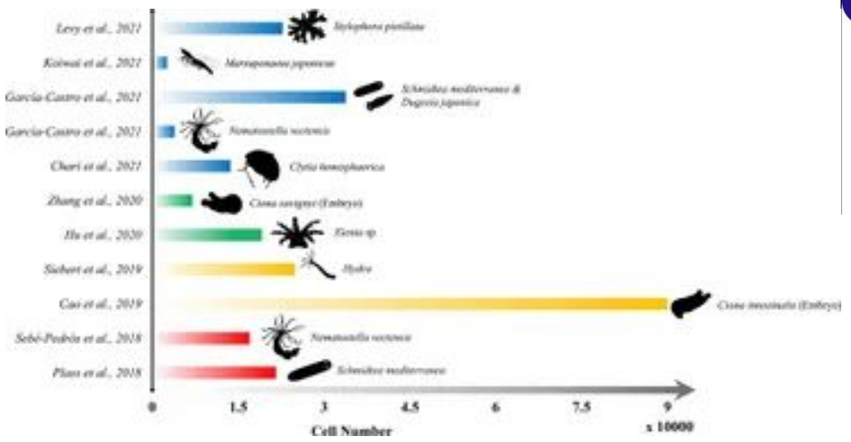
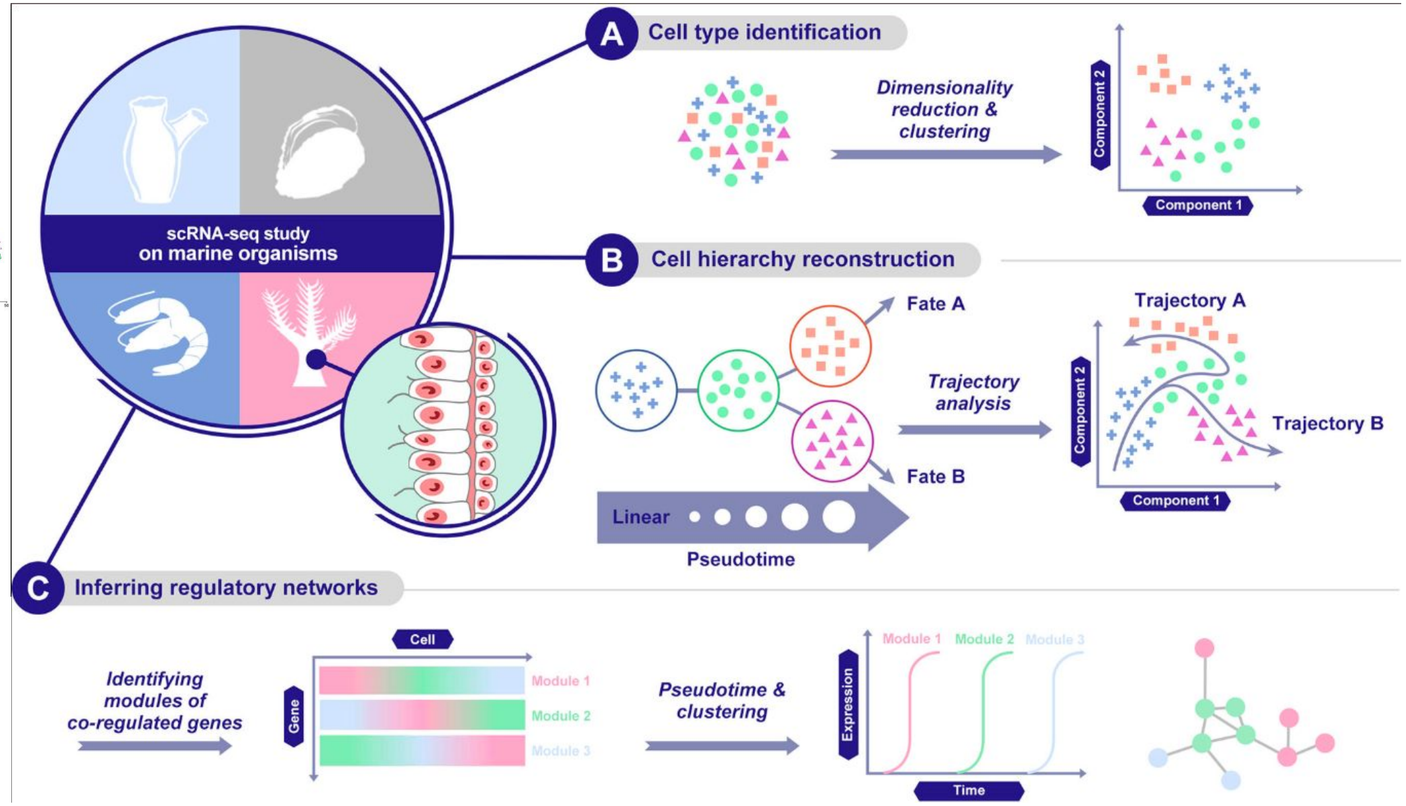
Transcriptomics

Single Cell Transcriptomics







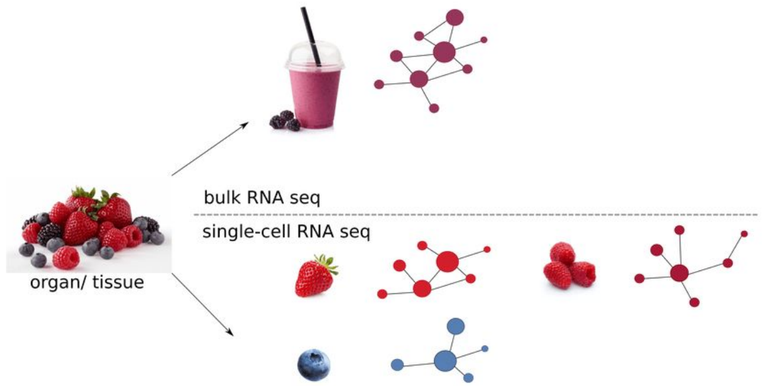
Single-cell RNA sequencing (scRNA-seq) is a technique that enables the analysis of gene expression at the level of individual cells, providing more detailed insights compared to conventional RNA-seq, which only captures average expression across entire cell populations.

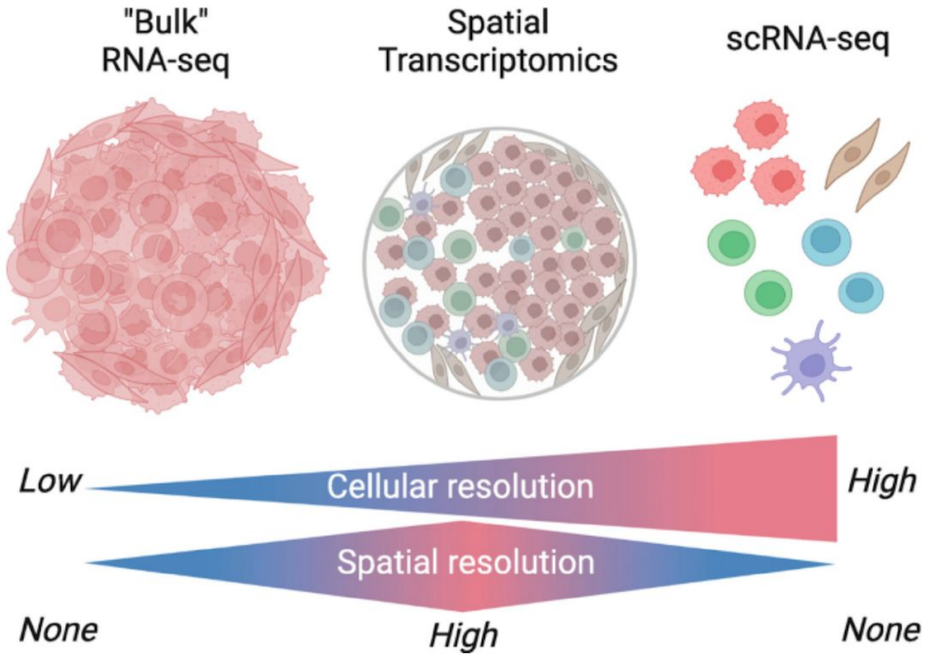
Co-funded by the European Union

Li et al. 2022

Transcriptomics

Single Cell Transcriptomics





Approach	Advantages	Disadvantages	Applications
Bulk	- Provides an overall view of gene expression in a cell population. - High throughput. - Cost-efficient and suitable for large-scale sample analysis.	- Only provides average gene expression profiles, thus failing to capture single-cell heterogeneity. - Does not provide spatial information of tissues.	- Transcriptome profiling. - Tumor diagnosis and prognosis. - Biomarker discovery, identification of functional genes, and studies on large sample cohorts.
Single cell	- Enables gene expression analysis at the single-cell level. - Allows tumor heterogeneity analysis. - Identification of unique cell types and rare cell populations.	- Higher cost. - Limited sensitivity and dependent on advanced techniques. - Does not provide spatial tissue information.	- Analysis of tumor and tissue heterogeneity. - Identification of cell types and evolutionary transitions. - Understanding immune responses and tumor microenvironment characteristics.
Spatial	- Provides spatially localized transcriptomic information. - 10X spatial transcriptomics technology offers higher cellular resolution. - High gene detection capability with high multiplexing.	- High cost and workflow complexity. - Relatively new technology still under development.	- Spatial transcriptomics analysis. - Emerging technology for tumor microdissection. - Spatial biomarker discovery. - Optimization of immunotherapy.

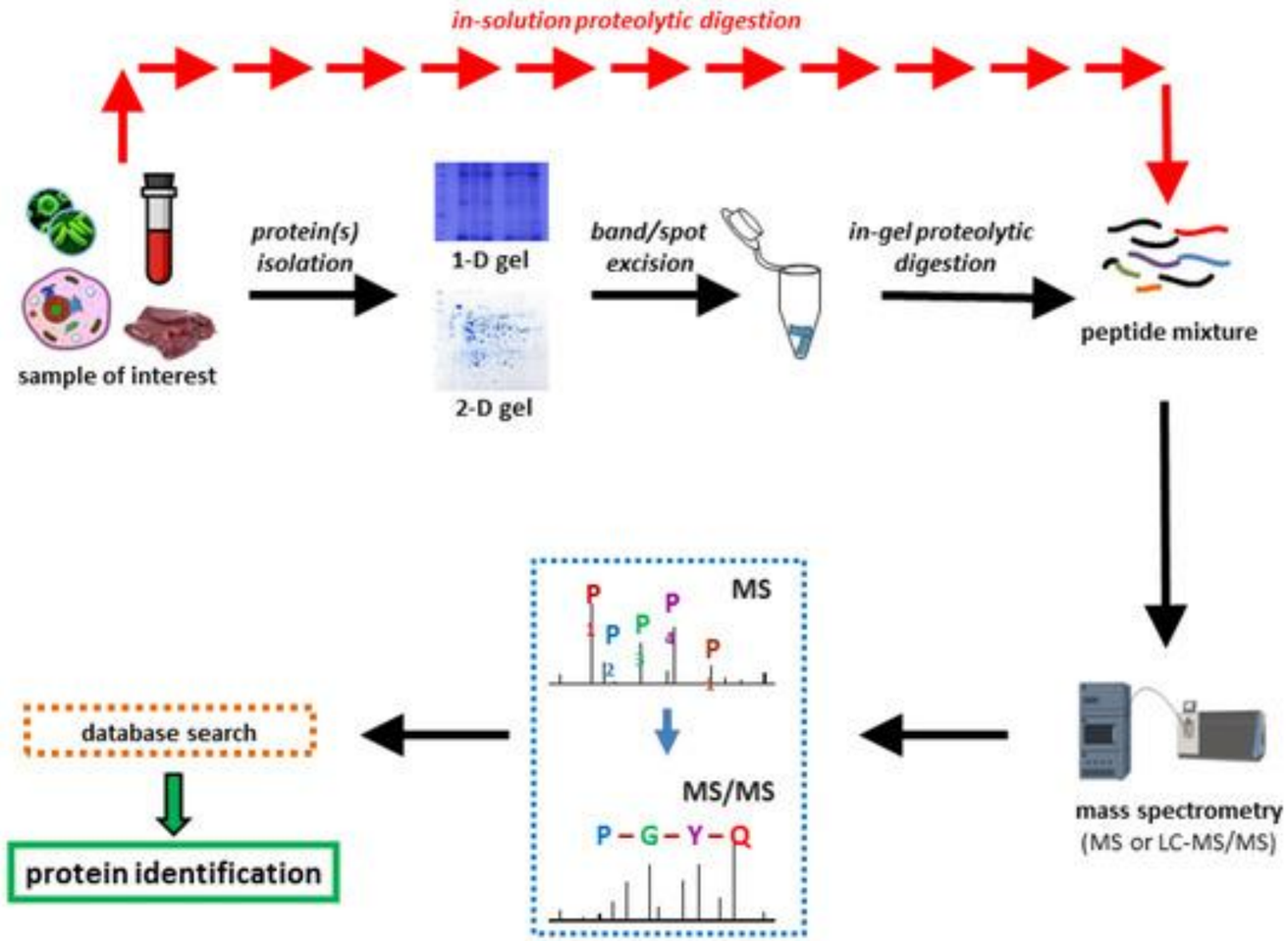
Co-funded by the European Union

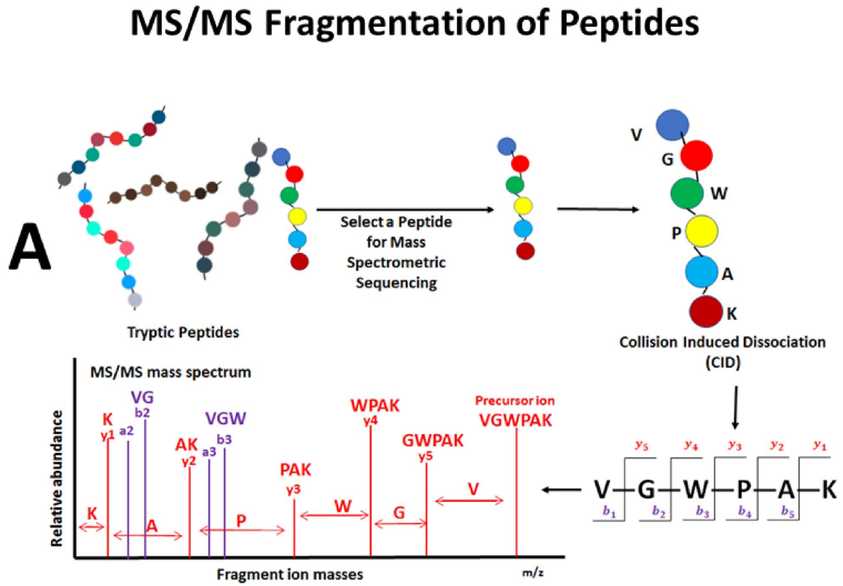
Steinheuer et al. 2021; Ospina et al. 2023; Yan et al. 2024

Proteomics

Proteomics

- **Proteomics** is the study of all proteins (the proteome) within an organism, including their variations due to post-translational modifications (PTMs), protein-protein interactions, and expression levels.
  Bottom-up proteomics is the most widely used primary approach, in which proteins are digested into small peptides and then analyzed using liquid chromatography and mass spectrometry for protein identification and quantification.



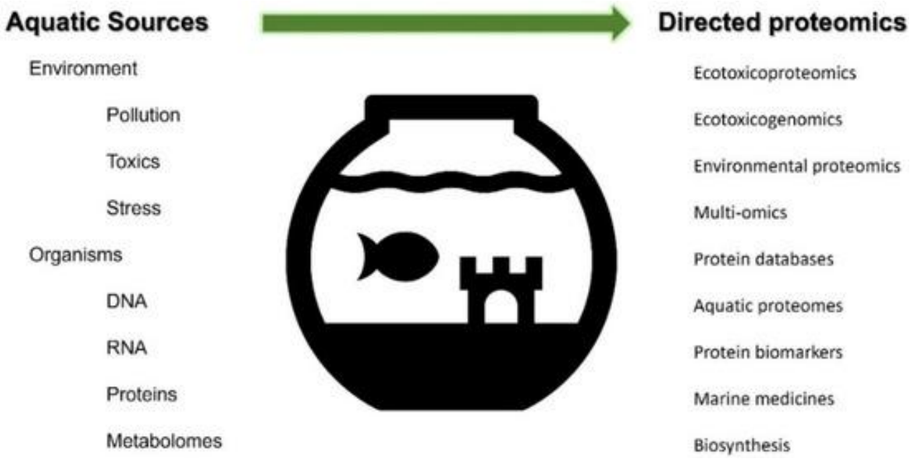


Co-funded by the European Union

Dupree et al. 2020

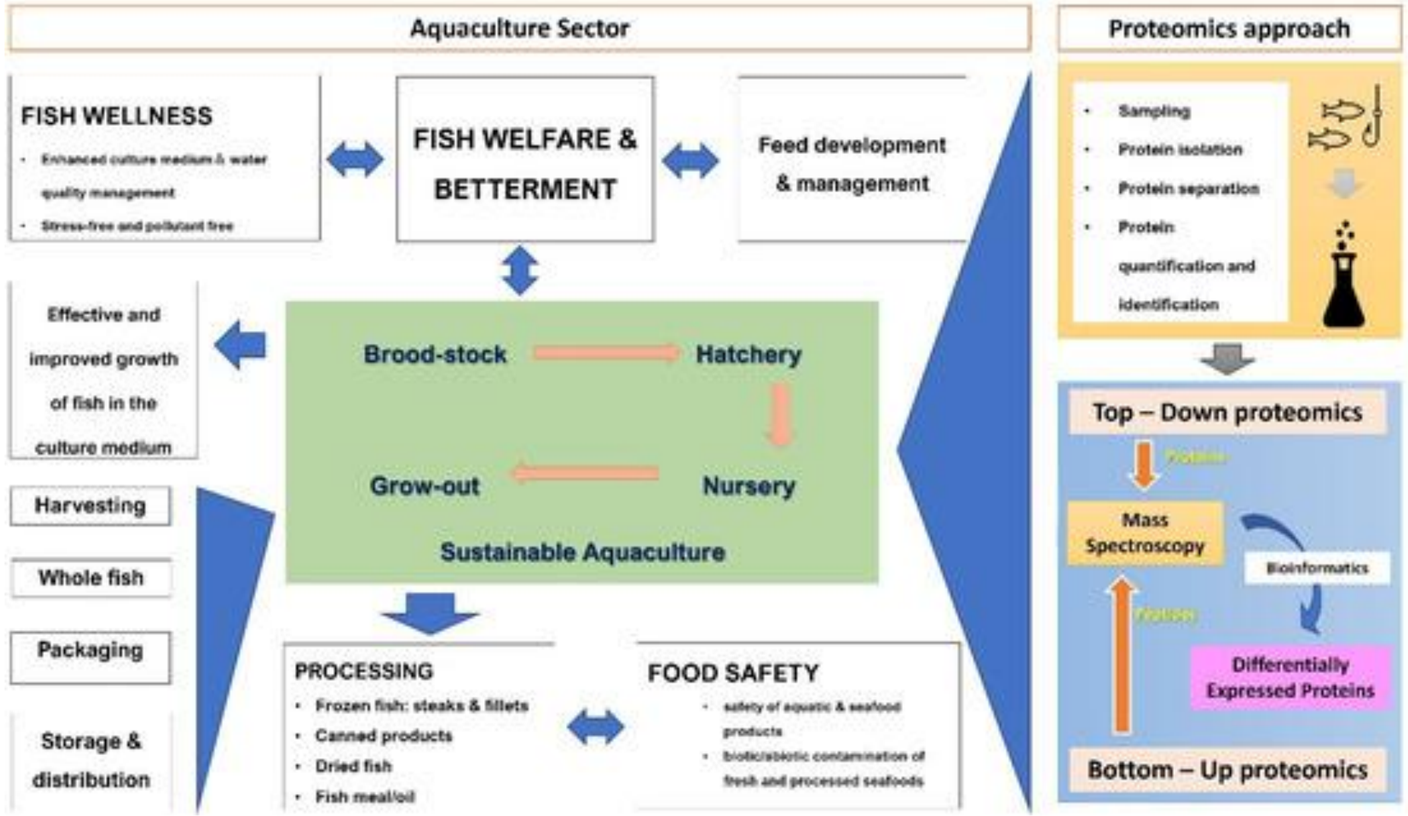
Proteomics

Proteomics in Biodiversity Study



There are three main areas of proteomic research in the context of biodiversity:

- **Aquaculture and fisheries**: Assessing fish growth, stress, and health; developing efficient and environmentally friendly fish feed; ensuring food safety; and more.
- **Environmental pollution monitoring** and its effects on biodiversity.
- **Identification of marine natural products and pharmaceuticals** (natural compounds).



