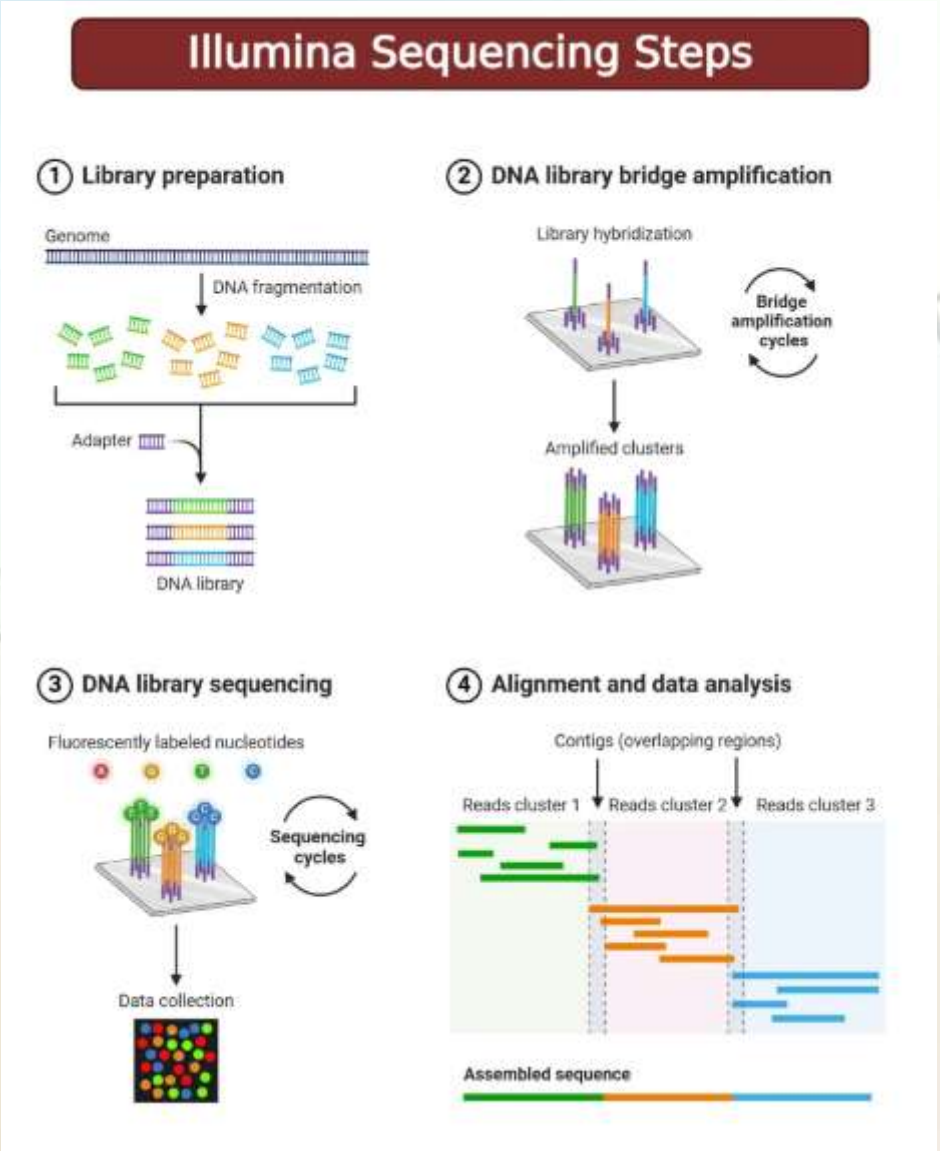
II. Technologie du Single Cell (Microbiologie, Edition Dunod 2015, page 132)

Single cell sorting- MMI CellEctor Plus.mp4

MIDAS animation.

https://www.youtube.com/watch?v=WNM6A9h6GJI

Watch the video



IV. METAGENOMIC APPROCHE

Metagenomics and Metatranscriptomics

Metagenomics and metatranscriptomics are molecular ecology approaches that involve analyzing, without prior targeting, the entire DNA or RNA extracted from a complex sample containing a wide diversity of different organisms..

