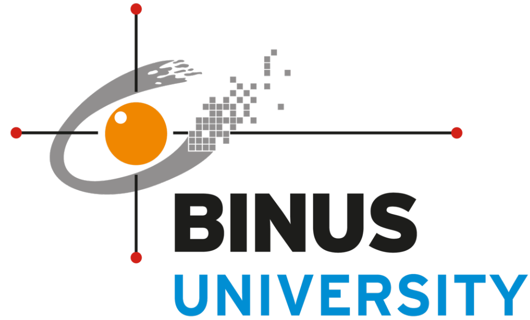

Culture-independent methods for qualitative and quantitative microbiological detection and identification

Ilia Popov

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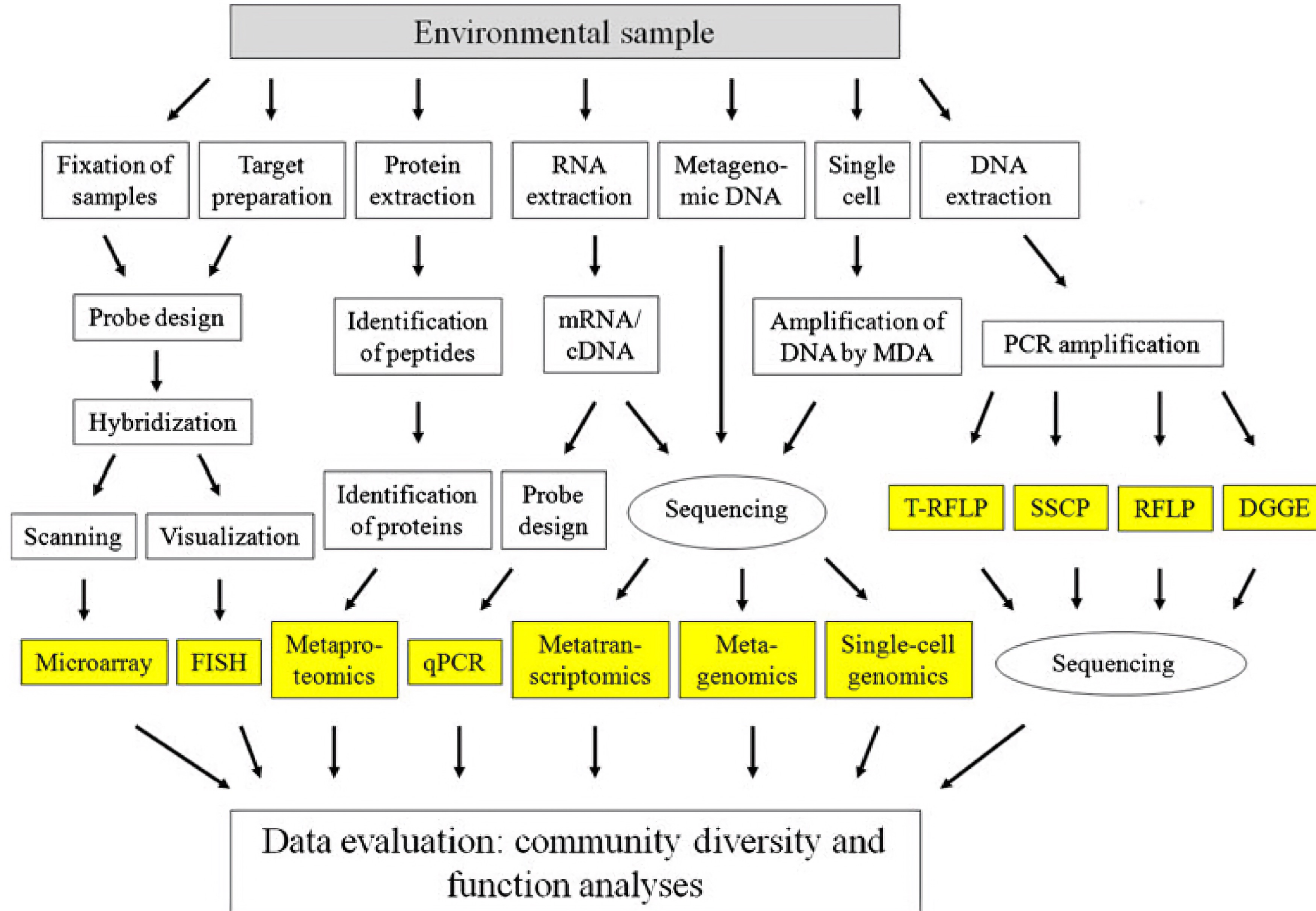
Introduction