Several proteomic techniques that can be used:

- 2D-Gel Electrophoresis
- LC-MS/MS
- Label-based (iTRAQ, TMT) and label-free quantification
- MALDI-TOF MS

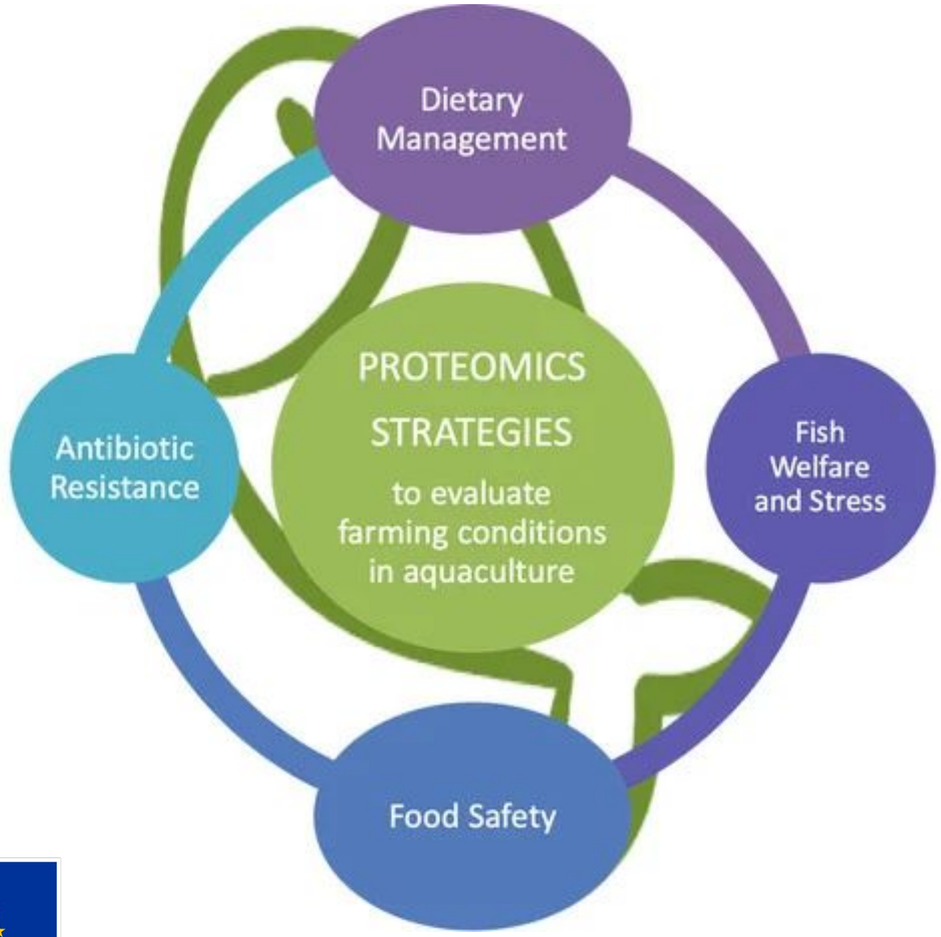
A major challenge: the lack of a universal extraction protocol for all aquatic organisms.

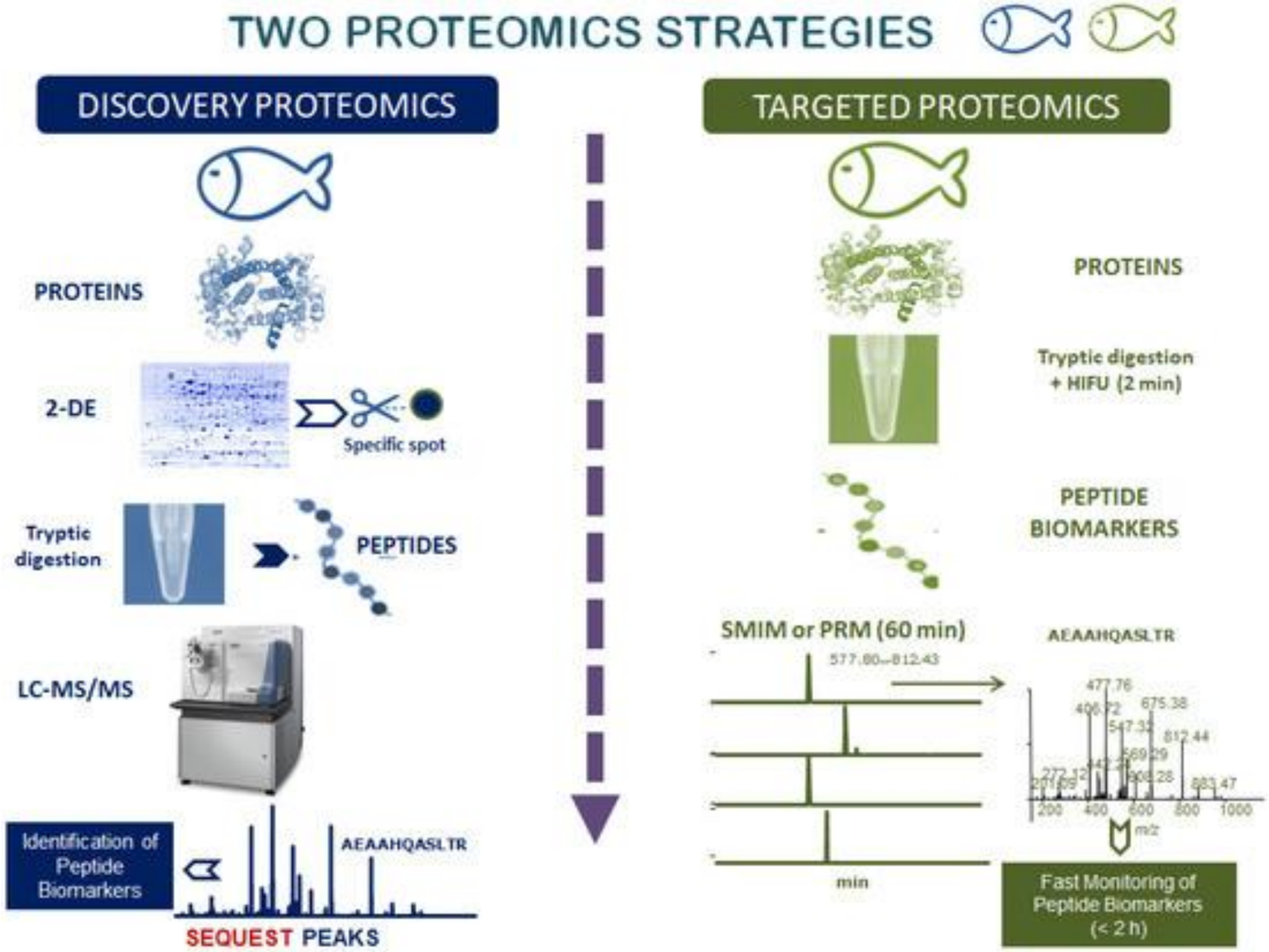
Co-funded by the European Union

Gamage et al. 2022

Proteomics

Proteomics in Biodiversity Studies





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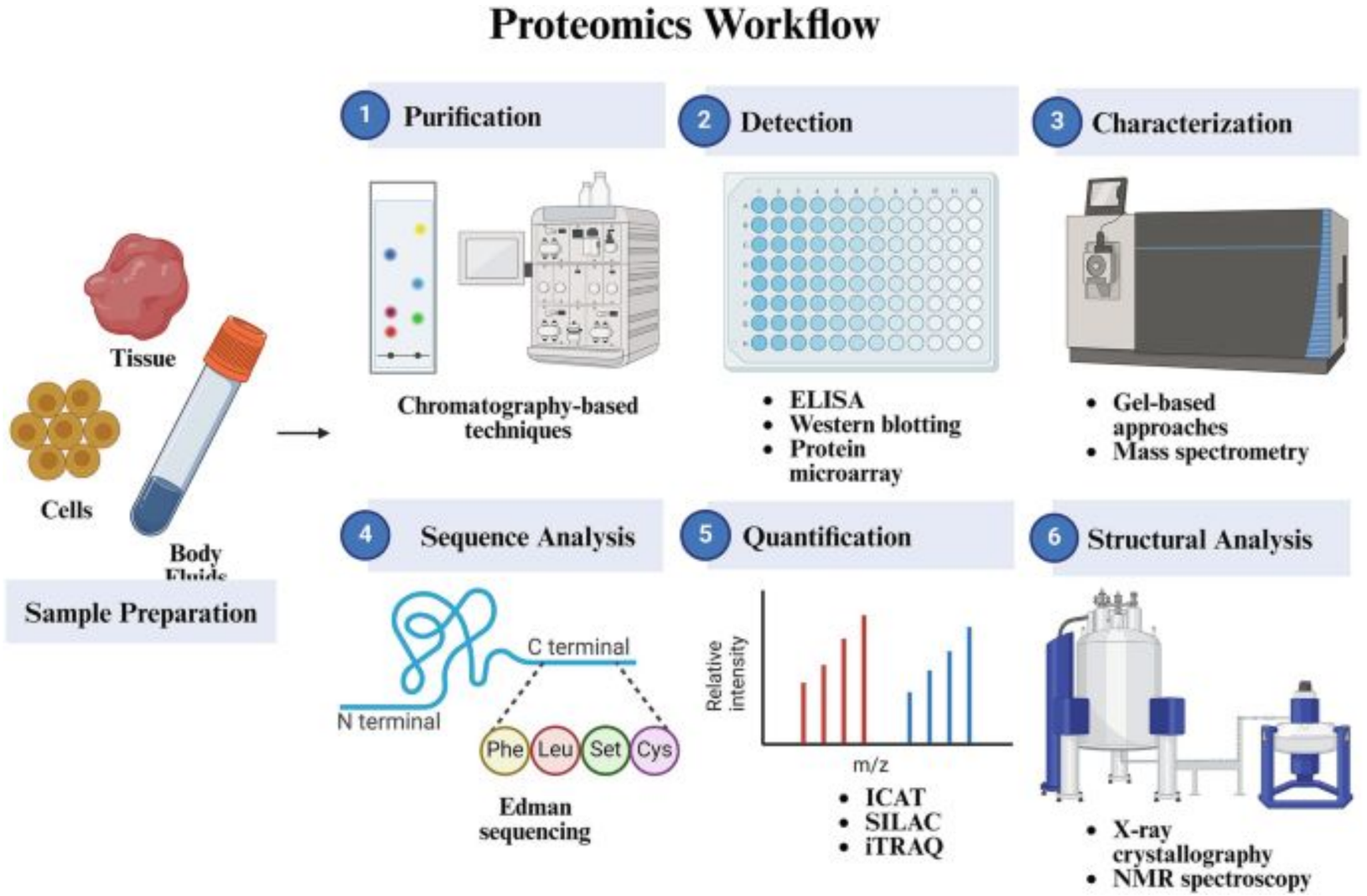
Gamage et al. 2022

Proteomics

Proteomics in Biodiversity Studies



Gamage et al. 2022

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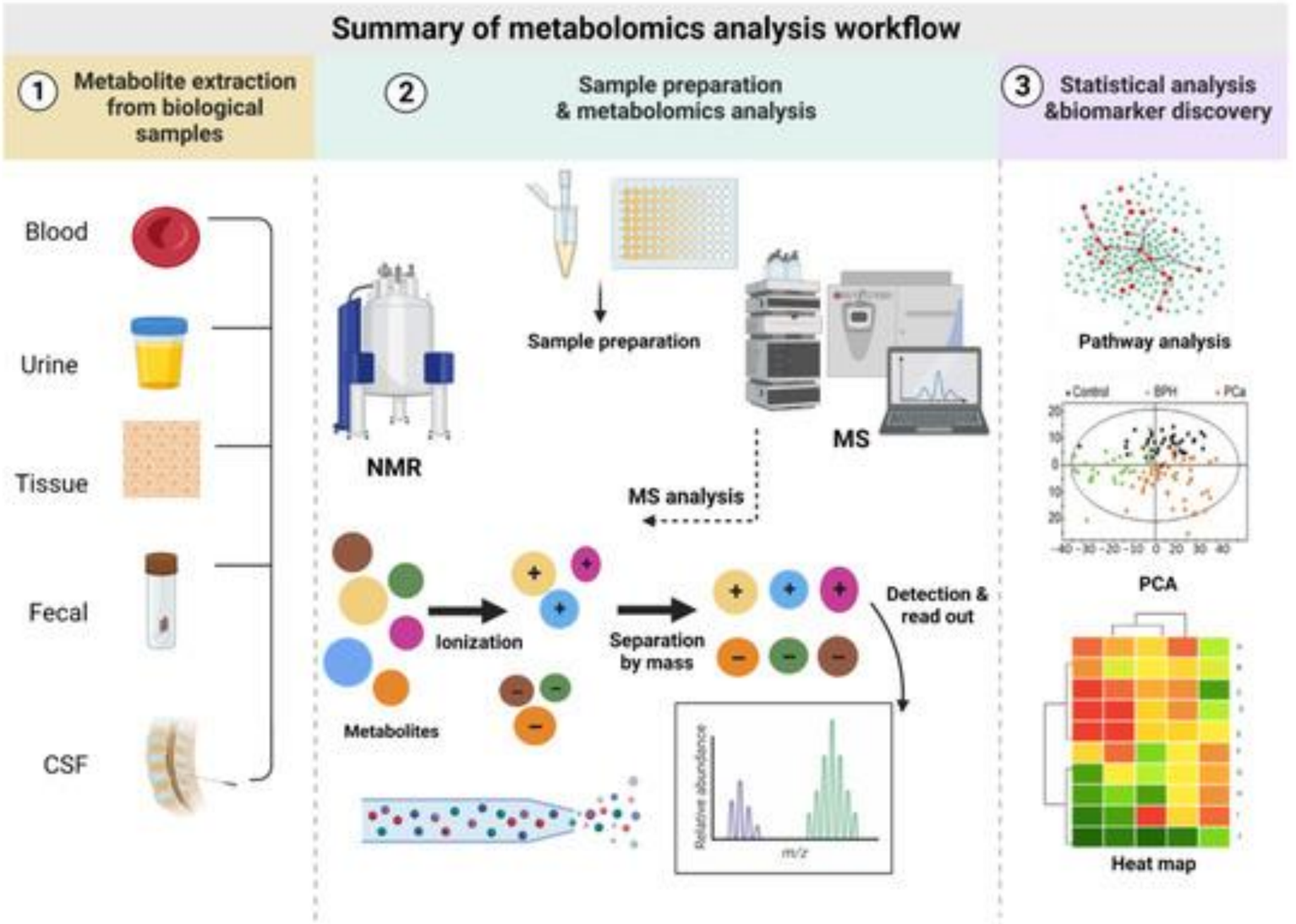
Metabolomics

Metabolomics

Metabolomics is the systematic study of small metabolites in biological samples, providing a direct snapshot of biochemical activity within the body.
It can be used to develop novel biomarkers.



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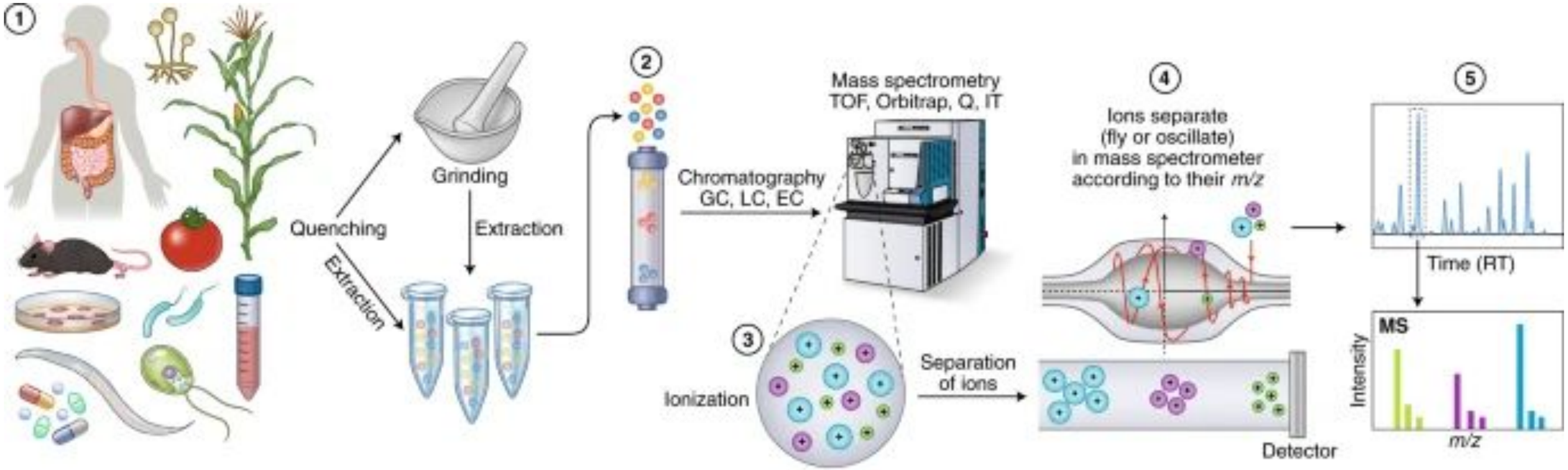
Al-Sulaiti et al. 2023

Metabolomics

Metabolomics



Sample preparation and extraction

- Avoid environmental perturbation during harvesting
- Control environment: harvesting at the same time and under the same conditions
- Snap-freezing in liquid nitrogen
- Enzyme quenching: completely terminate all enzyme activities
- Standards spiked into the quenching solvent
- Grinding, isolation of cells, fast-filtration or aspiration

Sample replication and randomization

- At least four biological replicates, preferably more
- Technical and analytic replicates are worthy of consideration
- Randomization of samples throughout workflows is essential
- In large-scale studies, quality-control samples and batch correction are essential

Chromatography–mass spectrometry

- Separation methods, composition of the mobile phase, column properties and injection volume
- Metabolites are within their range of detection
- Avoid ion suppression: dilution of extracts, sonication, filtration or centrifugation, recovery test
- Choosing ionization source and type of detection mode, MS method, scan number and speed, MS/MS and energy for fragmentation

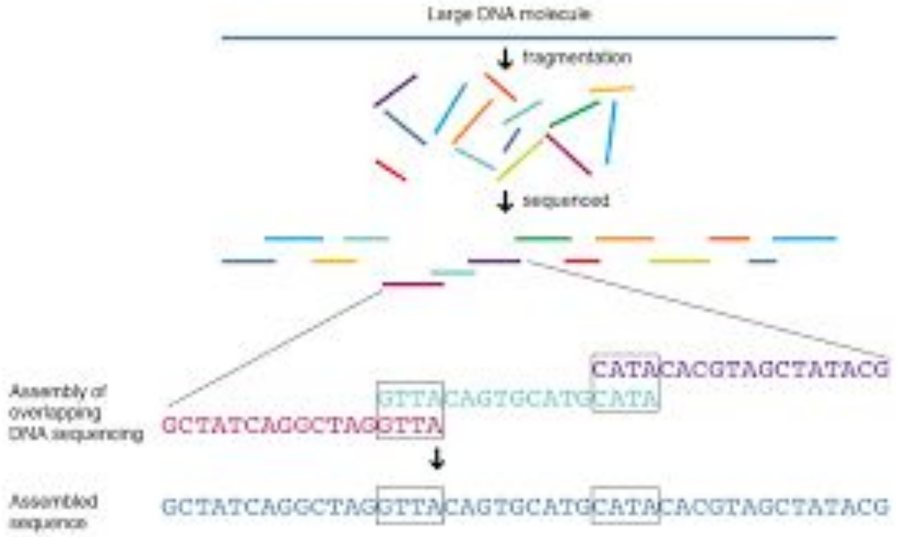
Co-funded by
the European Union

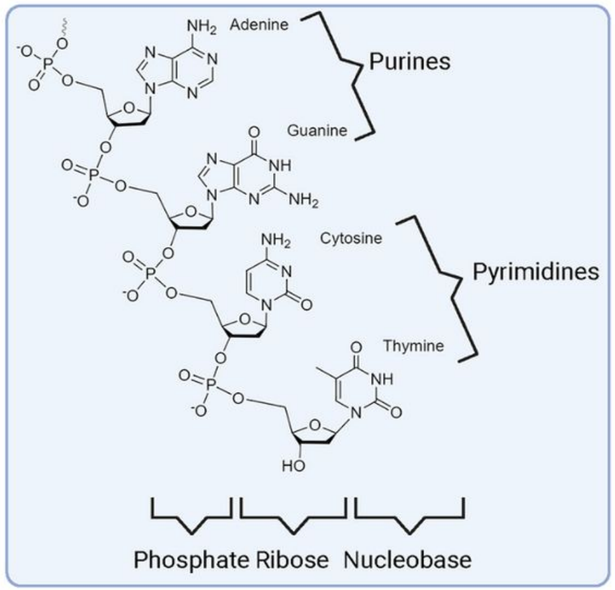
Alseekh et al. 2021

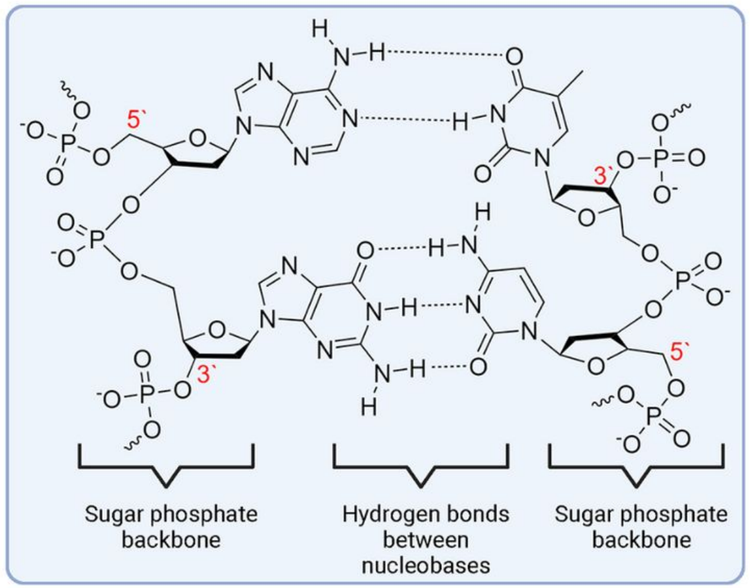
Next-Generation Sequencing

Sequencing Principle

- An **approach to determine the nucleotide sequence** in a DNA molecule.
  It is used to understand genetic information, identify genes and mutations, and support research in medicine, forensics, evolution, and biotechnology.