❖ Traditional microbial genomics

- Using cultures to isolate the microbe of interest
- Sequencing the genome of one organism at a time

Metagenomics

- Extracting sequence data from microbial communities as they exist in nature
- Bypassing the need for culture-based techniques
- Sequencing all the DNA present in the sample
- Selecting DNA based on a universal sequence

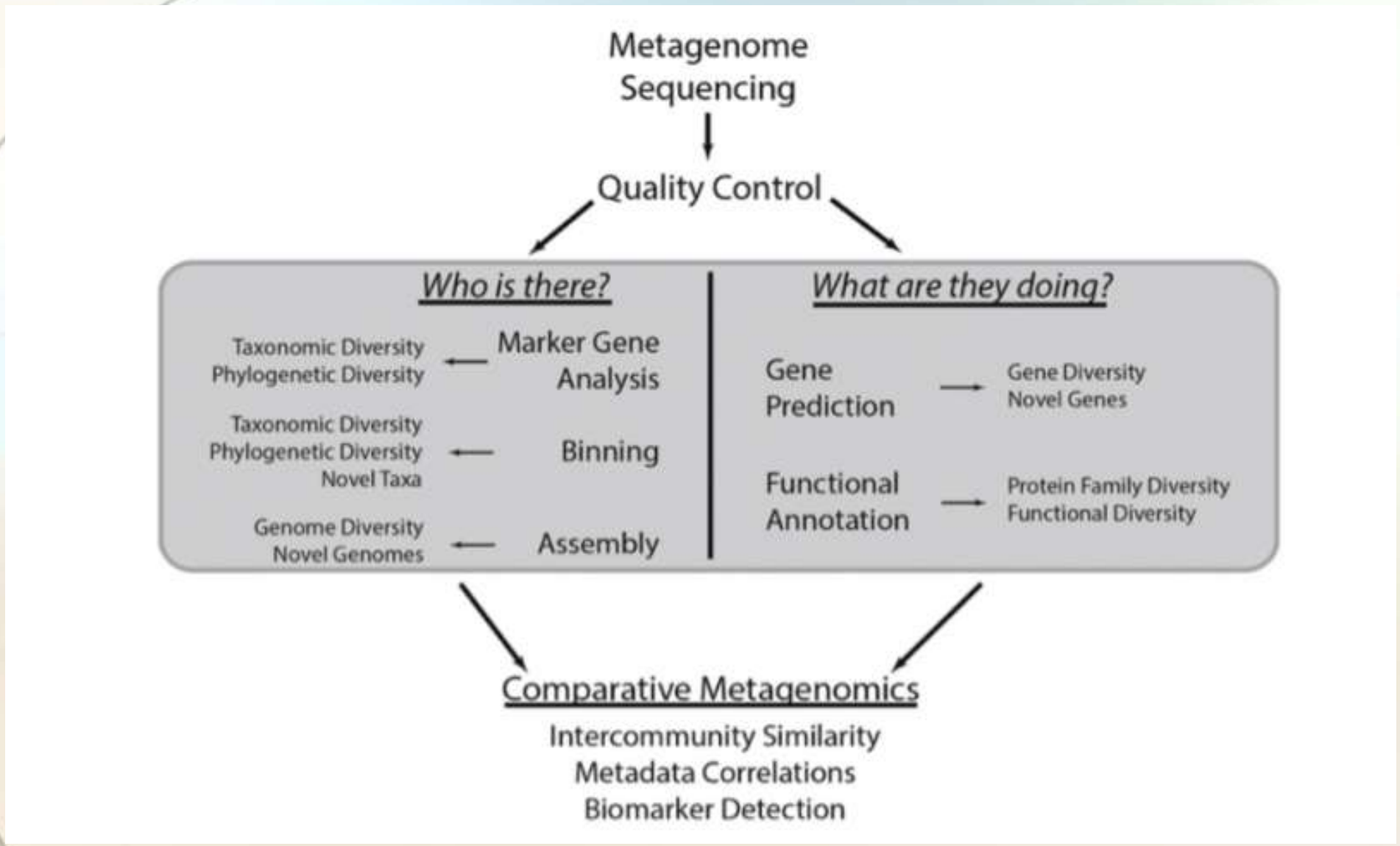

Metagenome Sequencing
Quality Control
Who is there?
What are they doing?
Taxonomic Diversity
Phylogenetic Diversity
Marker Gene Analysis
Taxonomic Diversity
Phylogenetic Diversity
Novel Taxa
Binning
Genome Diversity
Novel Genomes
Assembly
Gene Prediction
Gene Diversity
Novel Genes
Functional Annotation
Protein Family Diversity
Functional Diversity
Comparative Metagenomics
Intercommunity Similarity
Metadata Correlations
Biomarker Detection

Table 1: Historical events in the field of metagenomics.