Culture-Independent methods	Culture-dependent methods
Do not require culture on nutrient media	Based on the growth of colonies in culture
Rely on analysing the genetic material of microorganisms or their protein components ↓↓↓ Allows for **faster and more accurate results**	have some **limitations**, such as: • long culture times • the inability to detect and identify unknown or slow-growing microorganisms

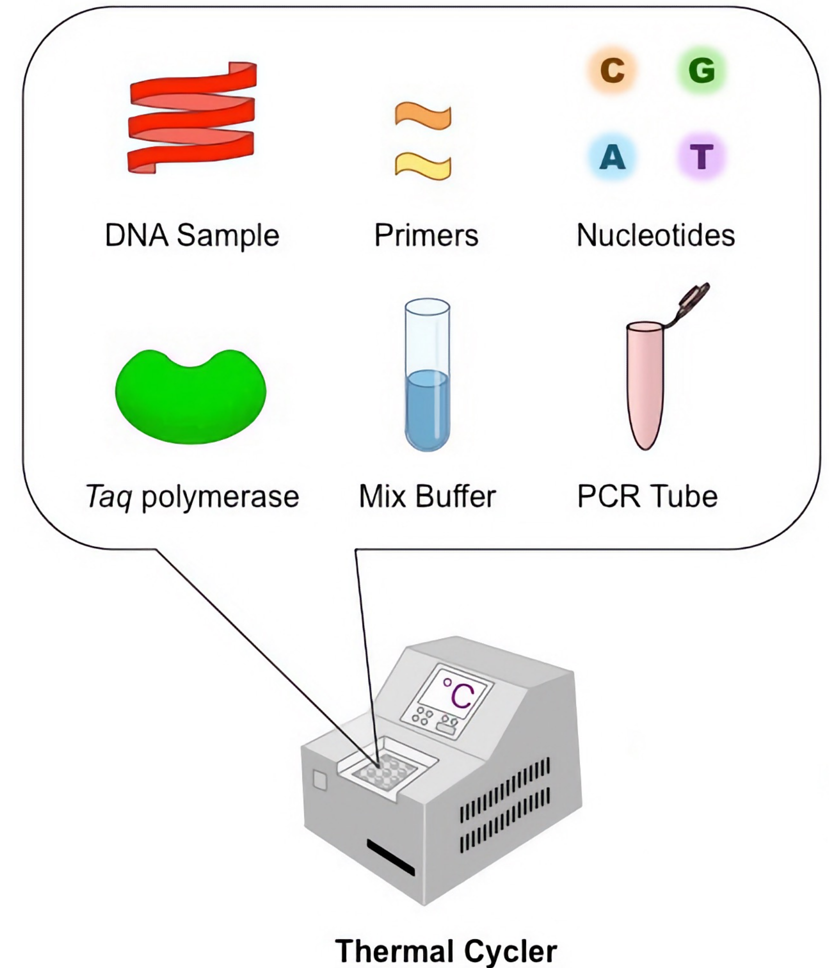
Overview of CIMs