Nitrogen base

Purin

Pirimidin

Pasangan basa:

Two hydrogen bonds

Three hydrogen bonds

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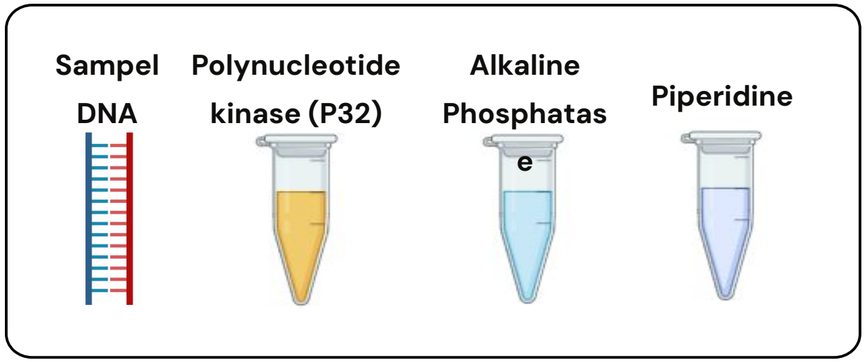
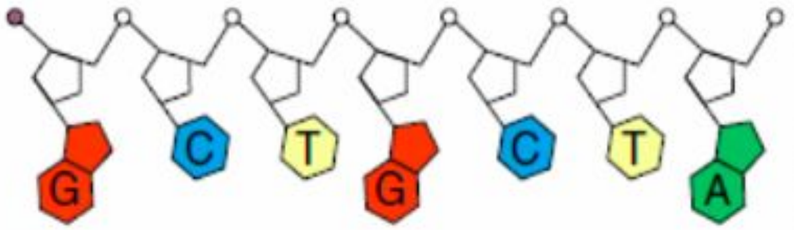
Adkar-Purushothama & Perreault 2020; Rupeika et al. 2024

Next-Generation Sequencing

Maxam-Gilbert Sequencing

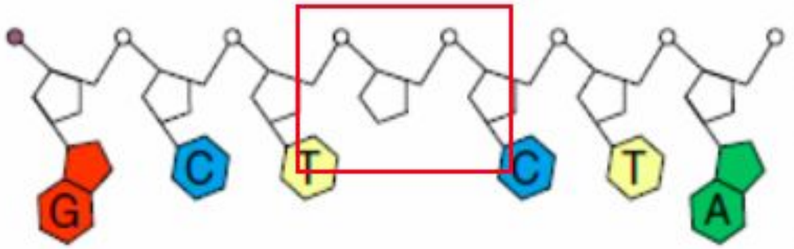


DNA is labeled at one end with P-32

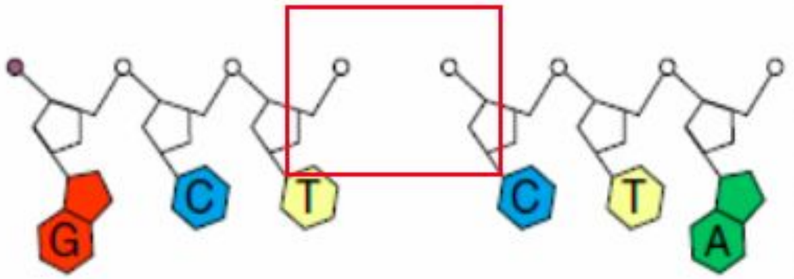


Base modification

Release of reactive bases



Strand cleavage

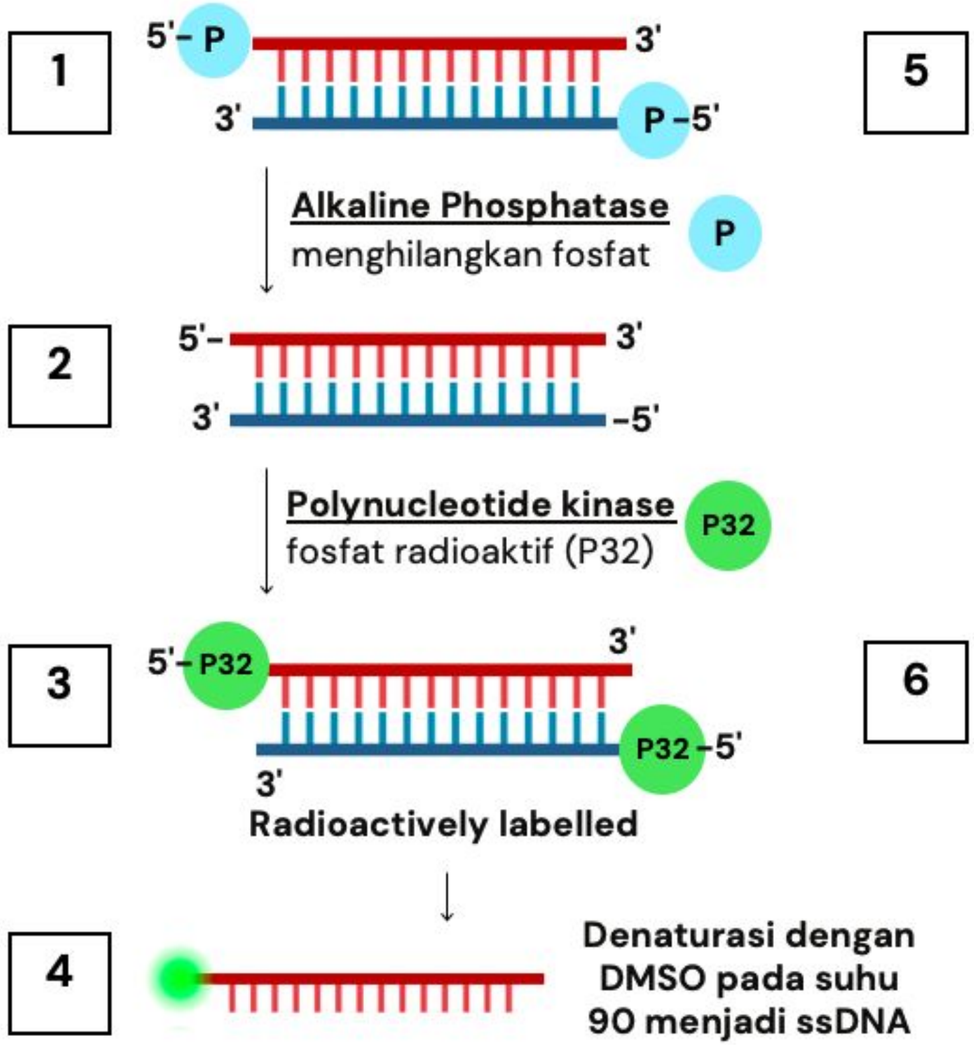


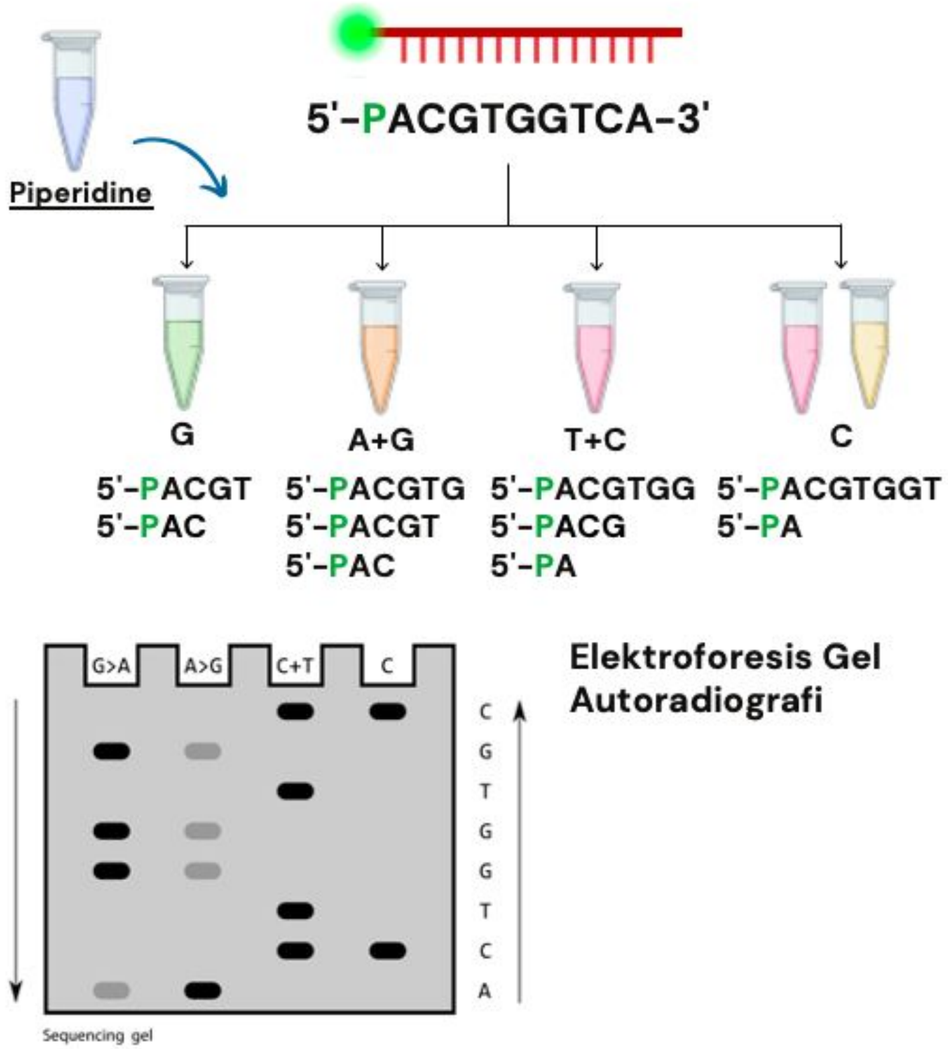
Co-funded by the European Union

Adkar-Purushothama & Perreault 2020; Rupeika et al. 2024

Next-Generation Sequencing

Maxam-Gilbert Sequencing





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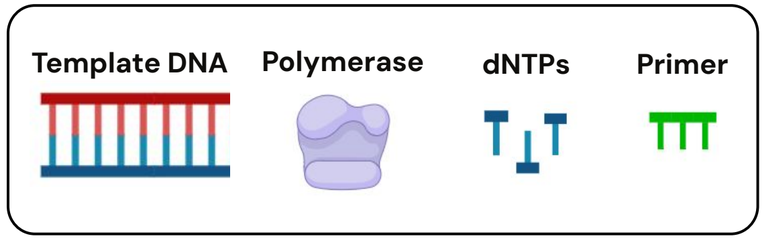
Ungelenk 2021; Eren et al. 2022; Madhumitha et al. 2024

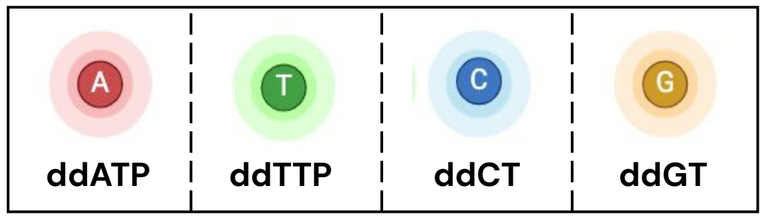
Next-Generation Sequencing

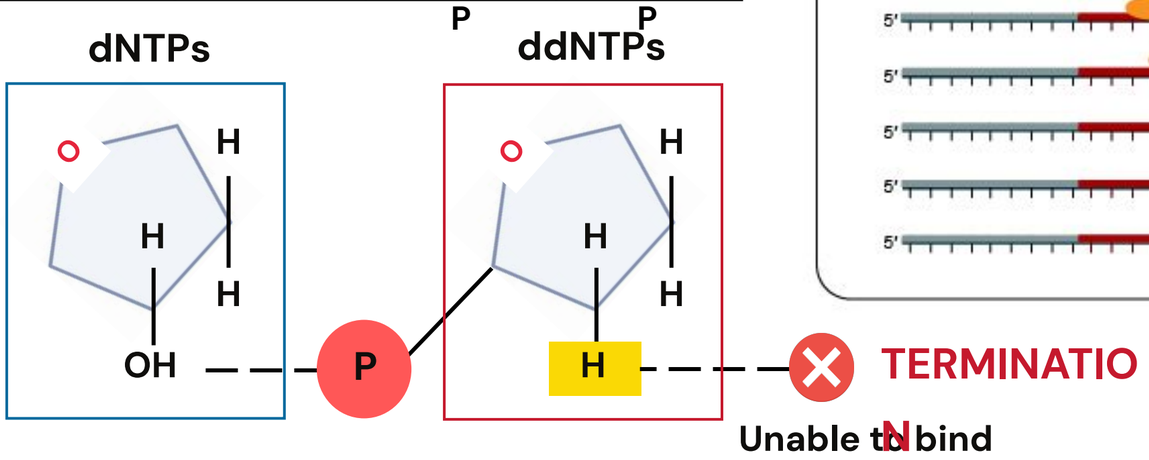
Sanger Sequencing

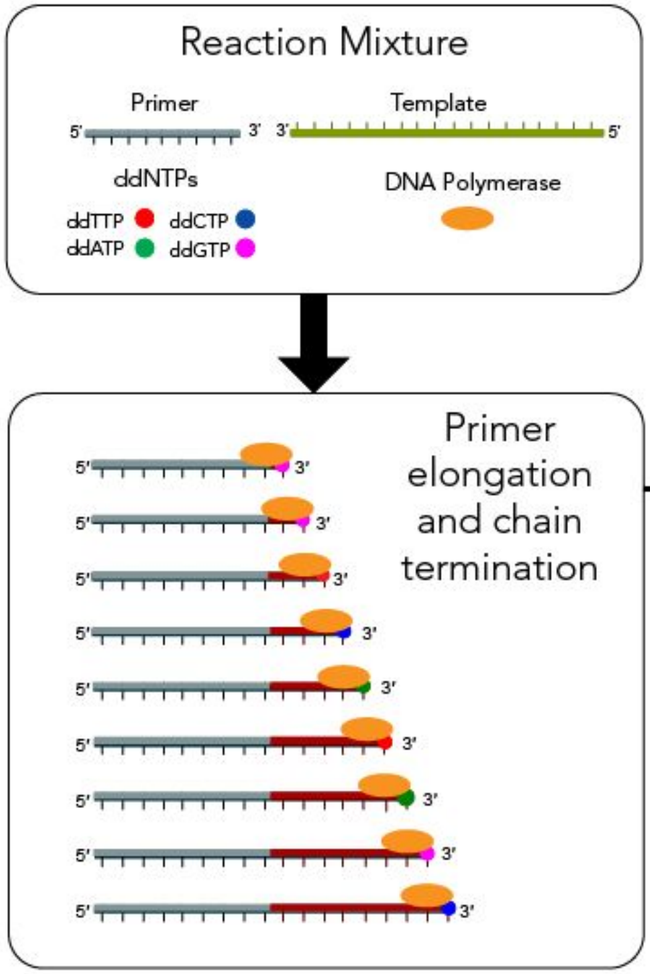
Component

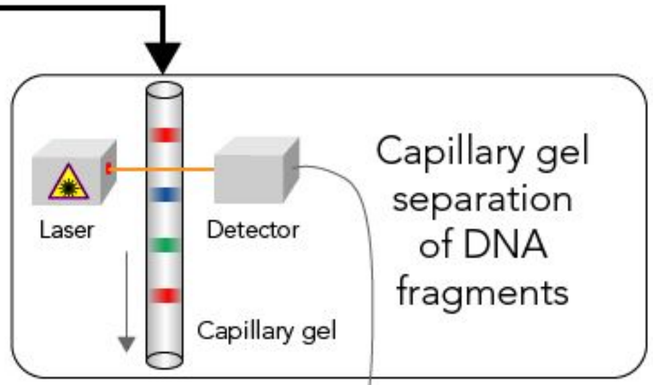


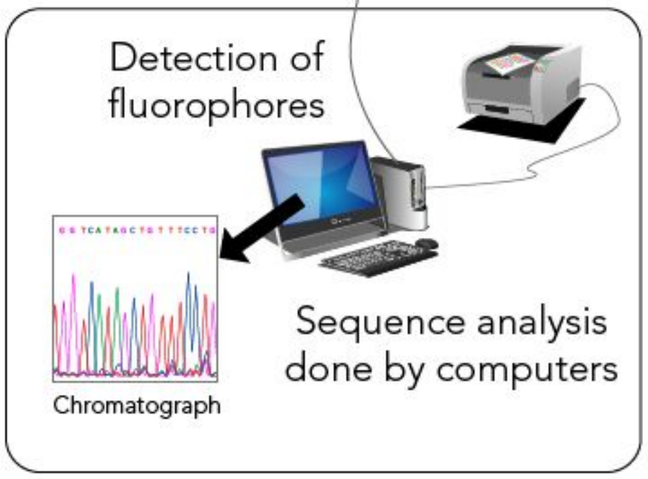
dideoksinukleotida (ddNTPs)











Co-funded by the European Union

McGovern 2015; Katara et al. 2024; Nafea et al. 2024

Next-Generation Sequencing

Next-Generation Sequencing

Step 1:

DNA Extraction

Step 2:

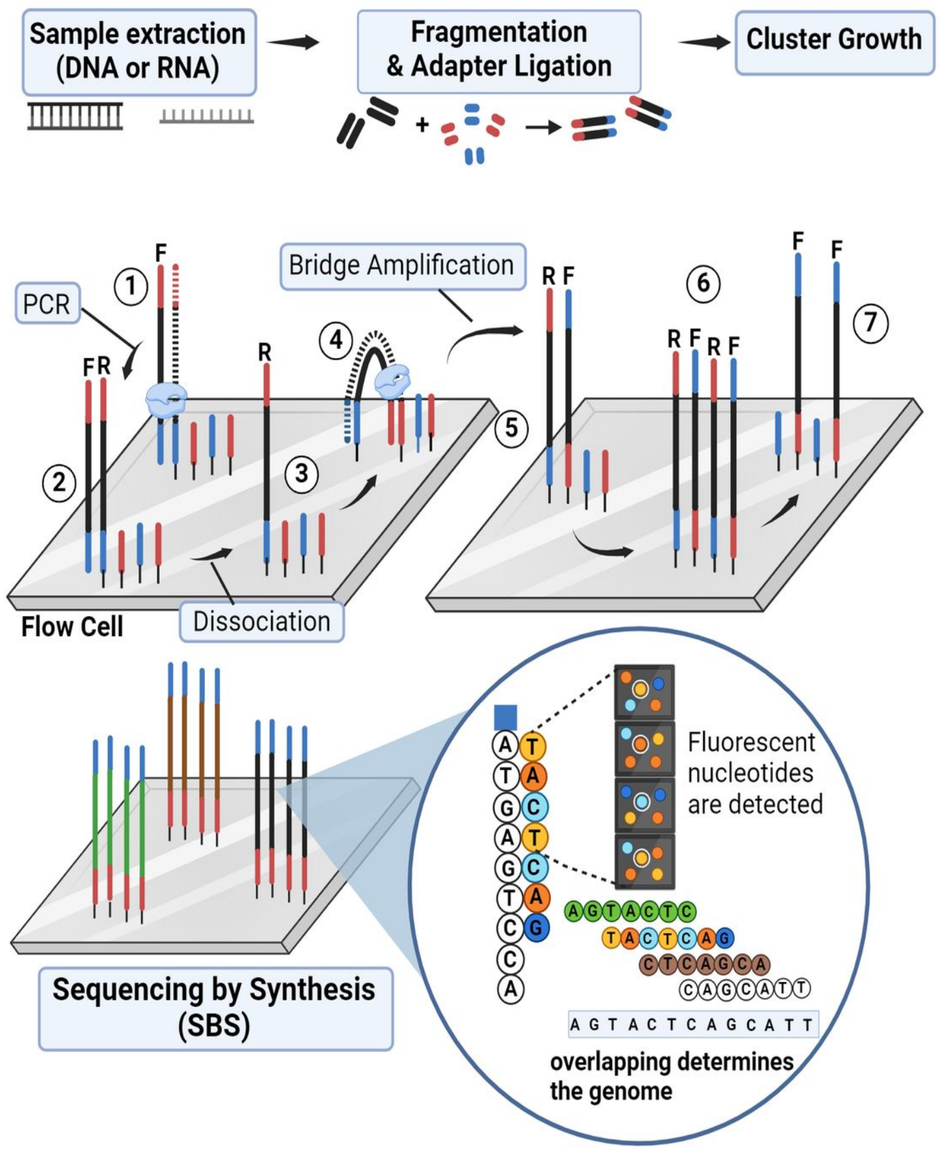
Library Preparation

Step 3:

Sequencing

Step 4:

Analysis



DNA fragments (+adapters) attach to complementary oligonucleotides on the flow ..