Year	Scientist(s)	Discovery
1988	Handlesman and Goodman et al.	Coined the term “metagenomics.”
1991	Pace et al.	Isolation and cloning of DNA from an environmental sample.
1995	Healy	Metagenomic isolation from Zoo Libraries.
2002	Breitbart et al.	The first time used shotgun sequencing and analysed 5000 different virus species from seawater.
2003	Venture C	Analyzed 2000 different microbial species from seawater.
2005	Schuster S	Published sequence data from an environmental sample.

Metagenomic technique

❖ 1. DNA Extraction

➢ The DNA extraction step is fundamental and its success is highly dependent on the type of sample being processed. Each sample matrix presents unique challenges that require adapted protocols to efficiently:

1. Lyse cells,
2. Remove contaminants,
3. Obtain high-quality, representative DNA.

Type of samples:

Environnemental samples	Biological samples
Soil, mud, water-pond, river, seawater decomposed biological samples	Oral cavity, stomach, stool, urine, animal-skin, intestine, gut, blood, cerebrospinal fluid (CSF) or (LCR in French)

Metagenomes are large

Soil contains up to 40,000 individual microbial species
The orders of magnitude of the soil metagenome are larger than the human genome.

- **Metagenome analysis**
  - Screening
  - Phylogenetic studies
  - Sequencing of uncultured organisms
  - Studying the metagenome under different conditions

Understanding Microbial Communities

❖ **Some questions that metagenomics can answer**

- Are certain adaptations observable along environmental gradients?

- How do different species interact with one another?

- Can lateral gene transfer be detected?