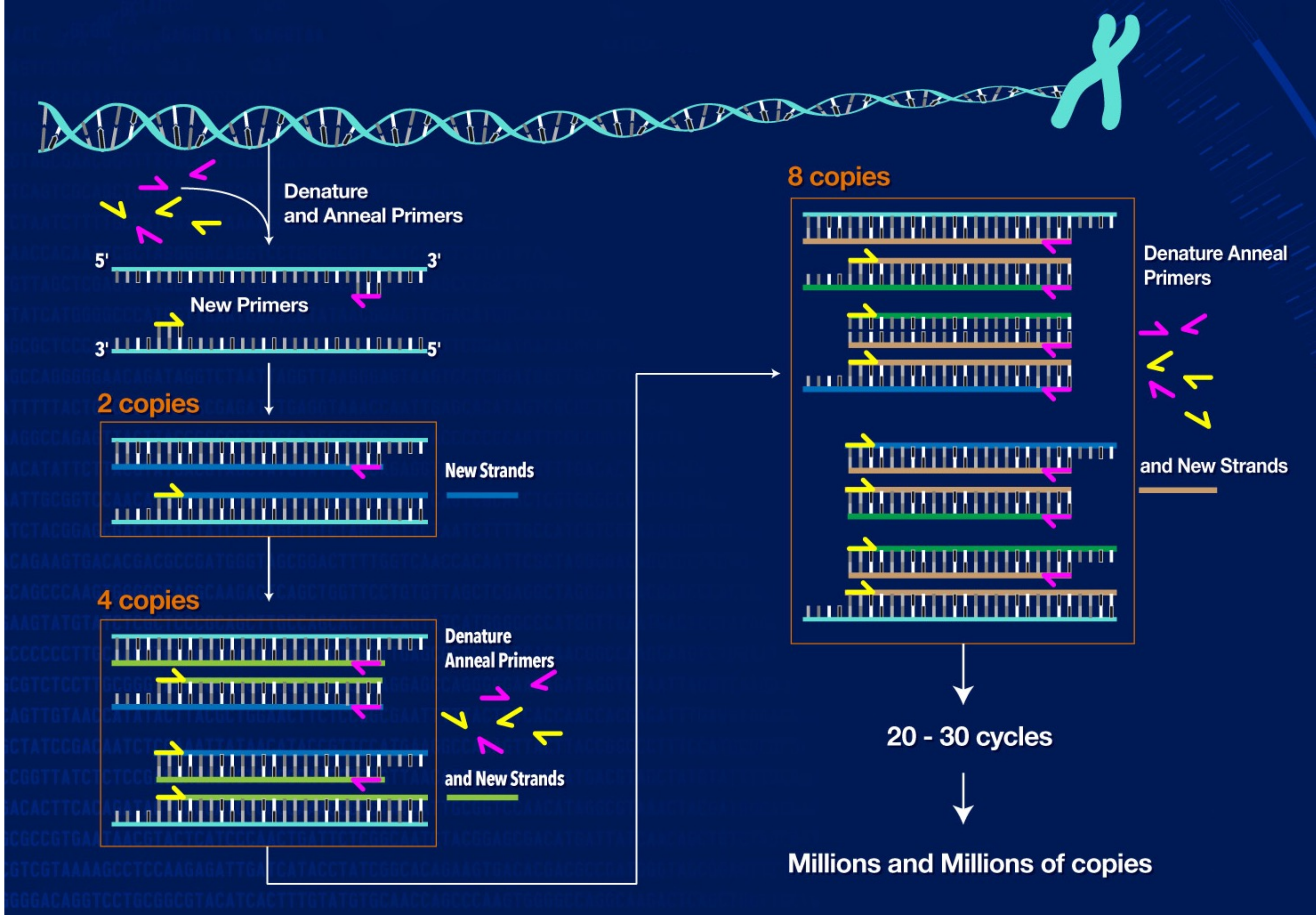
Can Su et al., Culture-independent methods for studying environmental microorganisms: methods, application, and perspective, Appl Microbiol Biotechnol, 2012

PCR

- Polymerase chain reaction is a technique that **allows specific regions of a microorganism's DNA to be amplified to detectable levels**
- It can be used to **detect and identify specific microbial species from unique sequences of their genetic material**



How does PCR work?