The reverse strand is synthesized, then the double-stranded DNA is denatured, and the original

The DNA strands are amplified through bridge amplification; the strand folds, and its free end binds to the nearest oligonucleotide, forming a double-stranded bridge, which is then denatured again.

After each amplification cycle, a laser scans the flow cell to activate fluorescent labels on the nucleotide bases.

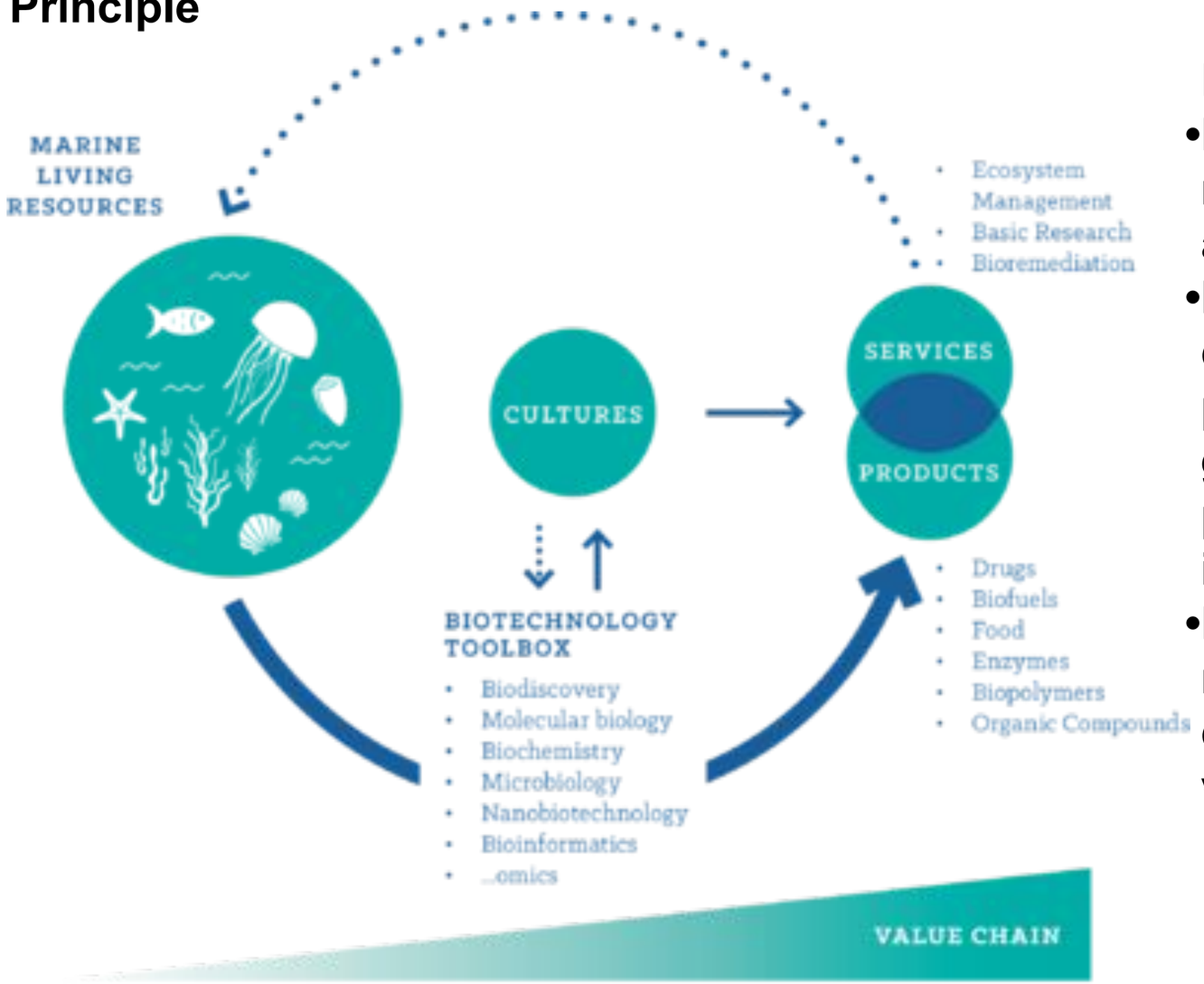
The light is detected by a computer, and the overlapping bases will determine the genome sequence.

Co-funded by the European Union

Young & Gillung 2019; Rupeika et al. 2024

Marine Biotechnology

Marine Biotechnology Principle



Coastal Wiki 2016; Rotter et al. 2021

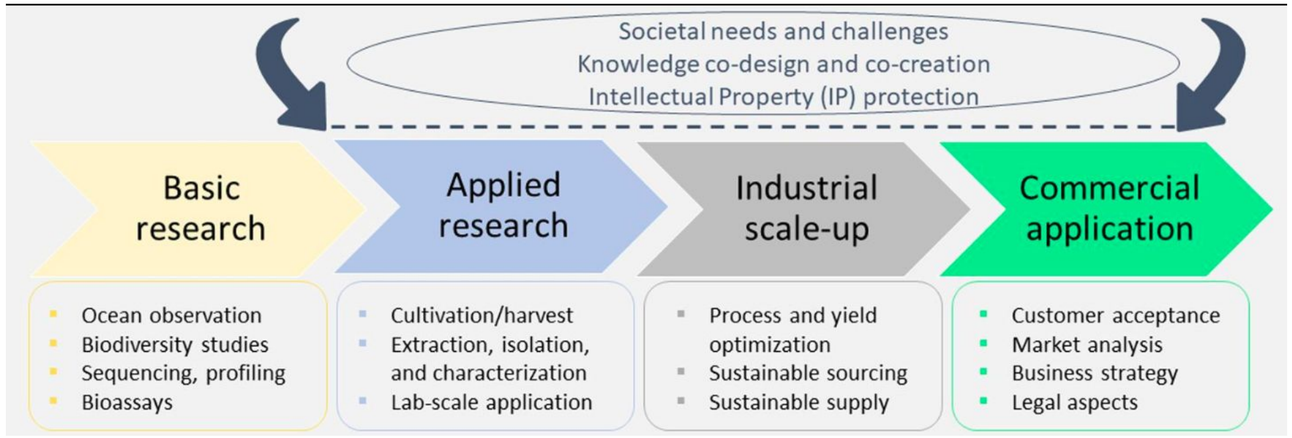
Key Concepts

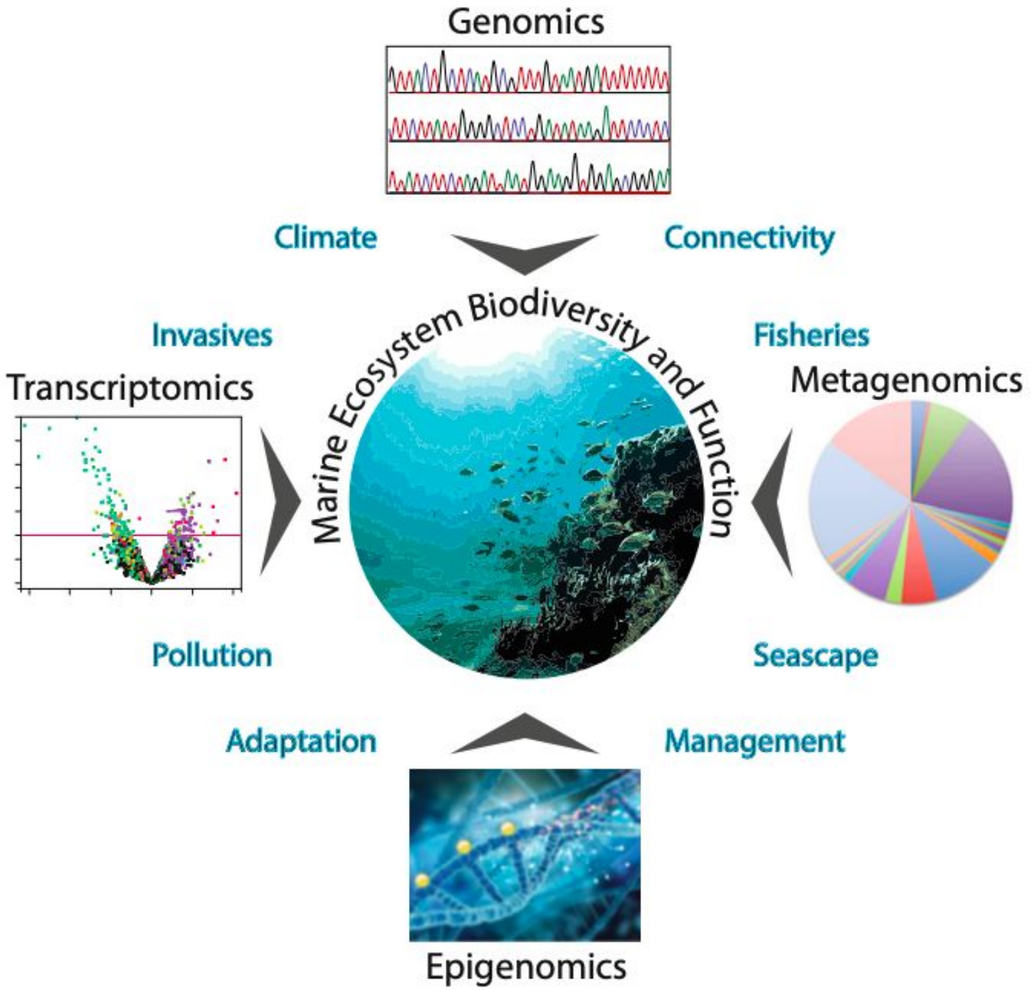
- **Bioeconomy** → the use of biological resources for sustainable production and energy.
- **Marine products** → bioactive compounds, enzymes, and polymers produced by marine organisms hold great potential for use in pharmaceuticals, cosmetics, food ingredients, and more.
- **Bioprospecting** → the exploration of marine organisms to discover compounds or genes with practical value.

Co-funded by the European Union

Marine Biotechnology

Marine Biotechnology Principle





Marine Biotechnology Workflow

- Exploration and bioprospecting
- Laboratory testing
- Market need validation
- Co-design and co-creation
- Public engagement
- Production scaling and commercialization

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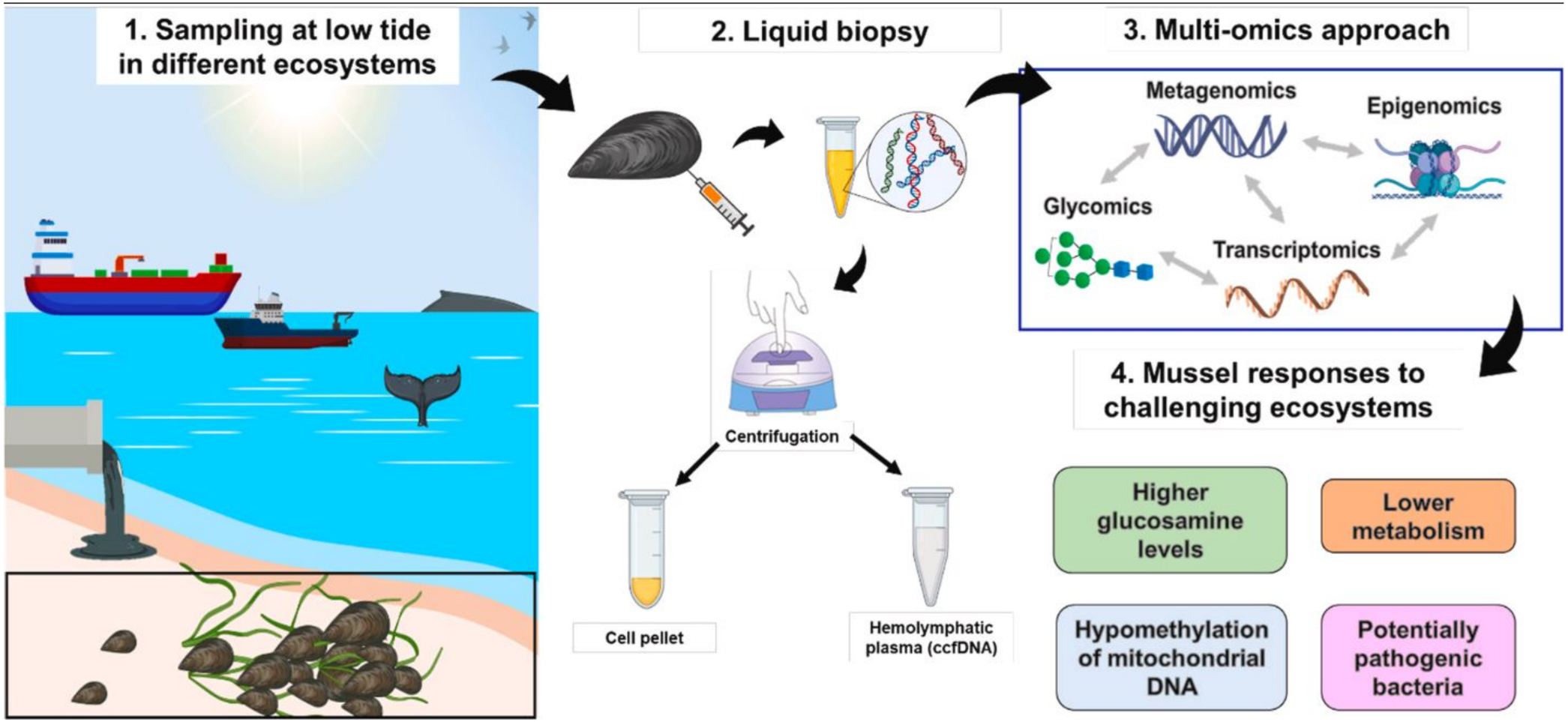
Coastal Wiki 2016; Rotter et al. 2021

Marine Biotechnology

Marine Biotechnology Principle



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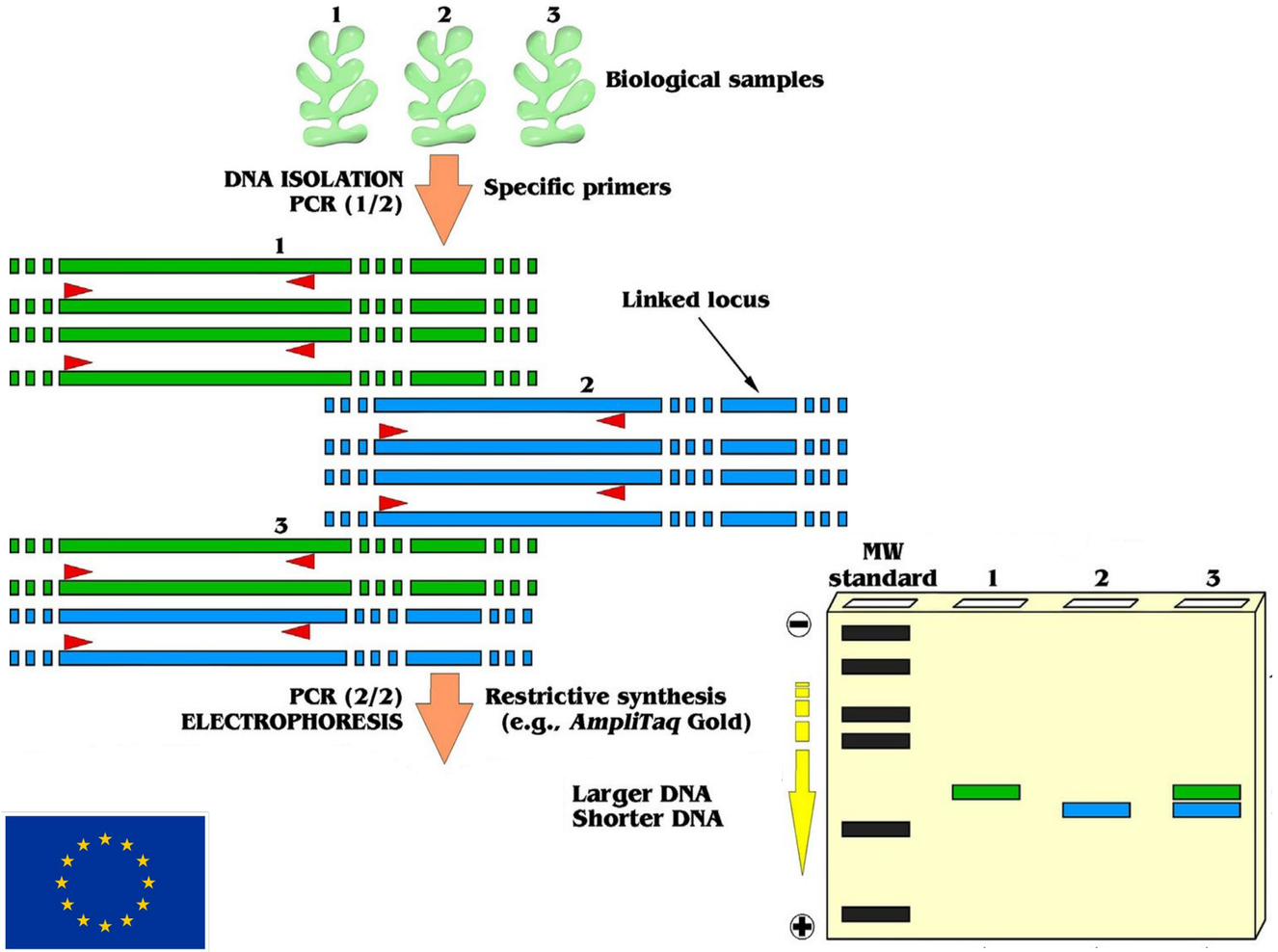
Coastal Wiki 2016; Rotter et al. 2021

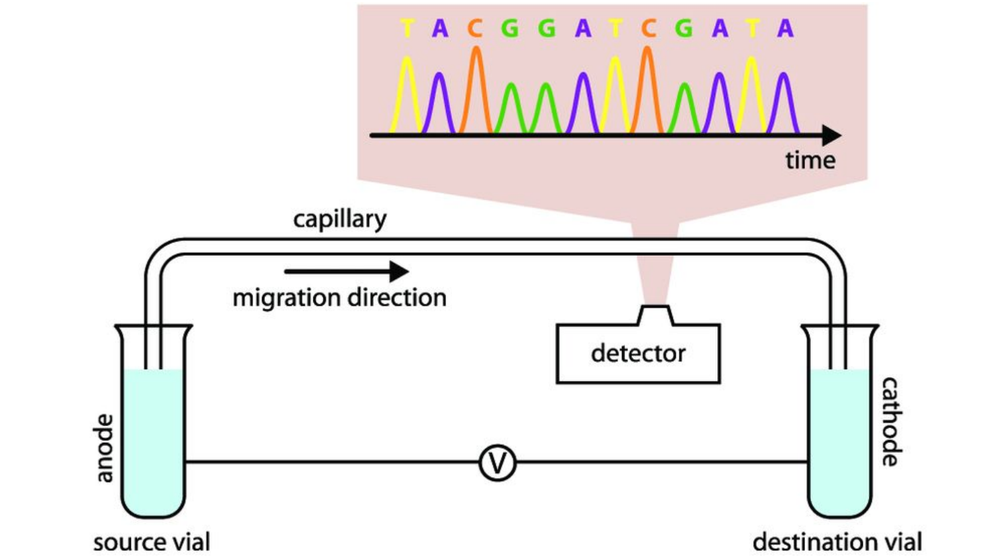
Marine Biotechnology

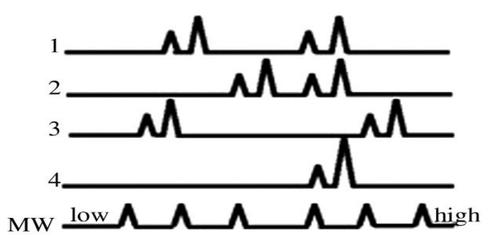
Microsatellite-based diversity detection