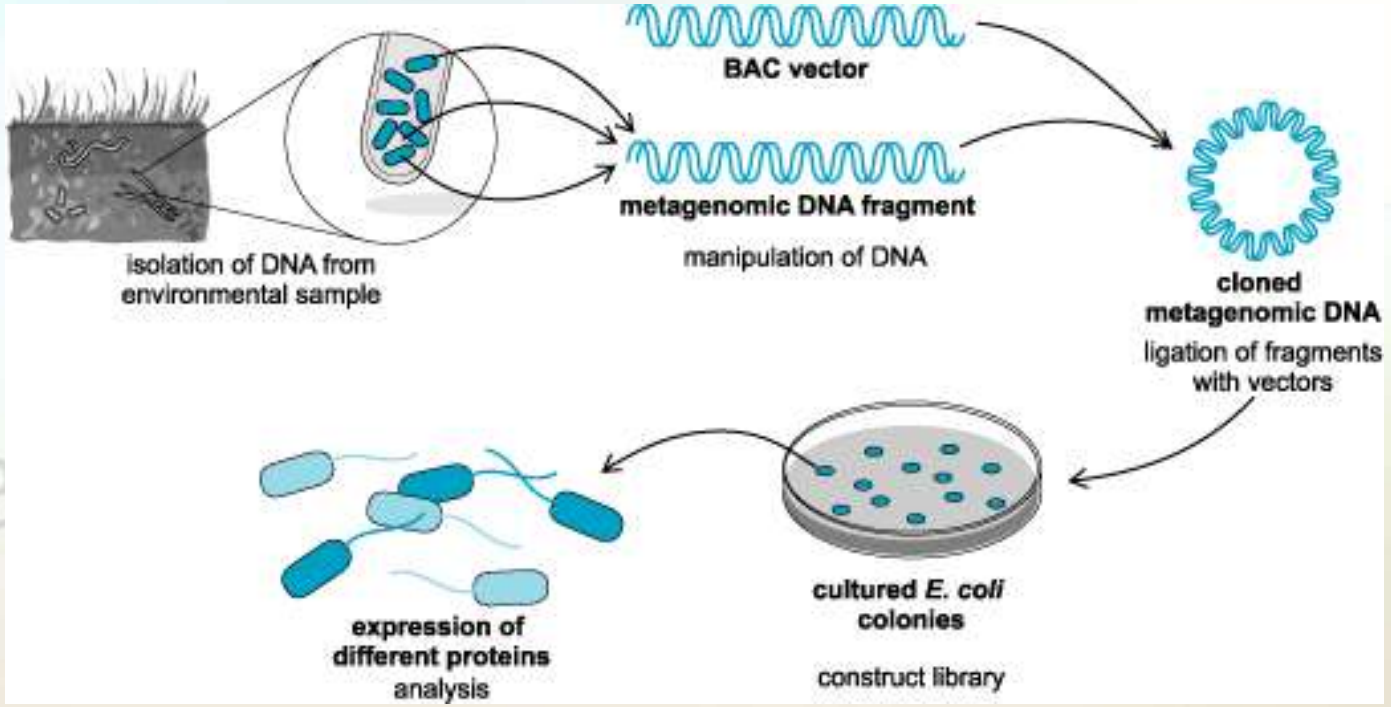
Metagenomic technique

I. Original Technique: metagenomics was often performed using a cloning-based method:

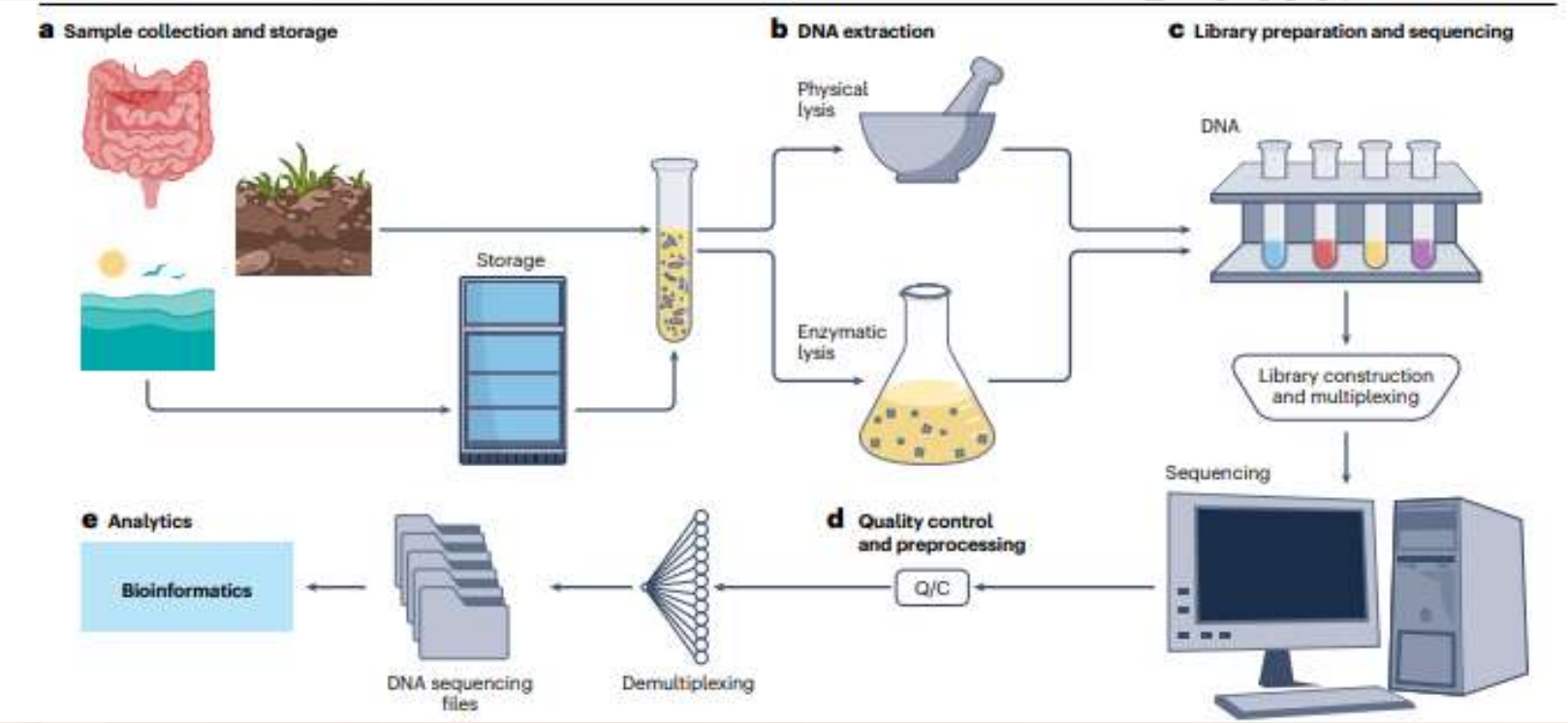