PCR: pros and cons

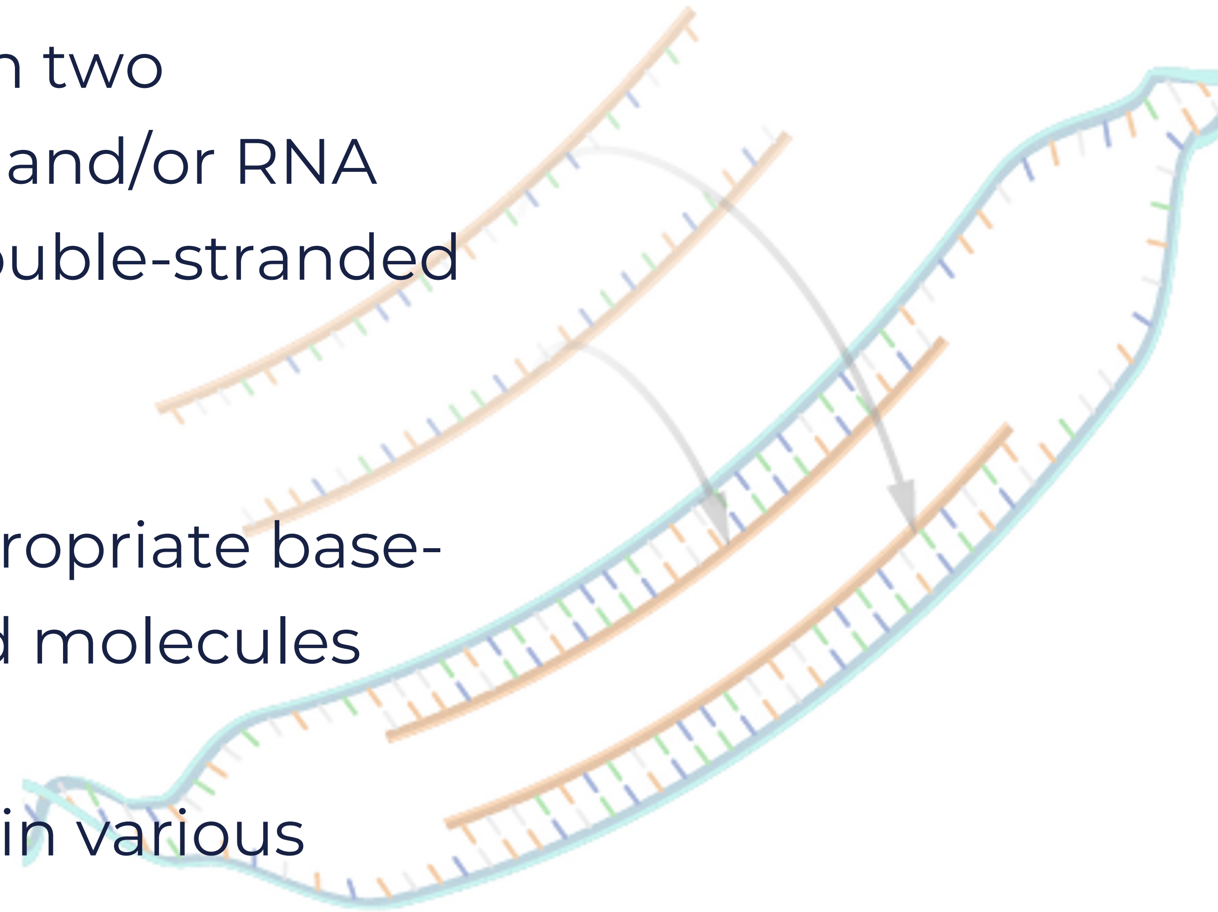
+	−
High sensitivity	Risk of contamination
High specificity	Amplification bias
Rapidity	
Multiplexing	Limiting the length of the fragment to be amplified