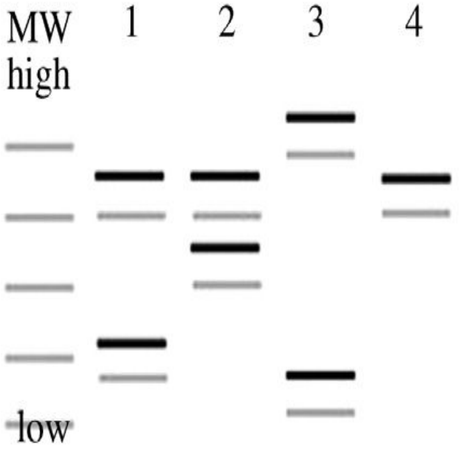
Polyacrylamide Gel Electrophoresis



Capillary Electrophoresis







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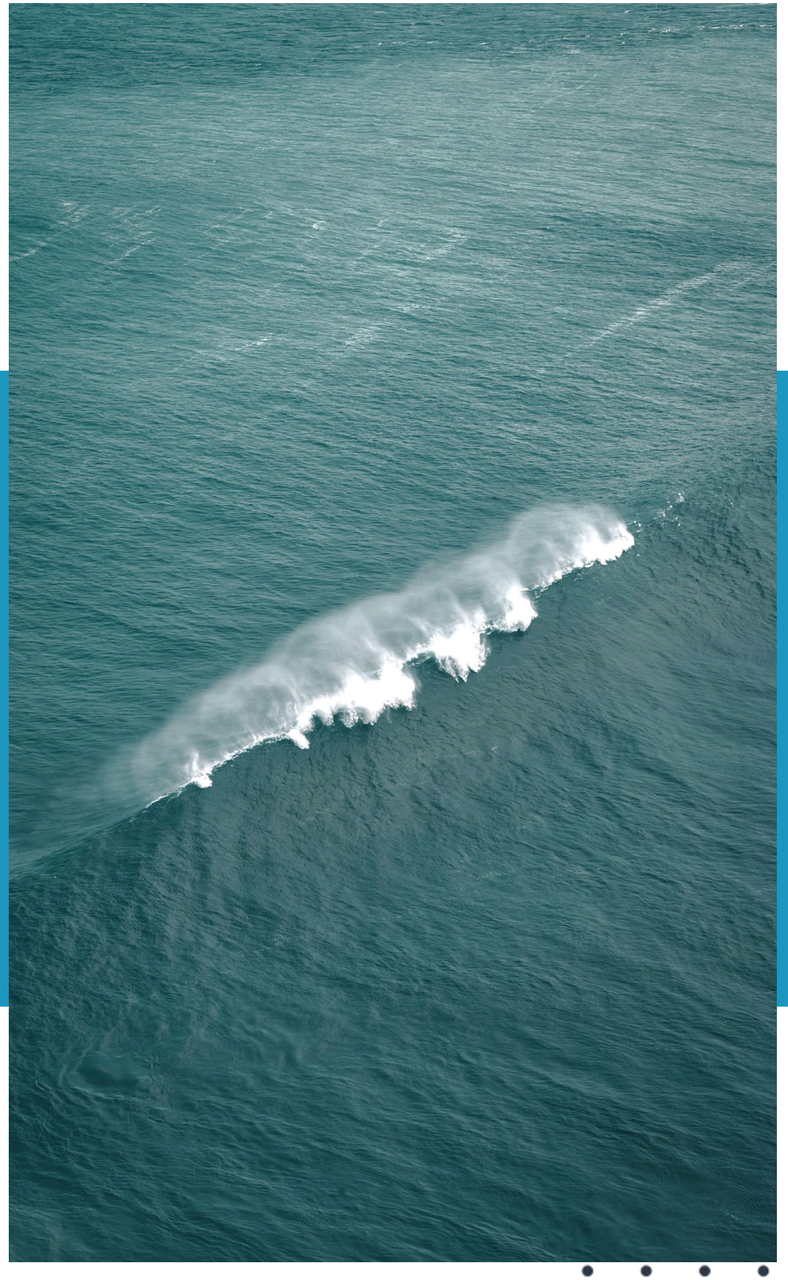
Dorado et al. 2015; Marwal & Gaur 2020

SustainaBlue
HEIs stands for Higher Education Institutions

02

DNA Barcoding Technology in Species Identification and Biodiversity Analysis



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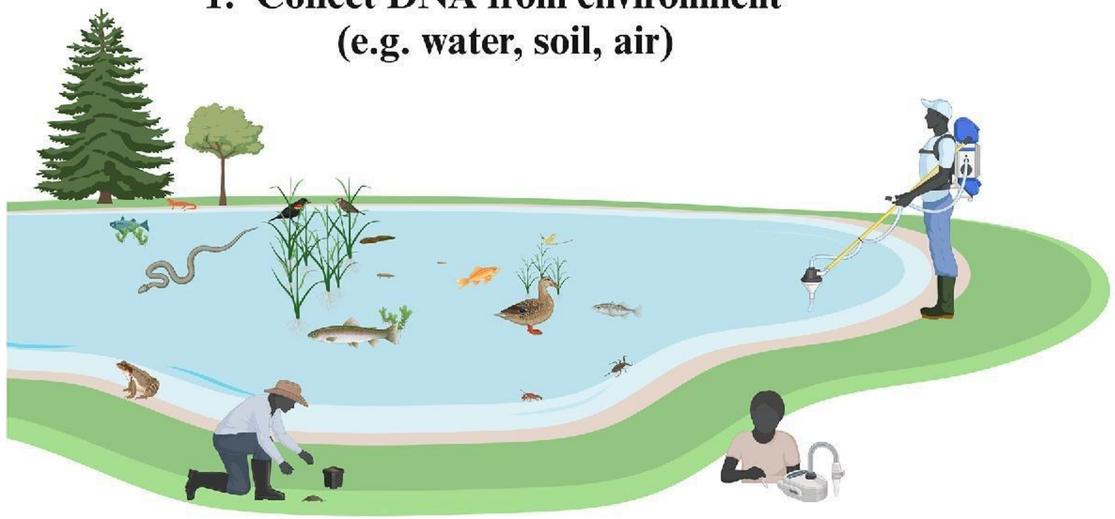
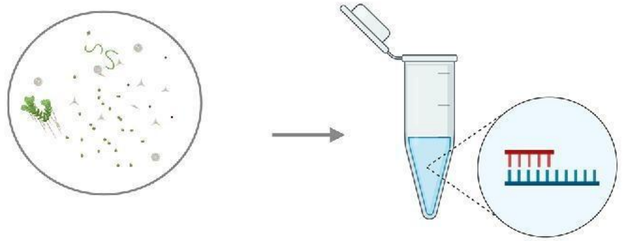
DNA *Barcoding*

Marine Biodiversity Exploration

1. Collect DNA from environment (e.g. water, soil, air)



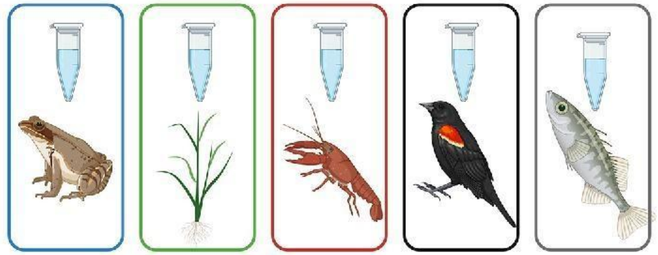
2. Extract eDNA from sample



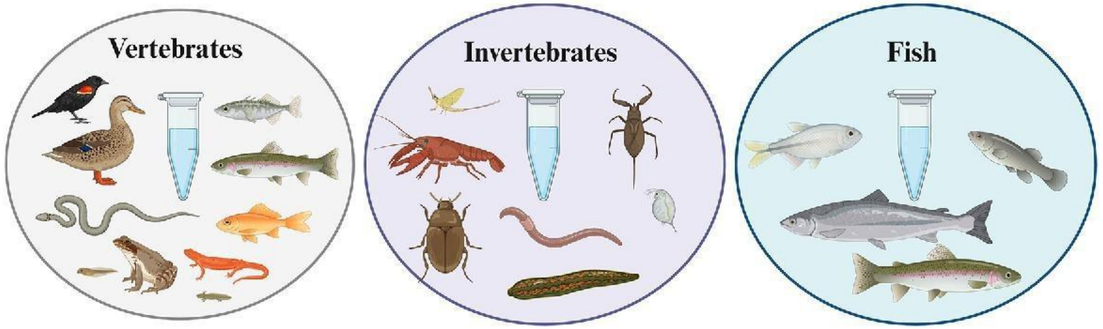
Environmental sample

eDNA in buffer

3. Analyze eDNA



Option 1: Targeted detection of a single species (qPCR)



Option 2: Targeted community detection (DNA metabarcoding)

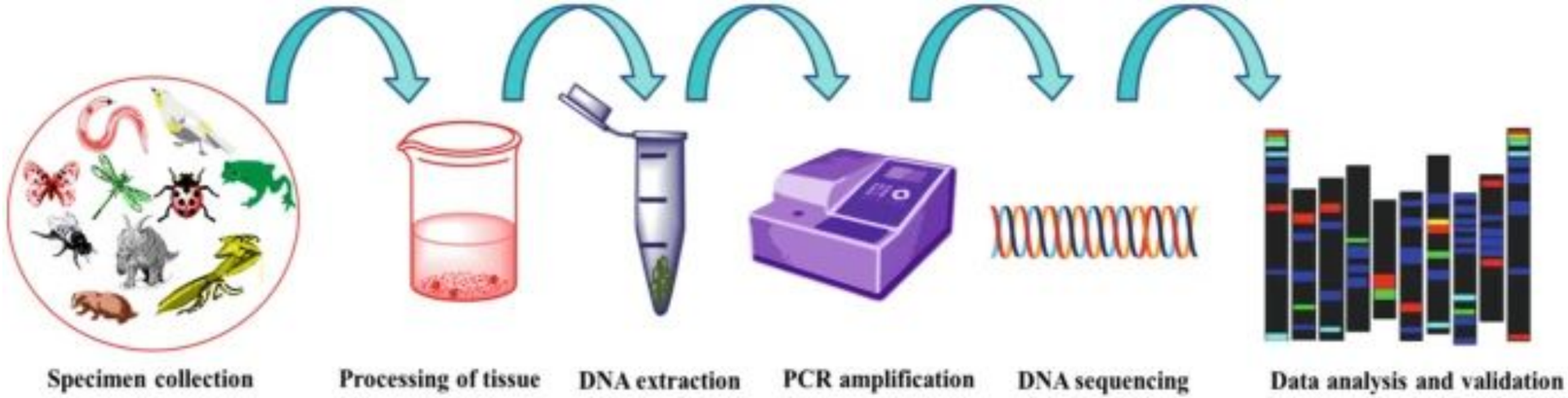
Co-funded by the European Union

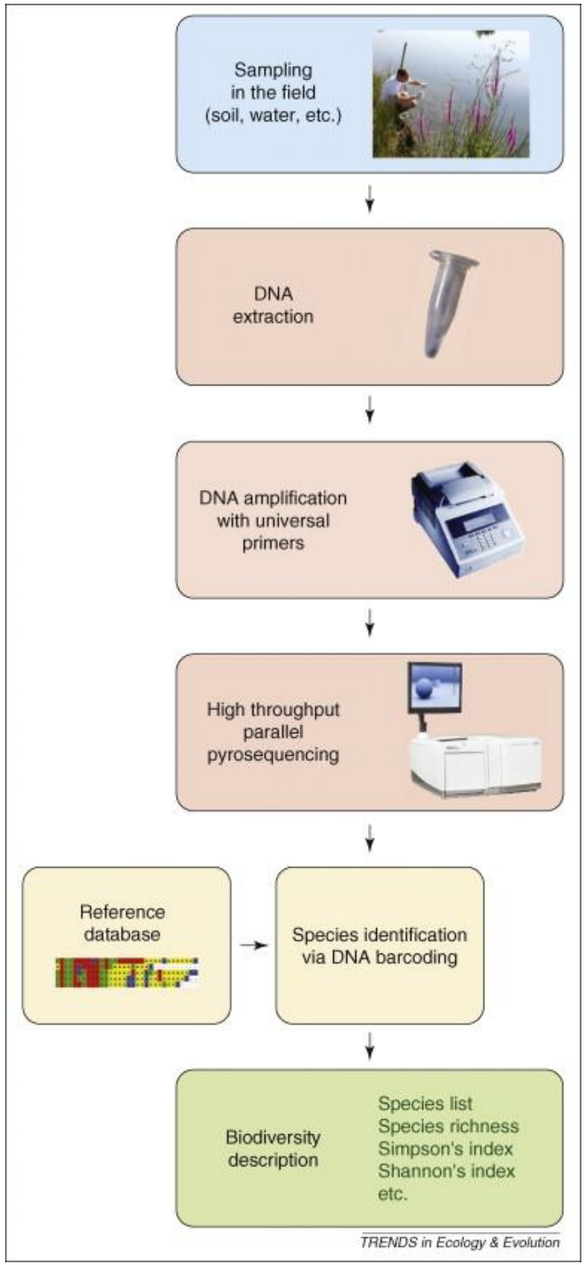
Nolan et al. 2024

DNA *Barcoding*

Marine Biodiversity Exploration

DNA barcoding is a method for identifying biological species using short, standardized segments of genetic DNA. This approach relies on specific DNA sequences that are conserved within a species but vary between species.





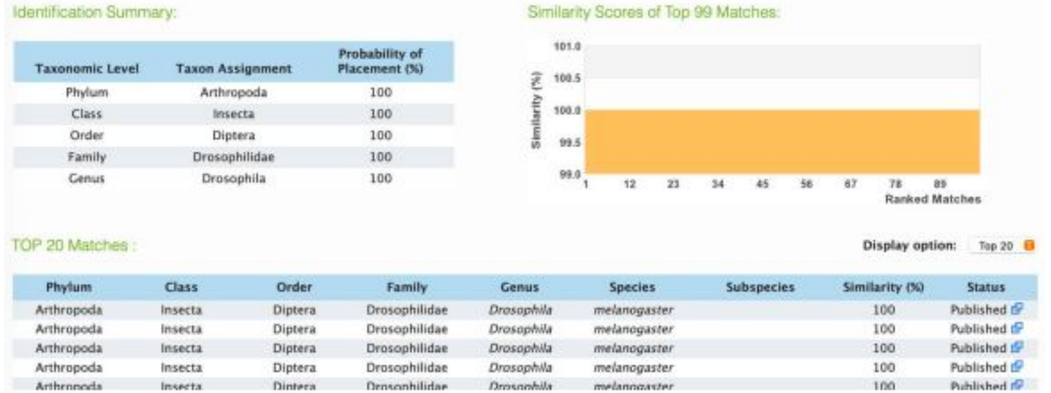
Co-funded by the European Union

Valentini et al. 2009; Suriya et al. 2020

DNA *Barcoding*

Marine Biodiversity Exploration

Animals	Cytochrome c oxidase subunit I (COI)	• Located in mitochondrial DNA (mDNA) • Standard marker for animal DNA barcoding
Plants	• Ribulose-1,5-biphosphate carboxylase large subunit (*rbcL*) • Maturase K (*matK*)	• rbcL and matK are in the chloroplast DNA (cpDNA) • rbcL is widely used for its broad utility across plant taxa. • matK shows greater variation than rbcL, making it useful for species-level identification.
Fungi	Internal transcribed spacer (ITS) region	Located between the 18S, 5.8S, and 28S ribosomal RNA genes
Bacteria	16S ribosomal RNA (16S rRNA)	Part of the bacterial ribosomal DNA.



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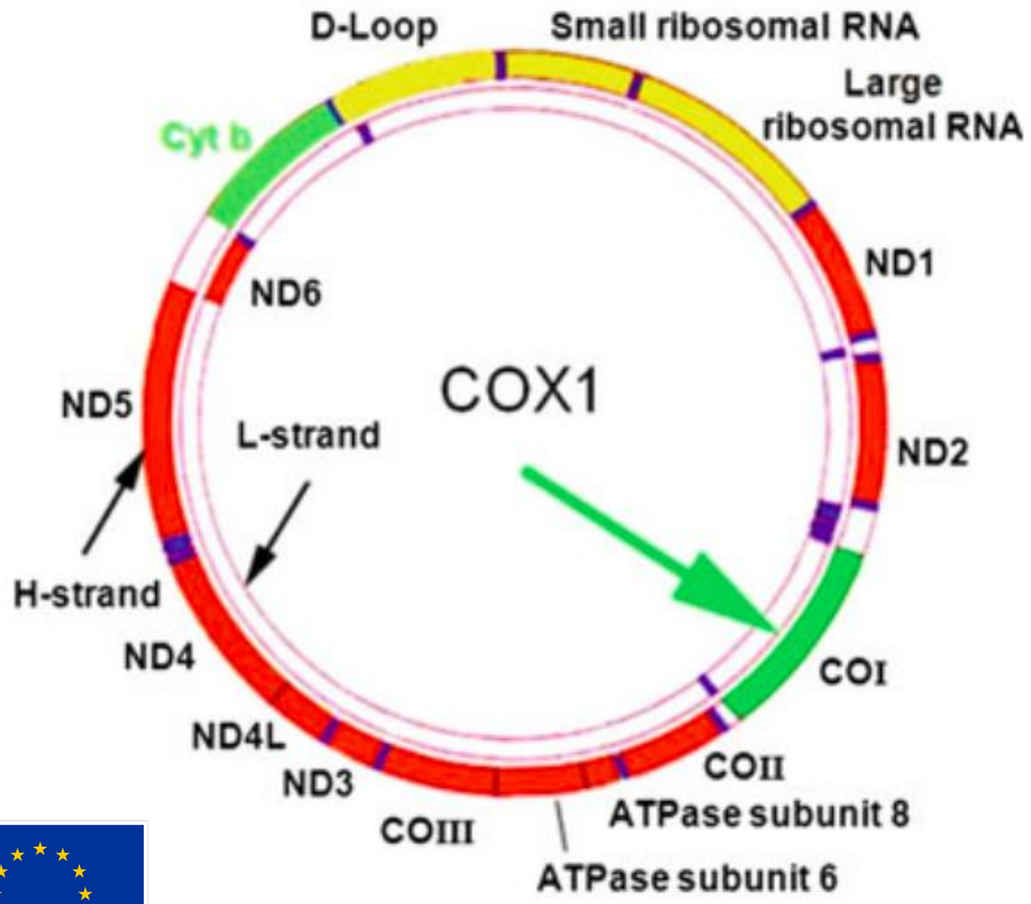
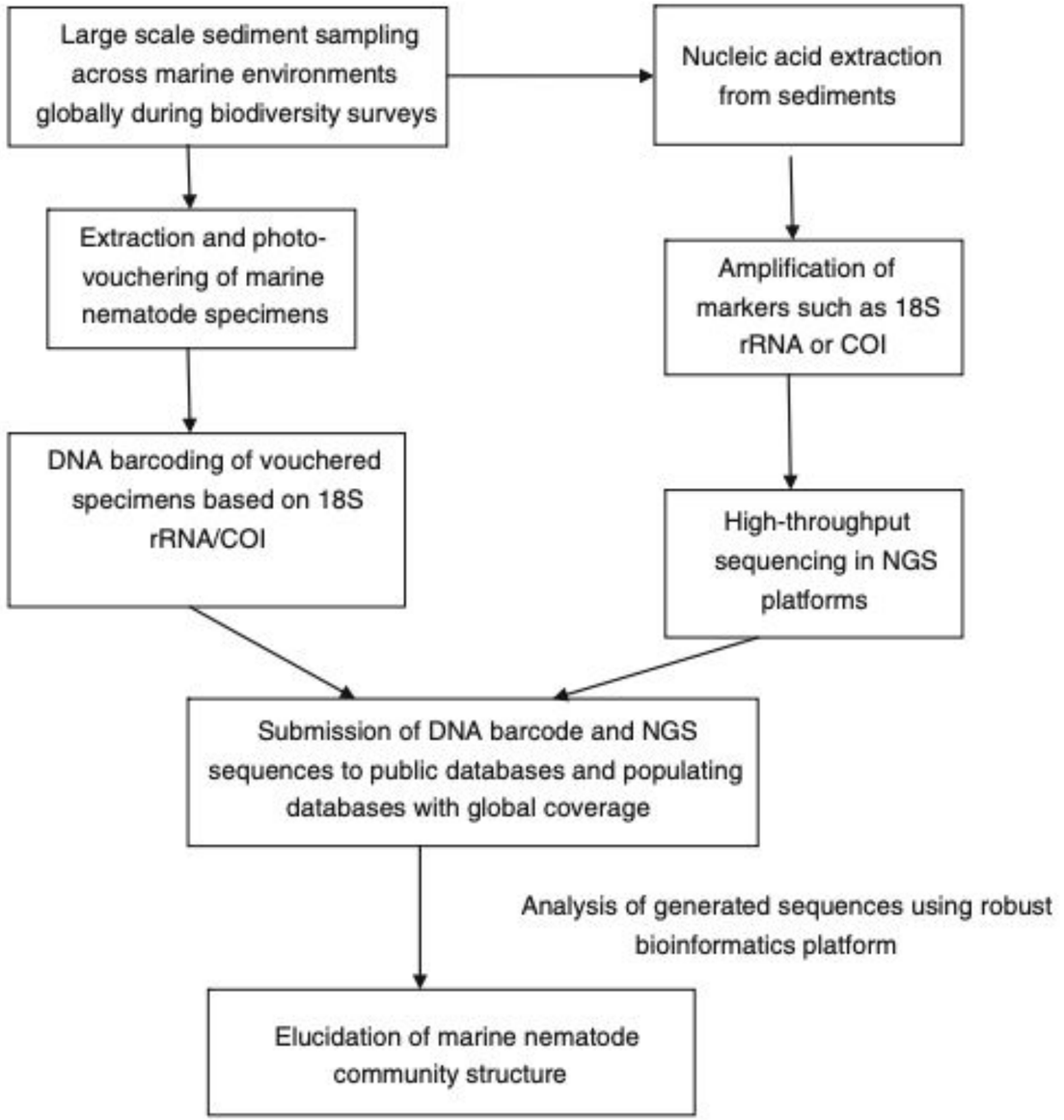
Mahadani et al. 2016