❖ 2. Clone the DNA

- Insert it into vectors
- Build a sample library

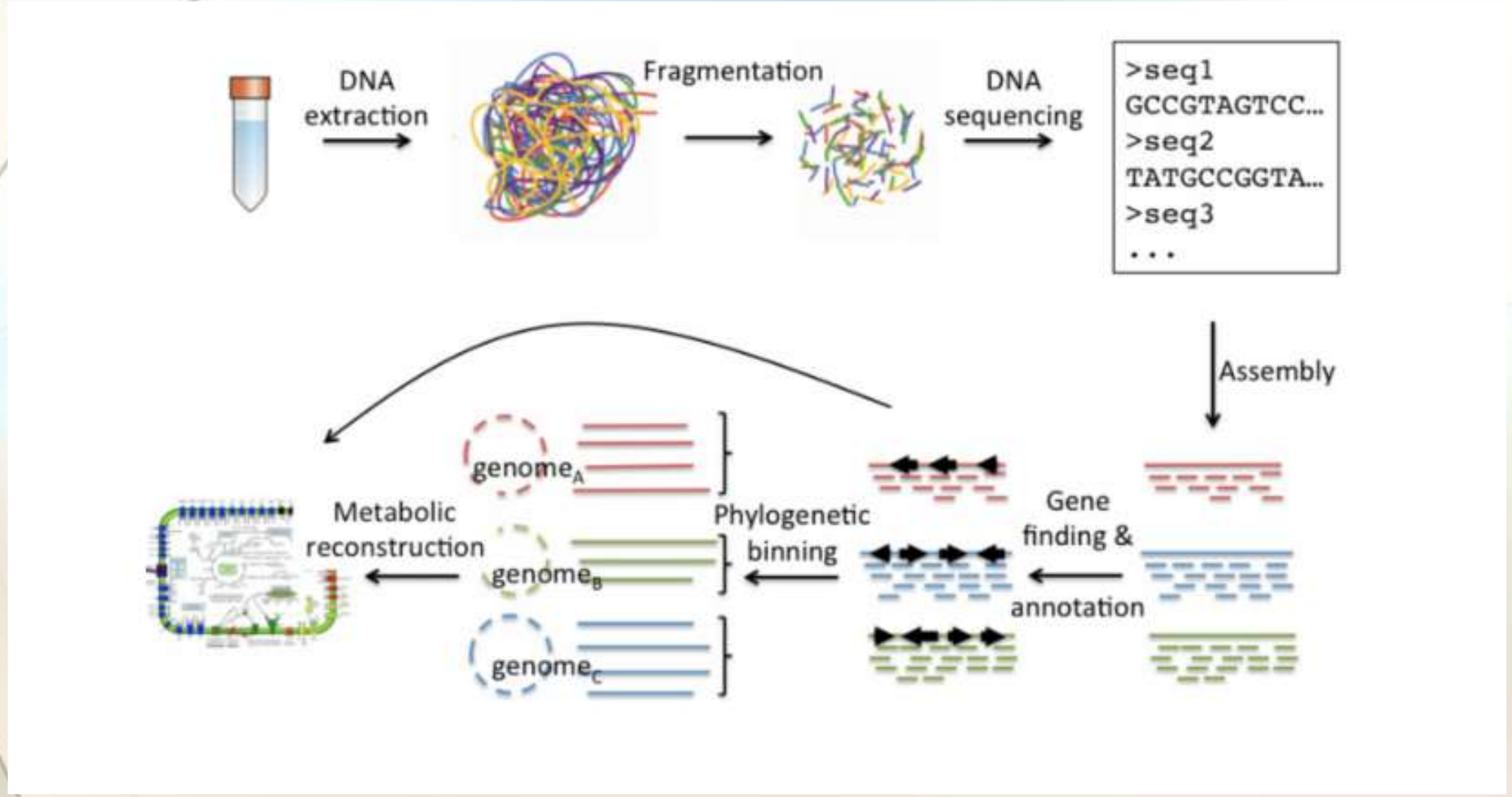
❖ 3. Screen or sequence

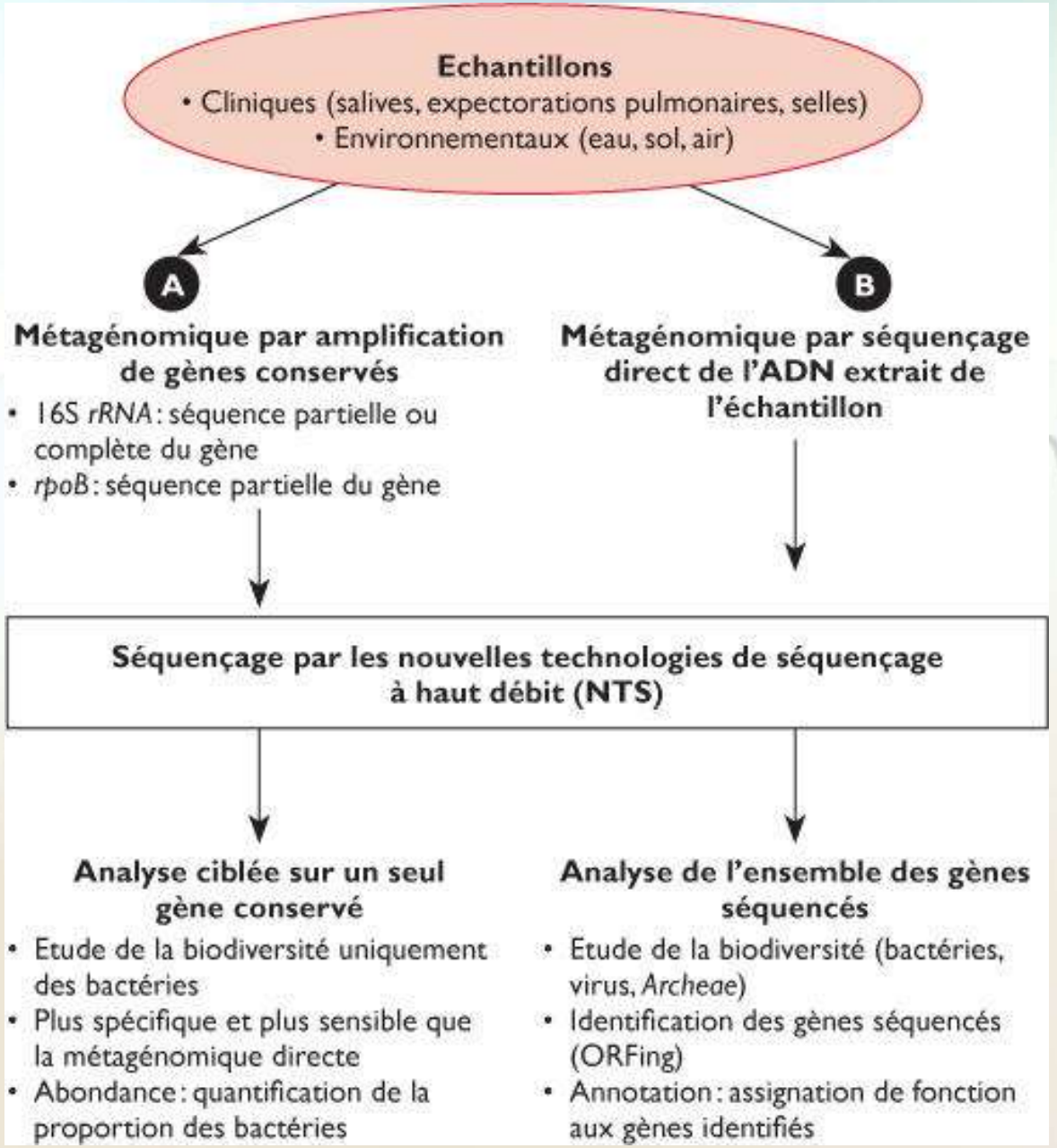


I. Shotgun metagenomics refers to sequencing all DNA fragments randomly from an environmental sample using next-generation sequencing (NGS) technologies, without cloning or targeting specific genes.