DNA/RNA Hybridization

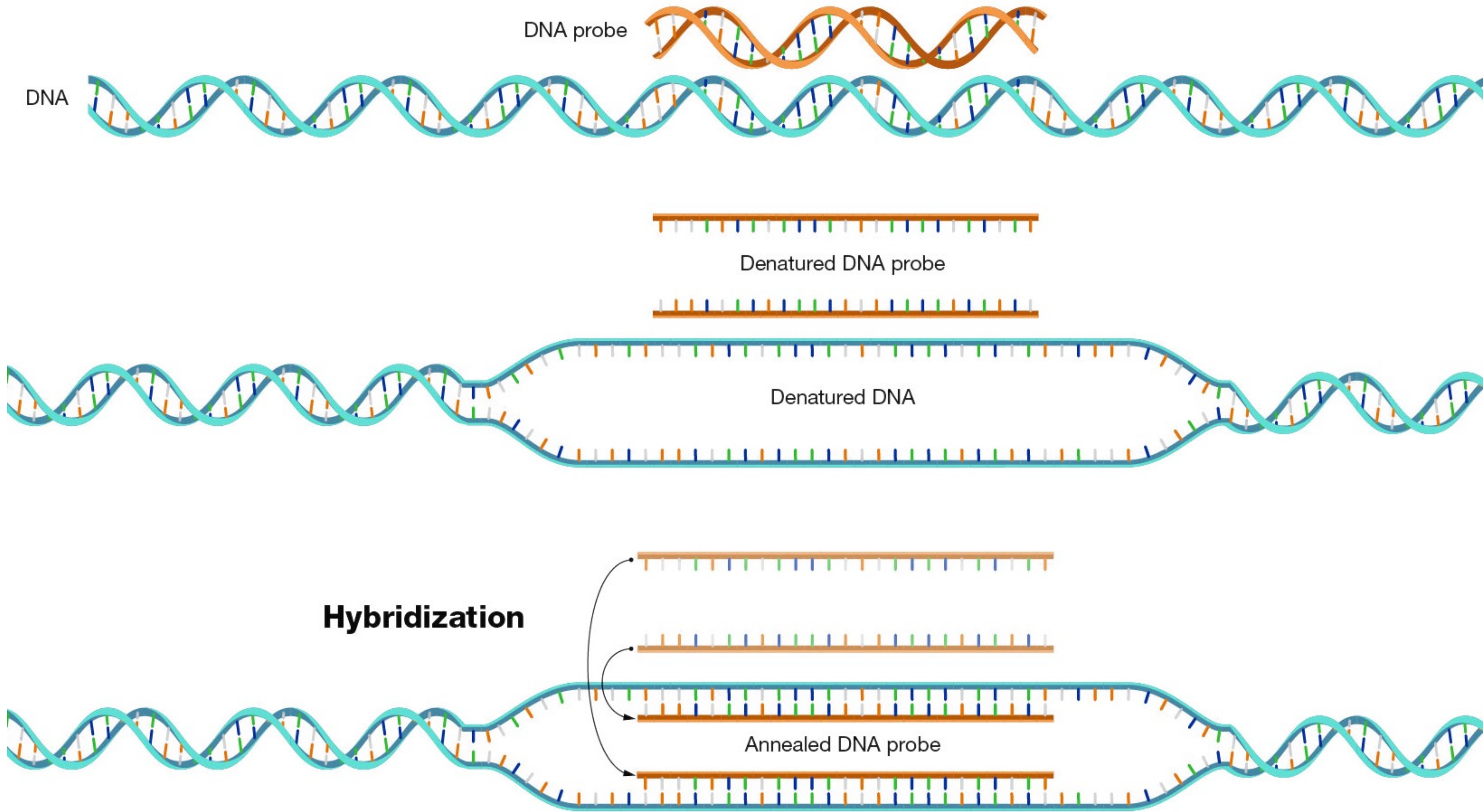
- **Hybridization** – is the process in which two complementary single-stranded DNA and/or RNA molecules bond together to form a double-stranded molecule

- The bonding is dependent on the appropriate base-pairing across the two single-stranded molecules

- Hybridization is an important process in various research and clinical laboratory techniques.



How does it work?