DNA *Barcoding*

Marine Biodiversity Exploration





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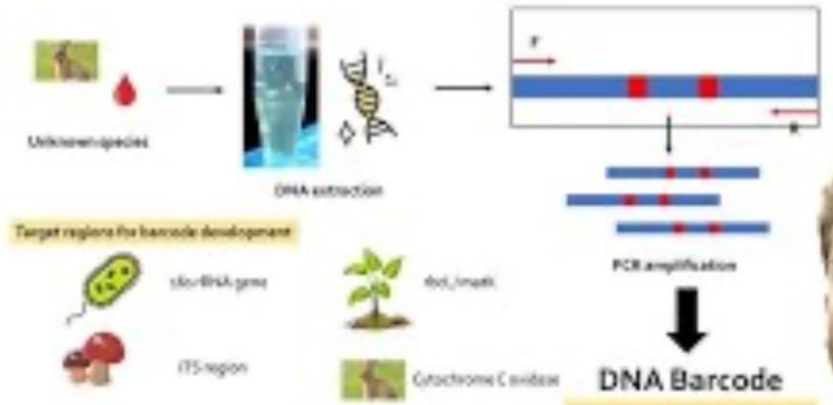
Bhadury 2016

DNA *Barcoding*

How DNA Barcoding works ?



https://youtu.be/HpRHhlbDFd8?si=ESU8z4tHc6F_bxb7

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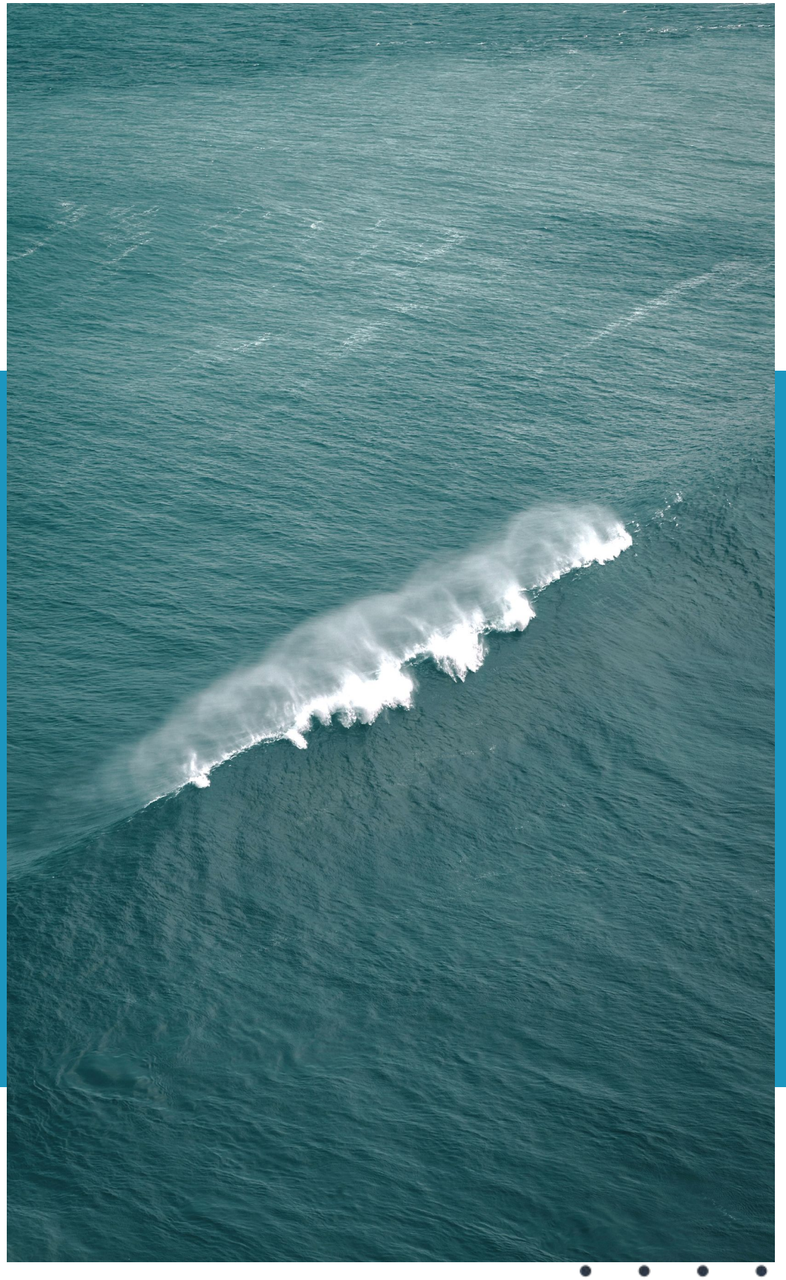
HEIs stands for Higher Education Institutions

03

Applications of Omics and DNA Barcoding in Conservation, Fisheries, and Bioprospecting



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Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health

Stressor	Coral species	Microbial response	Source
Thermal changes	*Acropora muricata*	Shift towards *Verrucomicrobiae- and α-Proteobacteria*-dominated community	Lee et al. (2015)
Pollution/ proximity to shore	*Orbicella faveolata, Porites astreoides; Orbicella annularis*	Increase in bacterial diversity	Morrow et al. (2012); Klaus et al. (2007)
Pathogens	*Diploria strigosa, Siderastrea siderea; Orbicella faveolata*	Increase in *α-Proteobacteria,* decrease in β- and *γ-proteobacteria;* increase in diversity and *Rhodobacterales*	Cárdenas et. al. (2012); Sunagawa et al. (2009)
Eutrophication	*Acropora hemprichii*	Increase in diversity	Jessen et al. (2013)
Salinity	*Fungia granulosa*	Increase in abundance of *Rhodobacteraceae*	Röthig et al. (2016)

The sensitivity of microorganisms and their ability to show clear changes in community composition and abundance in response to specific stressors highlight their potential as indicators of changes in coral reef ecosystems and coral host health.

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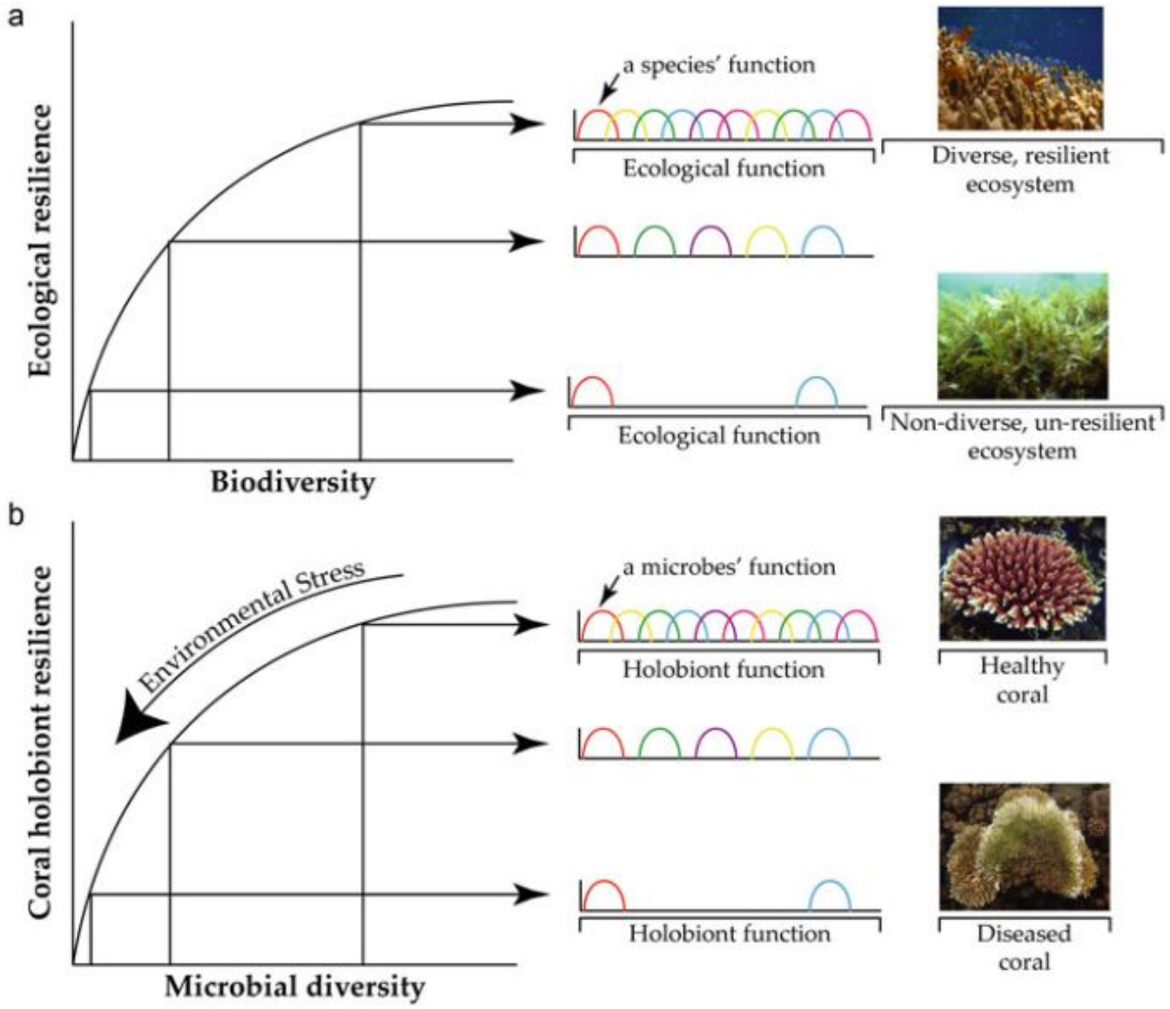
Roitman et al. 2020

Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health



Roitman et al. 2020

Rivet Hypothesis
Biodiversity within an ecosystem creates redundancy and functional complementarity due to the limited number of ecological niches available.
Because of overlapping functions, ecosystems rich in biodiversity tend to be more resilient to change — the loss of one or two species will not significantly impact the overall ecosystem.

The **structure and function of microorganisms** in the **coral holobiont** can reflect the role of each species in a highly diverse system.
The **coral microbiome** can also experience environmental stress and respond to it — leading to reduced diversity and decreased resilience of the coral host.

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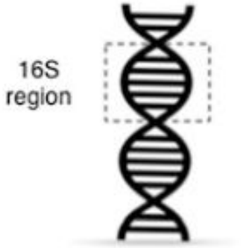
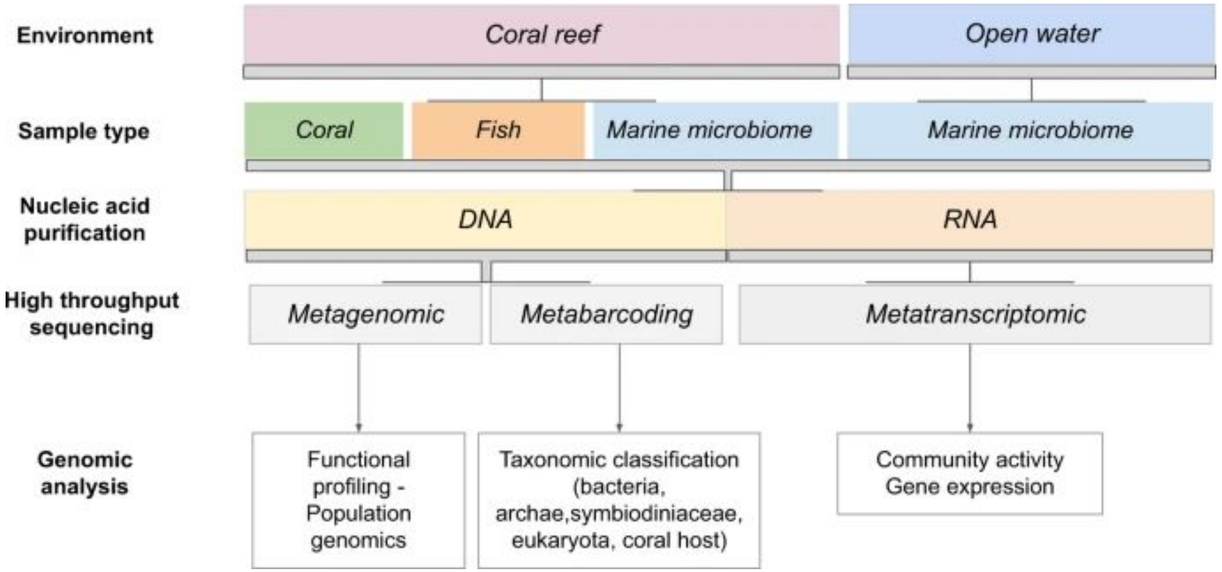
Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health

16S sequencing	Metagenomics	Metatranscriptomics
"Who is there?"	*"What can they do?"*	*"What are they doing?"*
 DNA region	 DNA	 RNA
Advantages: Relatively inexpensive Phylogenetic data **Disadvantages:** Low phylogenetic resolution Poor information on microbial function	**Advantages:** Assessment of entire holobiont Information on functional potential **Disadvantages:** Large amount of host genetic material can swamp microbial sequences	**Advantages:** Information on realized function, gene expression, response to changes **Disadvantages:** Relatively expensive Still a developing field



Various **omics approaches** and **DNA barcoding** can be used to analyze the **biodiversity of coral microbiomes** as a strategy for identifying **coral reef health**.

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Roitman et al. 2020; Belser et al. 2023

Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health

A Multimarker Approach to Identify Microbial Bioindicators for Coral Reef Health Monitoring—Case Study in La Réunion Island

Pierre-Louis Stenger[1,2] · Aline Tribollet[3] · François Guilhaumon[4] · Pascale Cuet[5] · Gwenaelle Pennober[6] · Philippe Jourand[4]

Received: 3 October 2024 / Accepted: 11 January 2025

Abstract

The marine microbiome arouses an increasing interest, aimed at better understanding coral reef biodiversity, coral resilience, and identifying bioindicators of ecosystem health. The present study is a microbiome mining of three environmentally contrasted sites along the Hermitage fringing reef of La Réunion Island (Western Indian Ocean). This mining aims to identify bioindicators of reef health to assist managers in preserving the fringing reefs of La Réunion. The watersheds of the fringing reefs are small, steeply sloped, and are impacted by human activities with significant land use changes and hydrological modifications along the coast and up to mid-altitudes. Sediment, seawater, and coral rubble were sampled in austral summer and winter at each site. For each compartment, bacterial, fungal, microalgal, and protist communities were characterized by high throughput DNA sequencing methodology. Results show that the reef microbiome composition varied greatly with seasons and reef compartments, but variations were different among targeted markers. No significant variation among sites was observed. Relevant bioindicators were highlighted per taxonomic groups such as the Firmicutes:Bacteroidota ratio (8.4%:7.0%), the genera *Vibrio* (25.2%) and *Photobacterium* (12.5%) dominating bacteria; the Ascomycota:Basidiomycota ratio (63.1%:36.1%), the genera *Aspergillus* (40.9%) and *Cladosporium* (16.2%) dominating fungi; the genus *Ostreobium* (81.5%) in Chlorophyta taxon for microalgae; and the groups of Dinoflagellata (63.3%) and Diatomea (22.6%) within the protista comprising two dominant genera: *Symbiodinium* (41.7%) and *Pelagodinium* (27.8%). This study highlights that the identified bioindicators, mainly in seawater and sediment reef compartments, could be targeted by reef conservation stakeholders to better monitor La Réunion Island's reef state of health and to improve management plans.

Keywords Microbiome · Bioindicators · Fringing coral reef · La Réunion Island

Stenger et al. 2025

Objectives

- To identify microbial bioindicators that can be used to monitor coral reef health.
- To analyze microbial communities (bacteria, fungi, algae, protists) from three ecosystem compartments (seawater, sediment, and coral rubble) in **La Réunion Island**, Indian Ocean.
- To use a **multi-marker approach** (16S, ITS, 18S, tufA) and **DNA metabarcoding analysis** for microbiome characterization.

The **coral microbiome** plays an important role in coral resistance and resilience → serves as a **bioindicator** → sensitive to environmental changes.

- It is **not limited to bacteria**, but also includes **fungi, algae, and protists**.

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Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health

Method

Samples were collected from three sites (**Toboggan, Copacabana, and Livingstone**) during two seasons (**summer and winter**).

Three ecosystem compartments were analyzed: **seawater, sediment, and coral rubble**.

DNA was extracted and characterized using specific genetic markers:

- **16S rRNA** for bacteria
- **ITS2** for fungi
- **18S rRNA** for protists
- **tufA** for microalgae

Analysis was performed using the **QIIME2 pipeline** and **Bayesian statistics** to identify **Amplicon Sequence Variants (ASVs)** as specific indicators.

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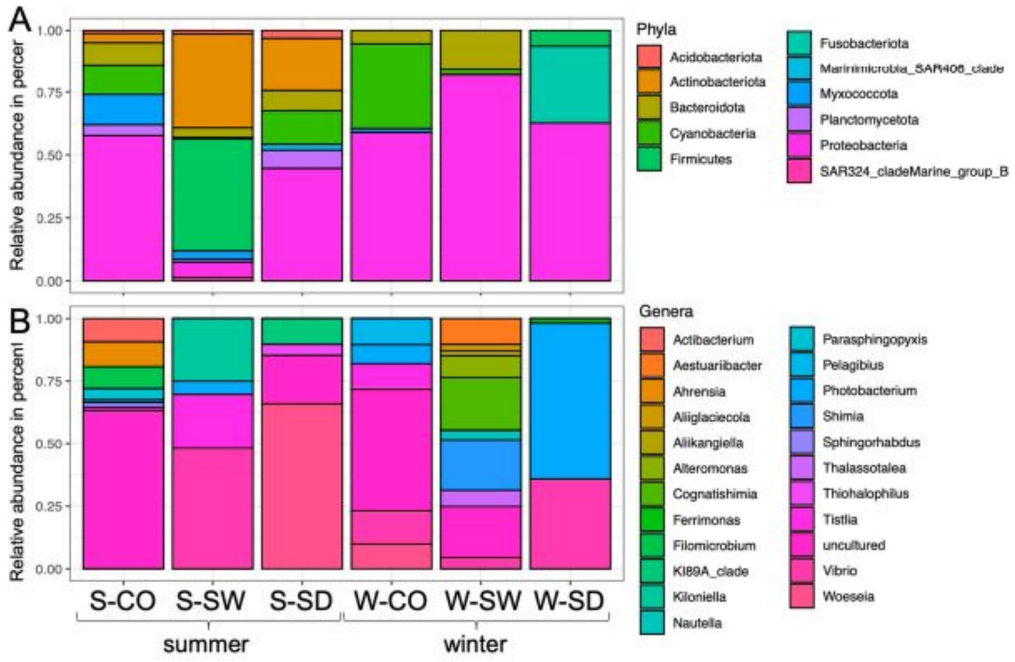
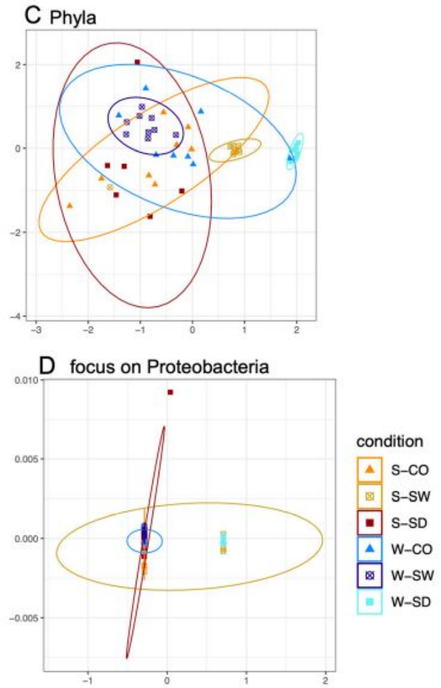
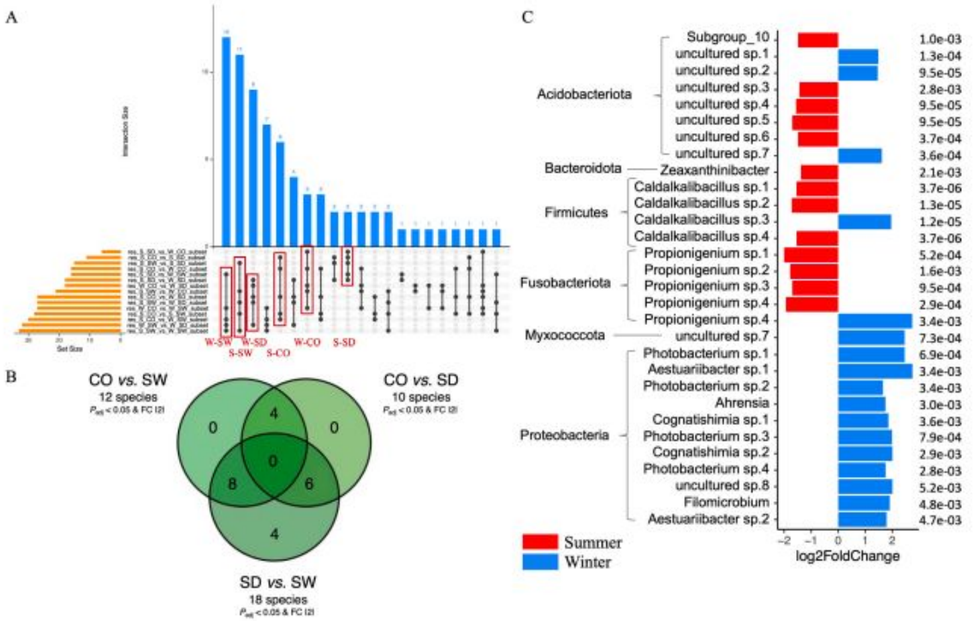
Stenger et al. 2025

Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health







Results

- The dominant bacterial communities across seasons and compartments were **Proteobacteria** and **Cyanobacteria**.
- A clear separation of microbial communities between **seawater and sediment** was observed depending on the season.
- **Potential bioindicators** include **Vibrio** and **Photobacterium**.
- The **Firmicutes-to-Bacteroidota ratio** may also serve as an indicator of coral health.