Experimental protocol for metagenomics experiments


DNA extraction
Fragmentation
DNA sequencing
>seq1
GCCGTAGTCC...
>seq2
TATGCCGGTA...
>seq3
...
Assembly
Gene finding & annotation
Phylogenetic binning
Metabolic reconstruction
genome$_A$
genome$_B$
genome$_C$


Echantillons
• Cliniques (salives, expectorations pulmonaires, selles)
• Environnementaux (eau, sol, air)
A
B
Métagénomique par amplification de gènes conservés
• 16S rRNA : séquence partielle ou complète du gène
• rpoB : séquence partielle du gène
Métagénomique par séquençage direct de l'ADN extrait de l'échantillon
Séquençage par les nouvelles technologies de séquençage à haut débit (NTS)
Analyse ciblée sur un seul gène conservé
• Etude de la biodiversité uniquement des bactéries
• Plus spécifique et plus sensible que la métagénomique directe
• Abondance : quantification de la proportion des bactéries
Analyse de l'ensemble des gènes séquencés
• Etude de la biodiversité (bactéries, virus, Archeae)
• Identification des gènes séquencés (ORFing)
• Annotation : assignation de fonction aux gènes identifiés


Metagenomics
Metatranscriptomics
Metaproteomics
Metabolomics

Advantages of metagenomics

- Allows the study of microorganisms that cannot be cultured in the laboratory
- Provides a comprehensive view of microbial diversity in an environment
- Helps identify functional genes and metabolic pathways
- Enables the study of microbial interactions and community structure
- Useful for environmental, medical, and industrial applications
- Allows detection of rare or low-abundance species

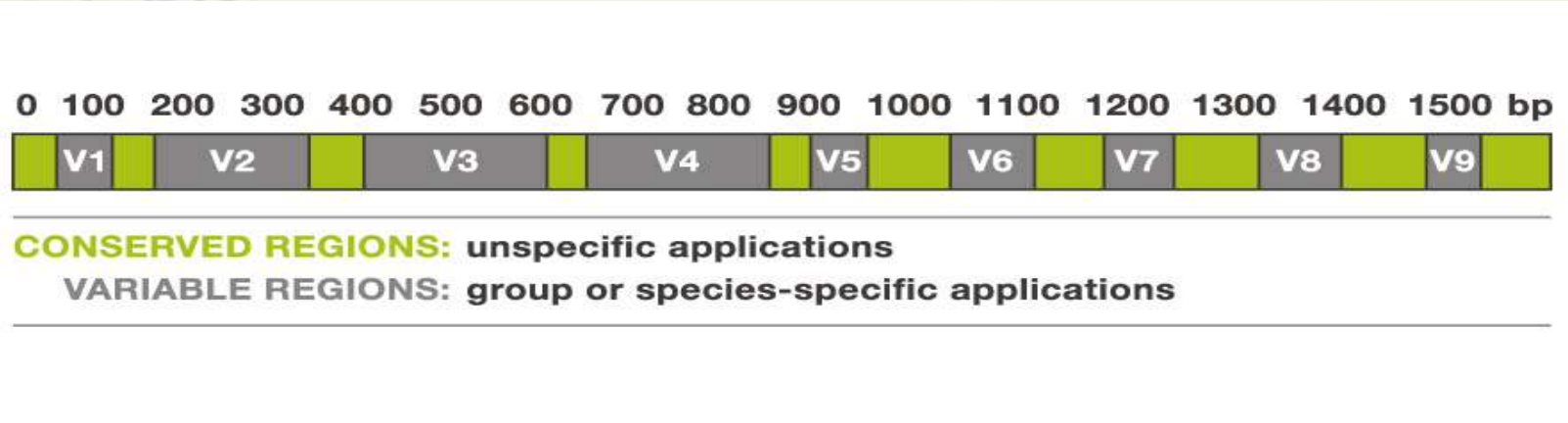
Disadvantages of metagenomics

- Data analysis is complex and requires advanced bioinformatics tools
- High cost of sequencing and data processing
- Difficulty in assembling complete genomes from mixed samples
- Results may be biased by DNA extraction and sequencing methods
- Limited ability to link specific functions to specific organisms
- Interpretation can be challenging due to large and complex datasets

Type of test: Ribotyping

Test description

The ribotyping technique is based on the presence, in bacteria, of several ribosomal RNA operons. These operons encode the 16S–23S–5S genes. The 16S and 23S genes are separated by an intergenic (non-coding) region of variable length. Ribotyping consists of amplifying these intergenic regions by PCR, which therefore vary in both number and size. The selected primers allow amplification from a segment of the 16S gene to another segment of the 23S gene. The resulting amplicons are then analyzed by capillary electrophoresis using a sequencer, or by NGS technologies.



16S rRNA gene illustrating the conserved (green) and variable (gray) regions