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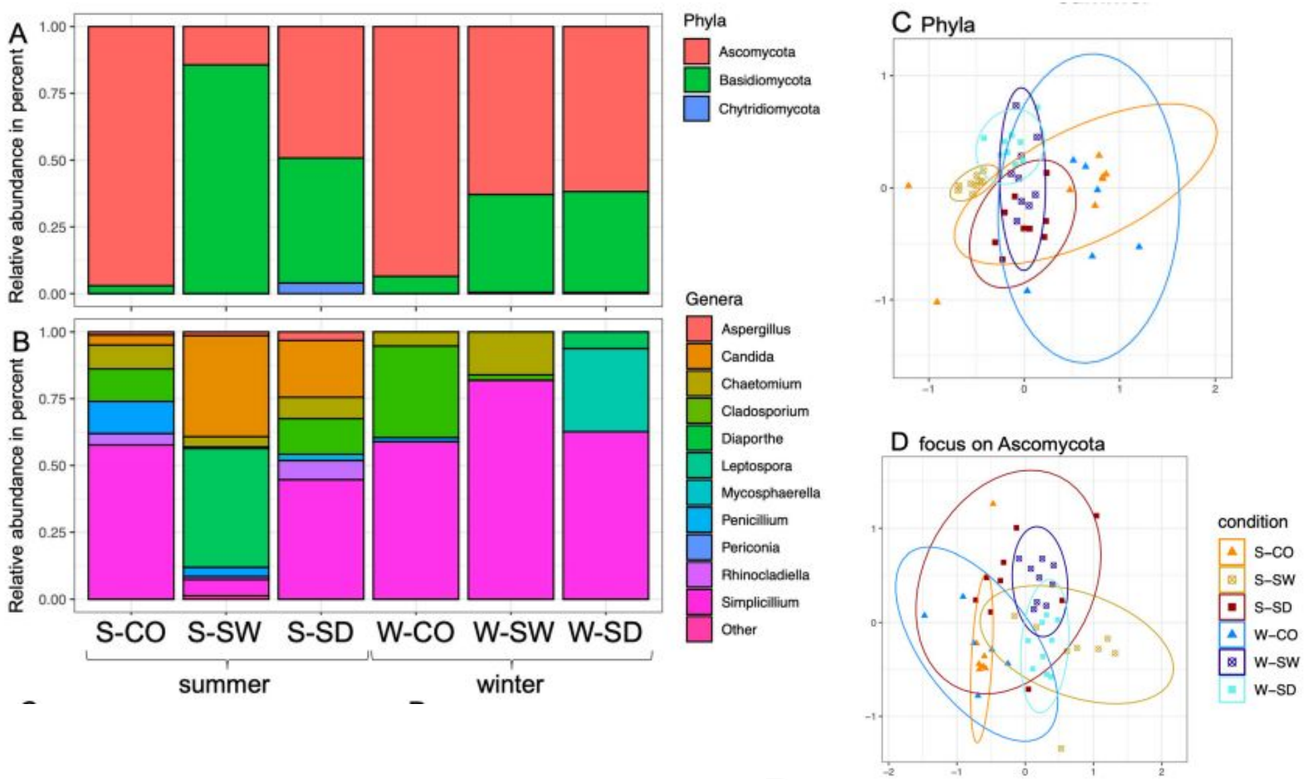
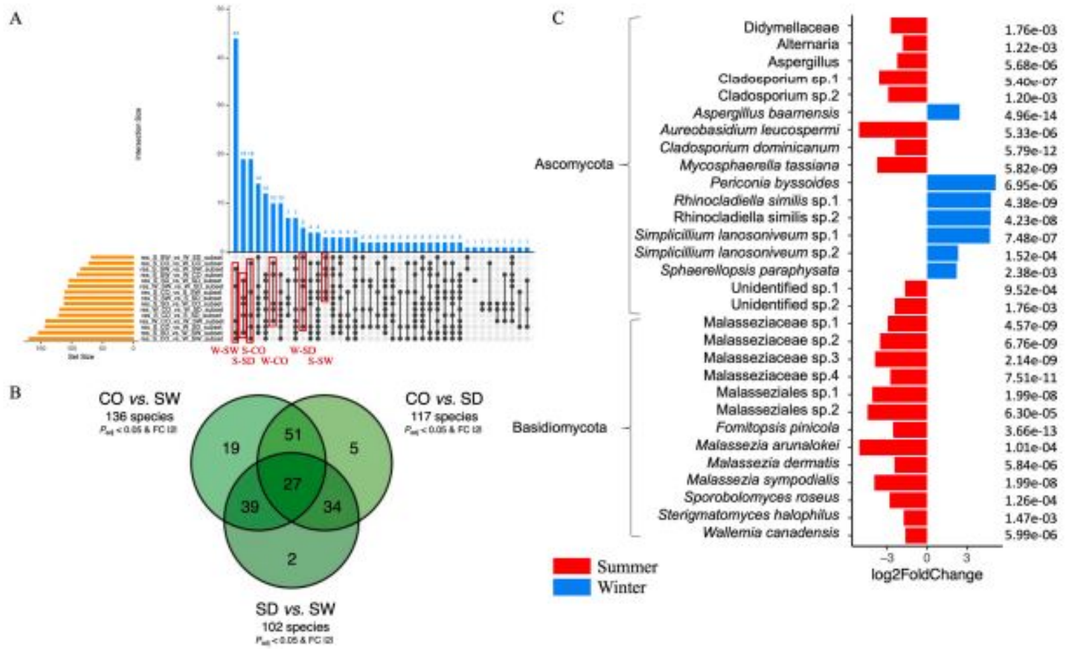
Stenger et al. 2024

Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health





Results

Fungal ASVs across seasons:

- **Summer:** *Aspergillus*, *Cladosporium*
- **Winter:** *Periconia*, *Simplicillium*

Potential bioindicators:

- *Aspergillus*, *Cladosporium*, and the **Ascomycota-to-Basidiomycota ratio**

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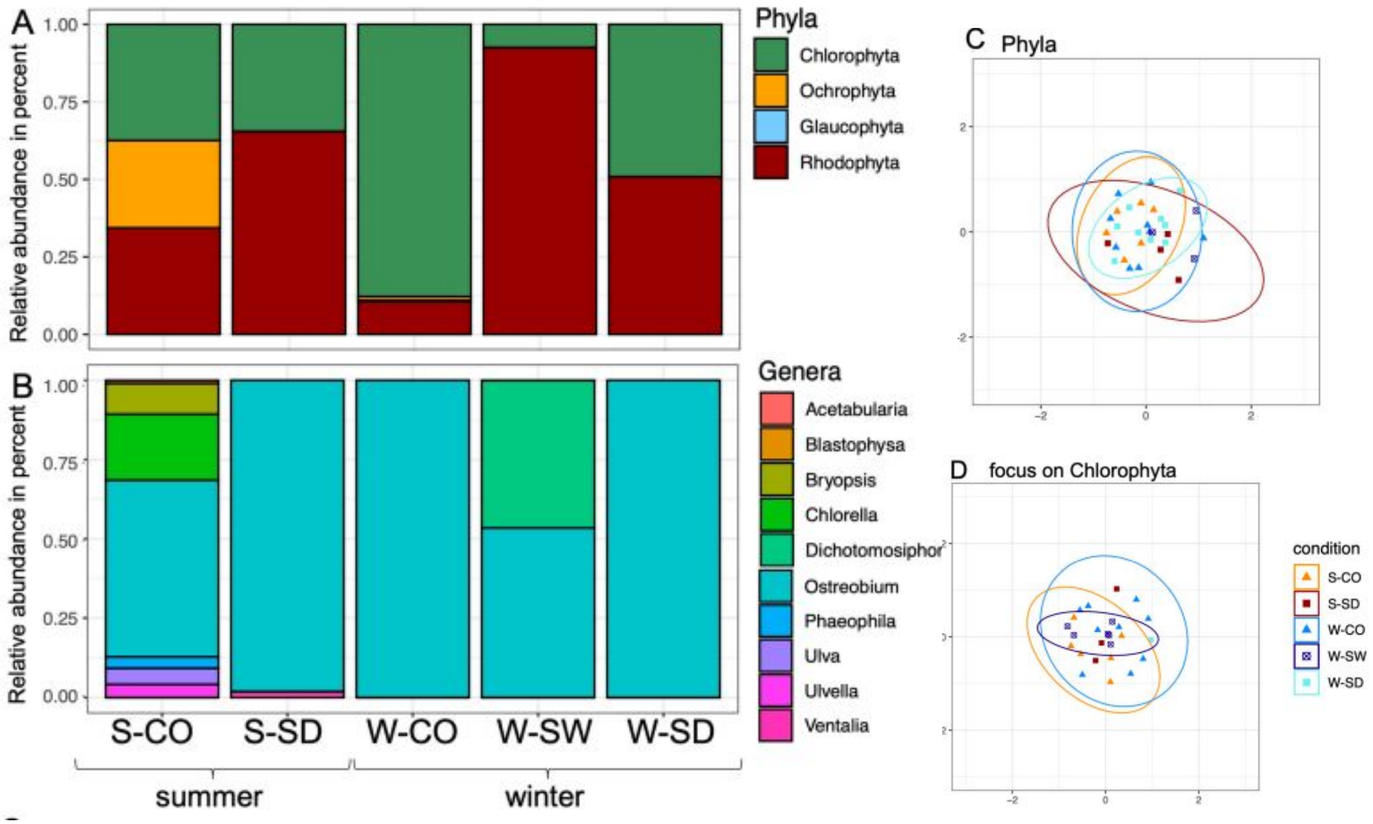
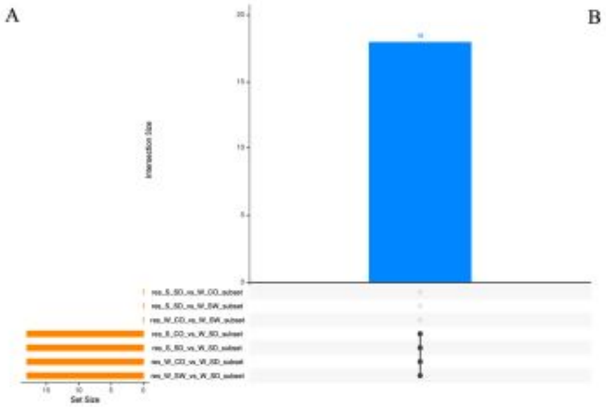
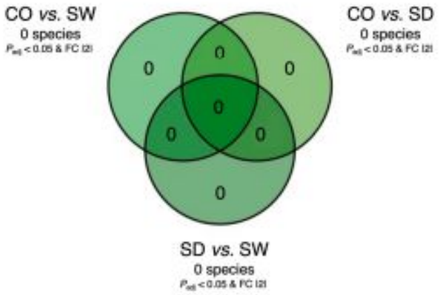
Stenger et al. 2024

Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health







Results

- **Dominant genus:** *Ostreobium*
- No significant ASV differences were found across conditions, compartments, or seasons → indicating that the **microalgal community is relatively stable** under varying conditions.

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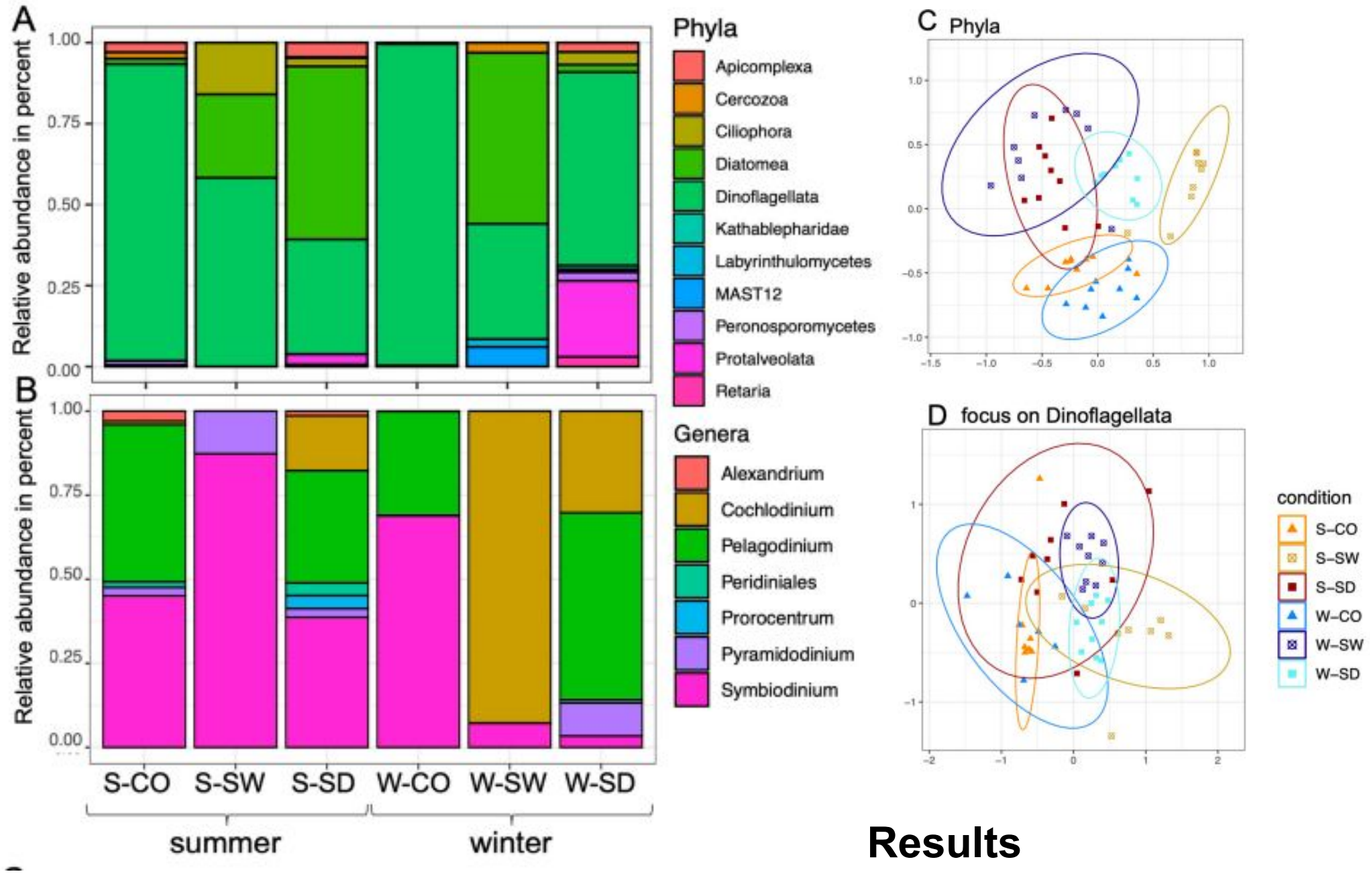
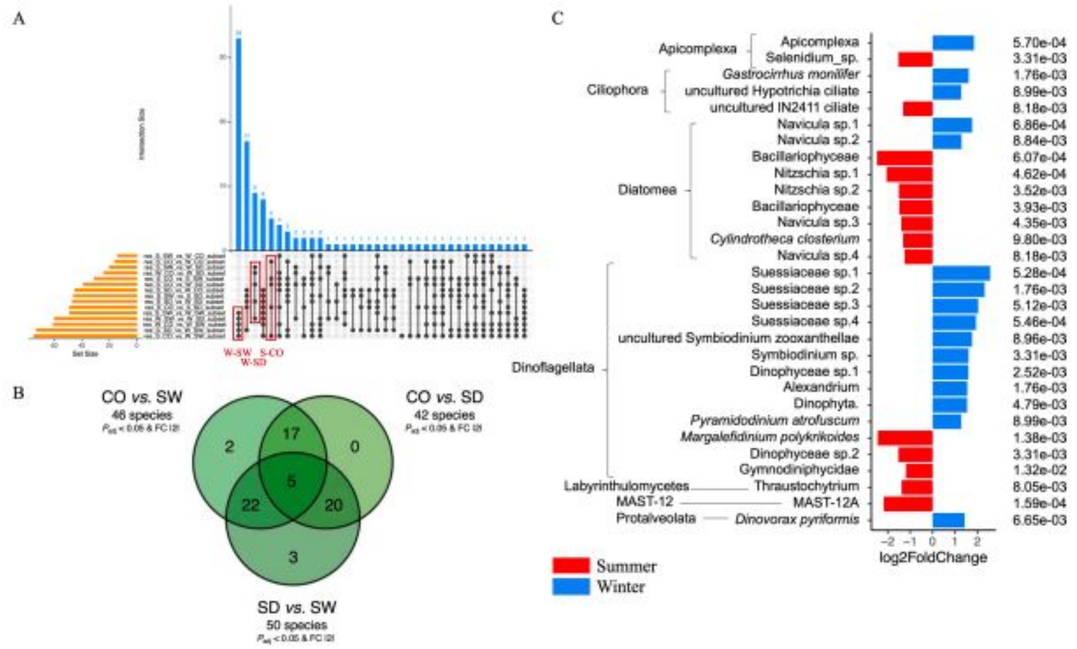
Applications of Omics Approaches and DNA Barcoding

Coral Microbiome Analysis as an Indicator of Coral Reef Health





Results

- **Dominant group:** *Dinoflagellates* — including *Cochlodinium*, *Plagodinium*, and *Symbiodinium*
- **Seasonal ASV variation:**
  - **Summer:** *Diatomea*, *Margalefidinium*
  - **Winter:** *Symbiodinium*, *Pyramidodinium*

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Stenger et al. 2024

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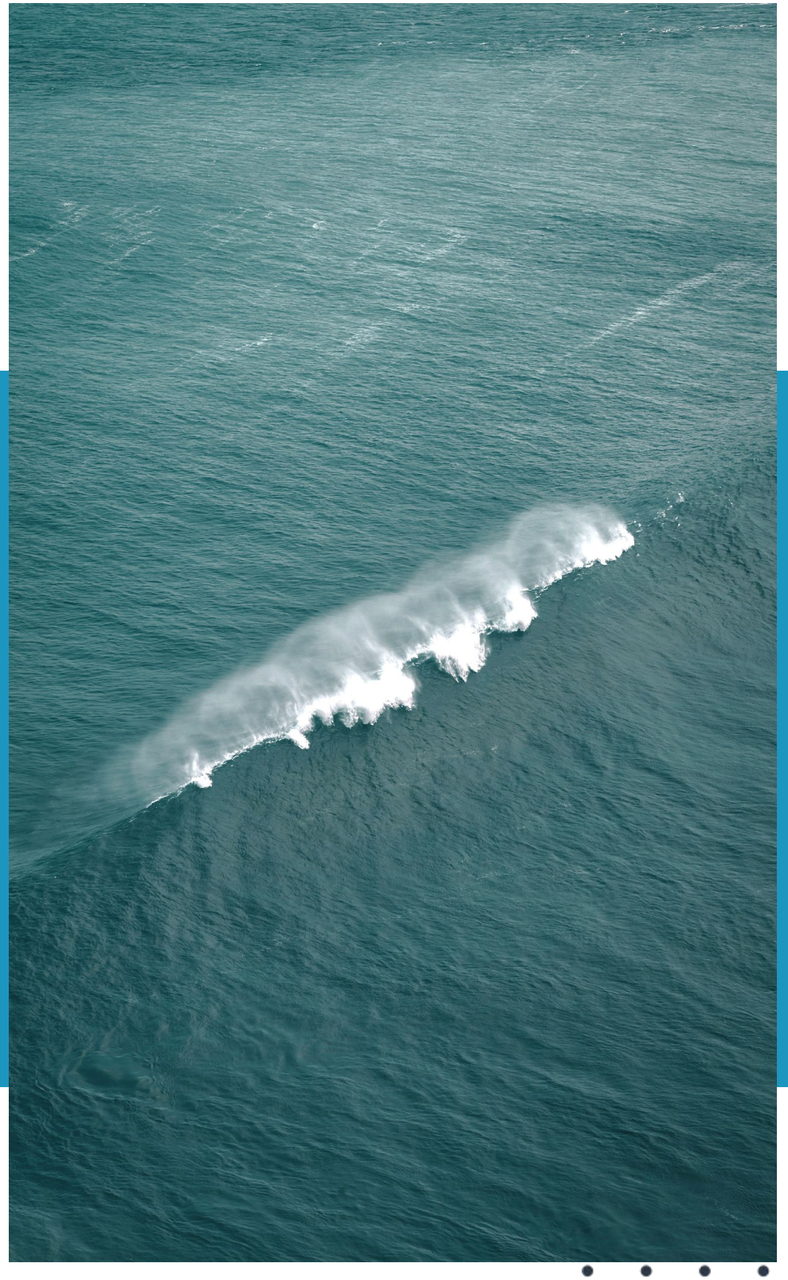
HEIs stands for Higher Education Institutions

04

GIS-based Integrative Biodiversity Analysis



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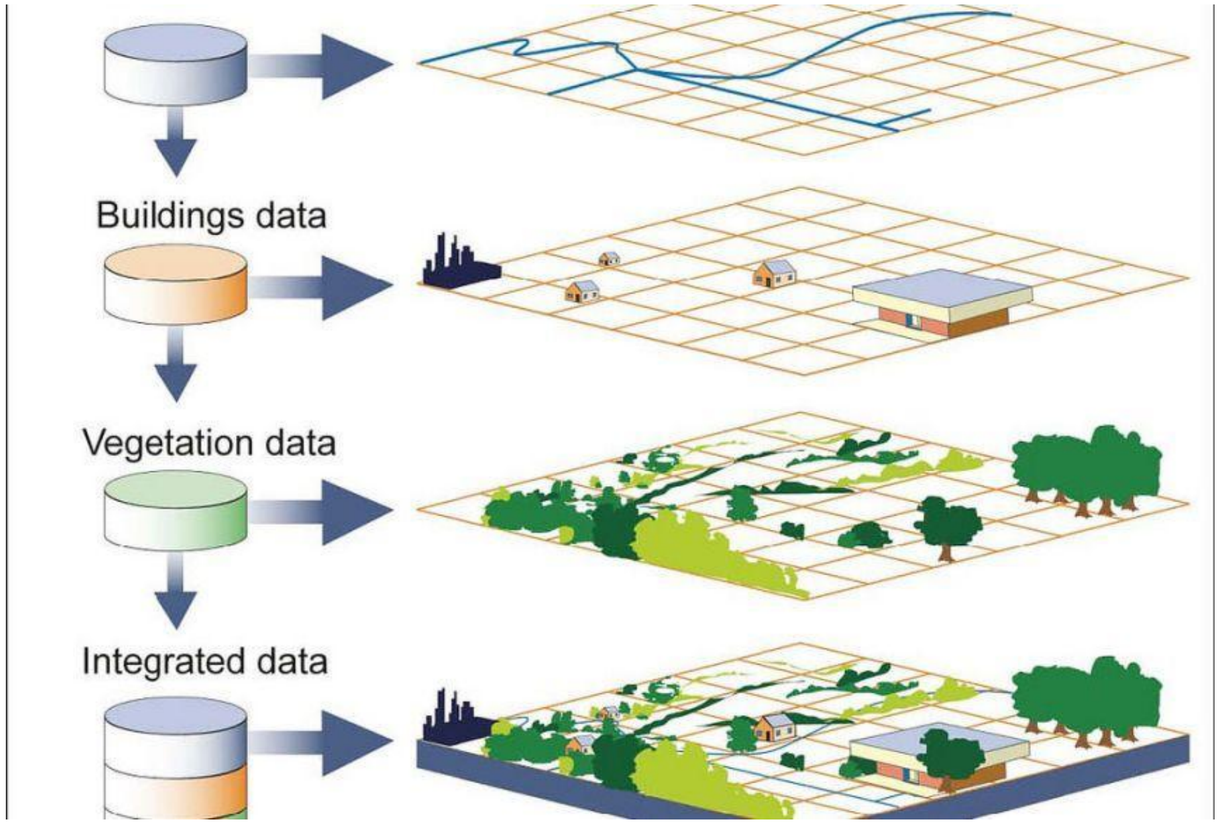
Geographic Information Systems (GIS)

Geographic System



Geographic Information System (GIS) is a computer-based system used to capture, store, examine, and display data that has a spatial reference on the Earth's surface, such as geographic coordinates or addresses.
It enables the analysis and visualization of spatial patterns and relationships between data that may initially seem unrelated.
GIS data is organized in **layers**, allowing users to combine and compare various types of information—such as demographics, infrastructure, vegetation, or rivers—on a single interactive map.

How GIS Works

- **Data input** → from GPS, satellite imagery, surveys, or digitized analog maps.
- **Verification and integration** → checking data quality and ensuring consistent formats.
- **Spatial analysis** → calculating distances, detecting spatial patterns, and identifying vulnerable areas.
- **Output** → interactive maps, analytical reports, and multi-layer visualizations.

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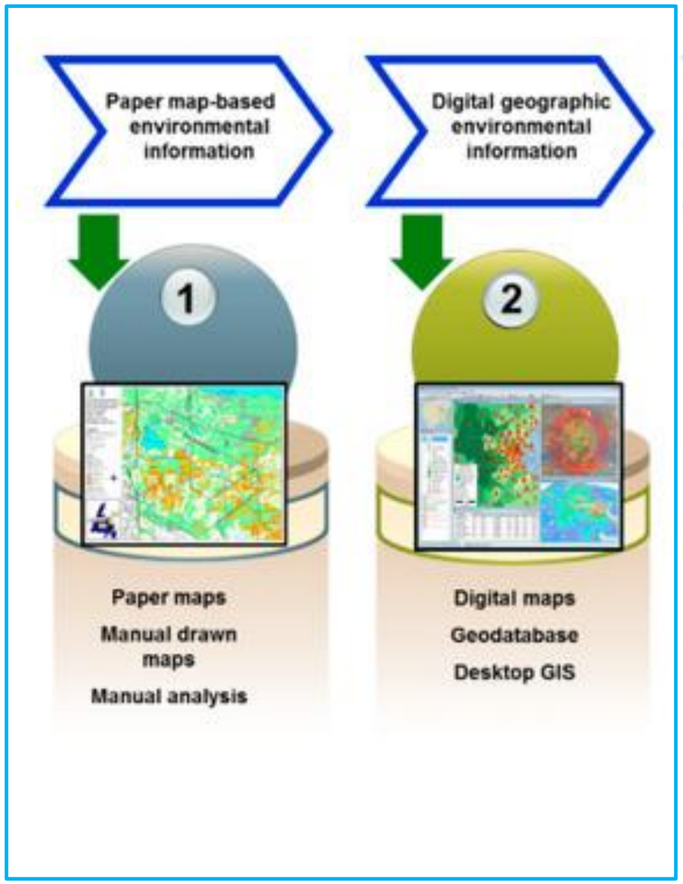
Geographic Information Systems (GIS)

Geographic Systems Evolution



- Paper-Based Maps

The use of physical maps to represent environmental data.
Limitations: Not dynamic, cannot be updated quickly, and typically used only by experts.

- Local Computer-Based GIS

Environmental data began to be collected and analyzed using GIS software on standalone computers.
Data was still collected by official institutions (authoritative sources).
Access remained limited to technical agencies.

- Web-Based GIS

GIS became accessible via the internet.
The general public could view the data, but could not yet contribute.
Data access improved, but interaction was still one-way (read-only).

- Geospatial Web 2.0 / Participatory GIS

The Web 2.0 era enabled two-way interaction: the public not only viewed but also contributed data (crowdsourcing).
This gave rise to the concept of VGI (Volunteered Geographic Information).
GIS became participatory and collaborative.

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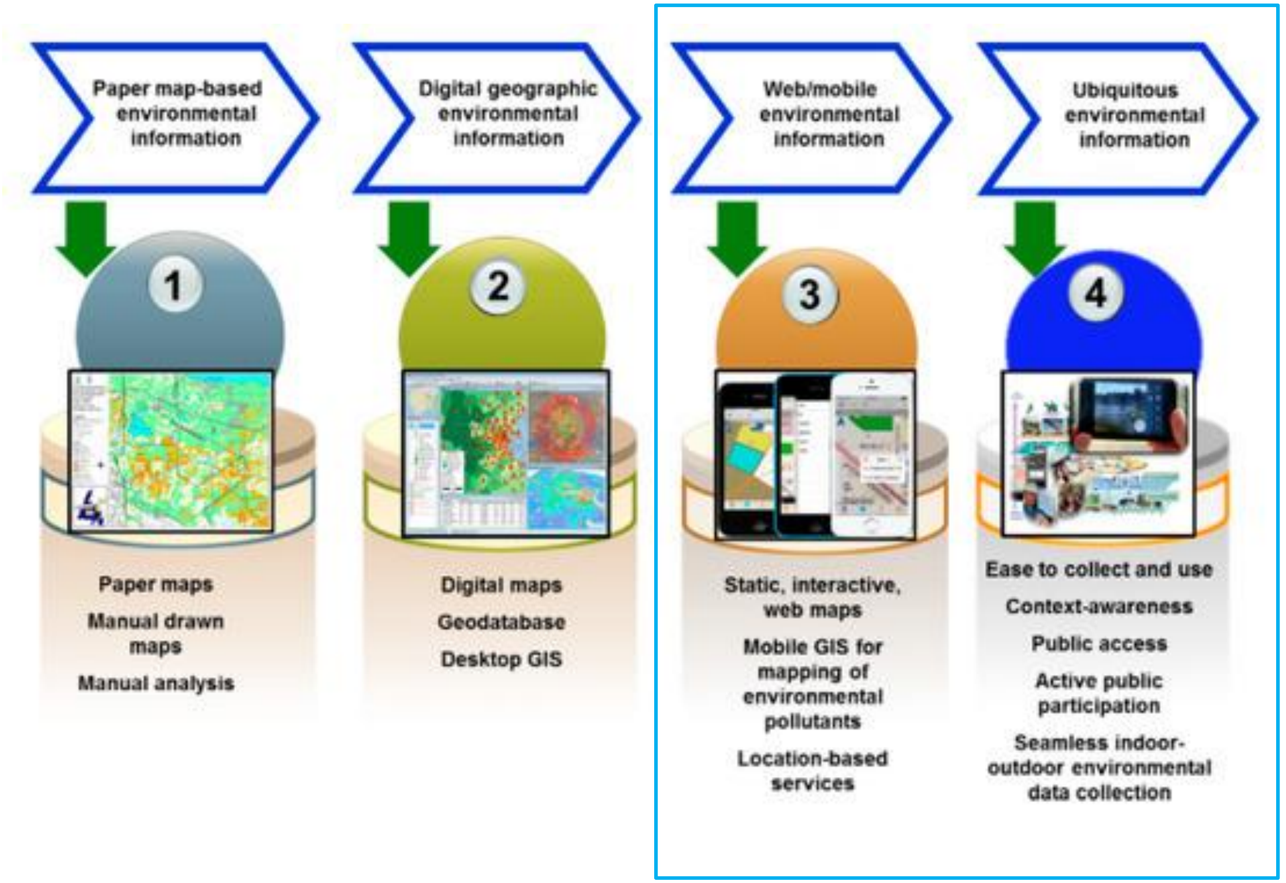
Sadeghi-Niaraki et al. 2020

Geographic Information Systems (GIS)

Geographic Systems Evolution



Sadeghi-Niaraki et al. 2020

Mobile GIS

The development of smartphones equipped with sensors (GPS, cameras) enables citizens to report directly from the location of events.
Data is real-time and location-specific.

Ubiquitous GIS

GIS can be used anytime and anywhere (ubiquitous). Technologies such as cloud computing, IoT sensors, and location-based services (LBS) support automatic and continuous environmental monitoring and reporting. Citizens become part of a distributed and active environmental monitoring system.

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Geographic Information Systems (GIS)

https://youtu.be/1ZnTczcikOY?si=wD17lecotT8BaMAg

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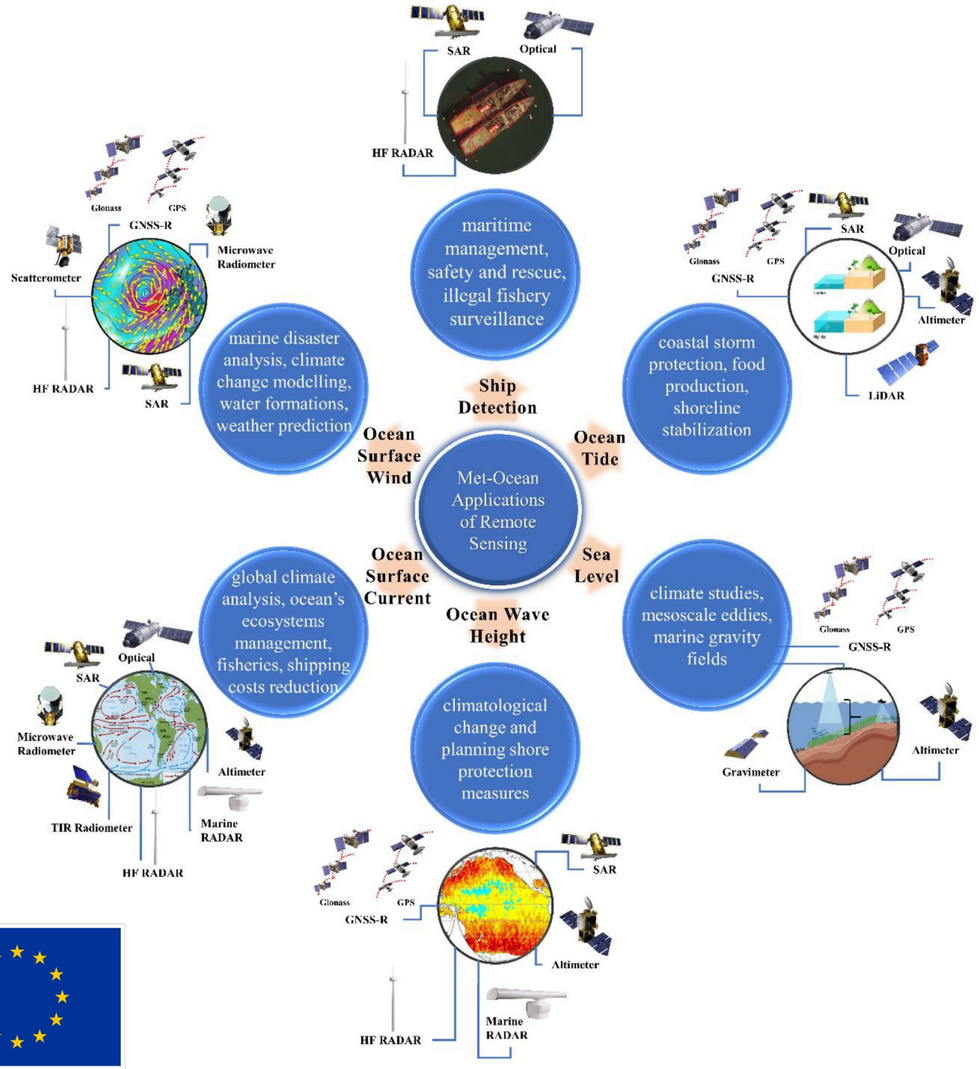
Geographic Information Systems (GIS)

Ocean Remote Sensing



Amani et al. 2022

Remote Sensing Systems in Marine Geographic Analysis

Passive Systems

- Rely on natural energy sources, such as sunlight or thermal radiation from the Earth's surface.
- Do not emit their own signals.
- Can only operate during daylight hours.
- Weather-sensitive.
- Examples: Optical sensors (*Landsat*, *Sentinel-2*), thermal infrared radiometer (TIR), microwave radiometer.
- Applications: Ocean color, sea surface temperature, monitoring of marine vegetation and coral reefs.

Active Systems

- Generate and transmit their own electromagnetic signals, then detect the reflected signals from Earth's objects.
- Operate both day and night, under all weather conditions.
- Suitable for areas with cloud cover or darkness.
- Examples: SAR, LiDAR, Scatterometer, Altimeter, HF radar, SONAR.
- Applications: Ship detection, ocean wave and current mapping, tide and sea level monitoring, seafloor mapping (bathymetry).

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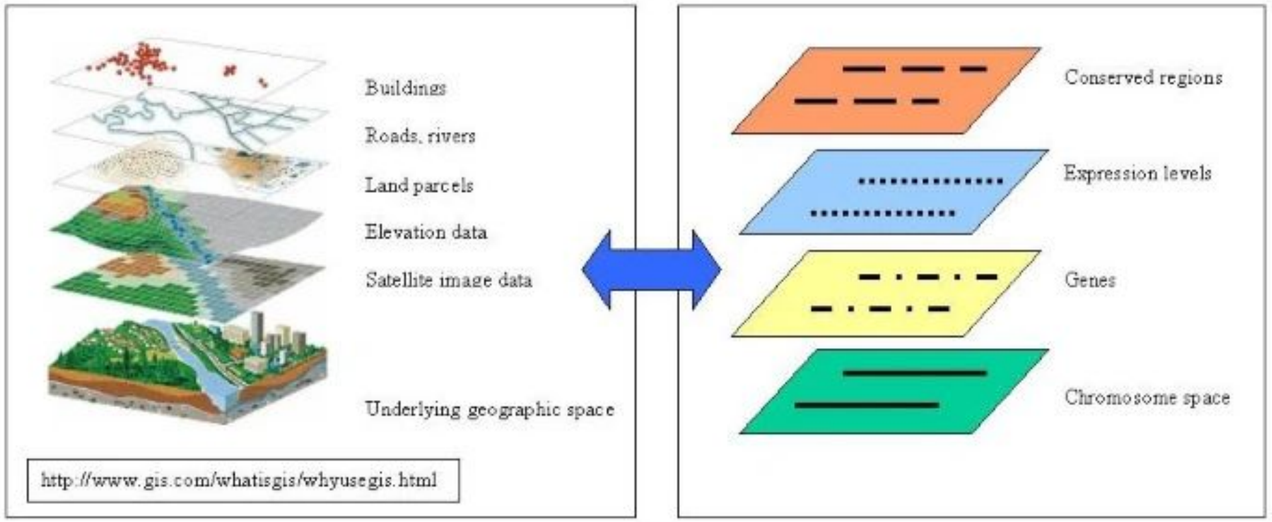
Geographic Information Systems (GIS)

Integration of Omics and GIS



Combining GIS, Remote Sensing, and Omics

Omics (e.g., metagenomics) studies the DNA of entire microbial communities without the need for isolation or culturing. When combined with spatial data from GIS, researchers can:

- Identify where specific organisms thrive.
- Analyze how organisms respond to environmental changes (e.g., sea temperature, pollution, salinity).
- Gain critical insight into the role of biodiversity in biogeochemical cycles, such as the carbon and nitrogen cycles.

Applications in Marine Resource Management

GIS maps and monitors the physical conditions of marine habitats (e.g., coral reefs, seagrass beds, seafloor), while omics provides in-depth biological data (e.g., microbial types, enzyme activity, pathogenic potential). This integration enables:

- Monitoring ecosystem health.
- Data-driven, integrative conservation planning.

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Dolan et al. 2006

Conclusion

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The **integration of omics and GIS** is a powerful modern approach for:

- Understanding the **roles of organisms within ecosystems**
- **Sustainably managing marine resources**
- **Predicting the impact of environmental changes** on microbial life—and vice versa

This approach combines the strengths of **molecular biology**, **geospatial analysis**, and **artificial intelligence** to support **environmental science** and **conservation policy**.

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Evaluation Answer Key: 1) True, 2) B, 3) False, 4) D

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Evaluation Answer Key: 1) True, 2) B, 3) False, 4) D

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THANK YOU

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