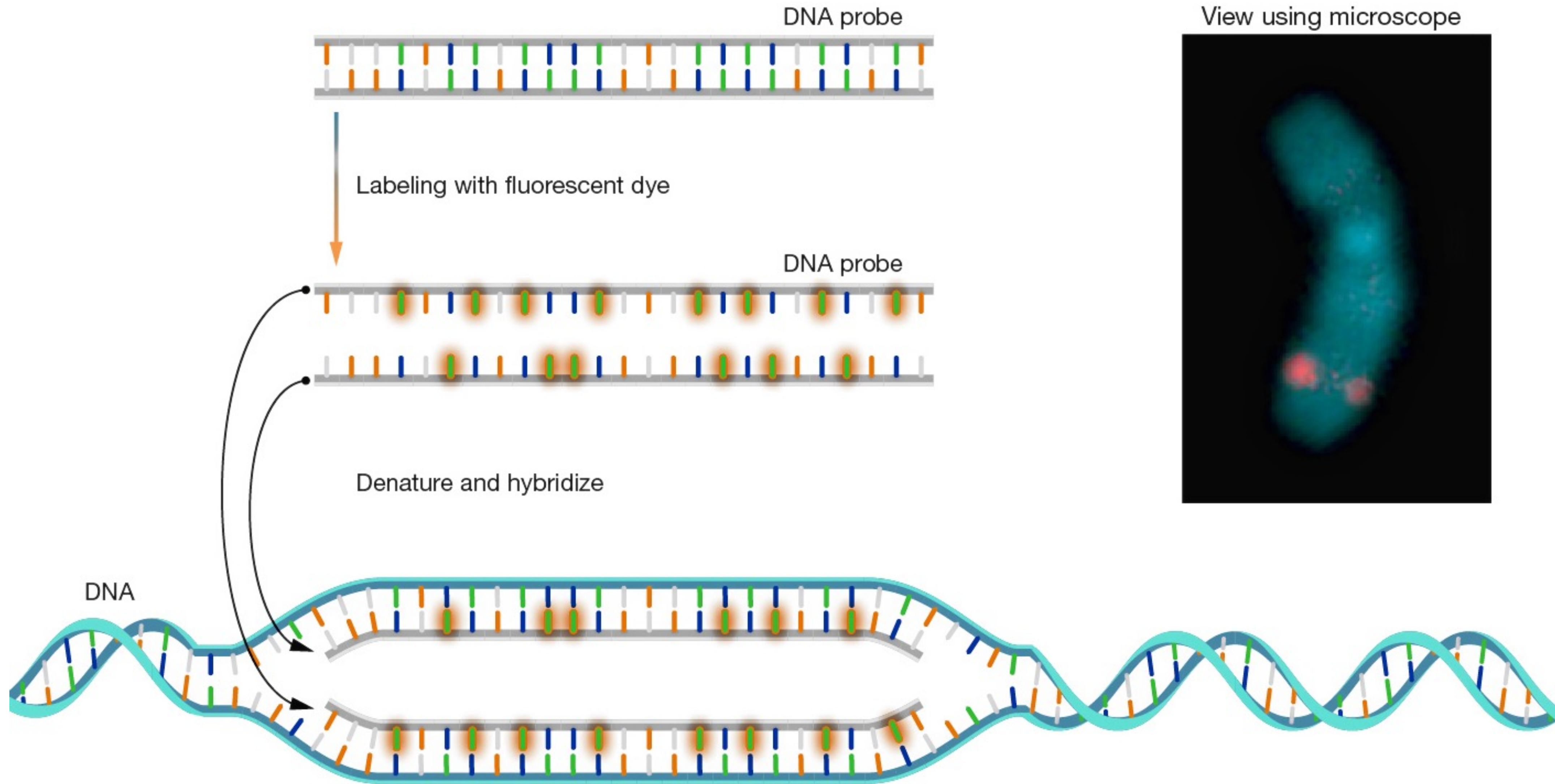
Hybridization: pros and cons

+	−
Specificity of detection	Limited method
High sensitivity	
Exploring the genetic structure	Risks of contamination
Genetic linkage analysis	

- **FISH:** This method uses probiotic molecules (samples) containing complementary DNA or RNA sequences to bind to specific genetic sequences in microorganisms

- Samples are **labeled with fluorescent tags**, allowing visualisation and identification of microorganisms under a microscope.

How does it work?



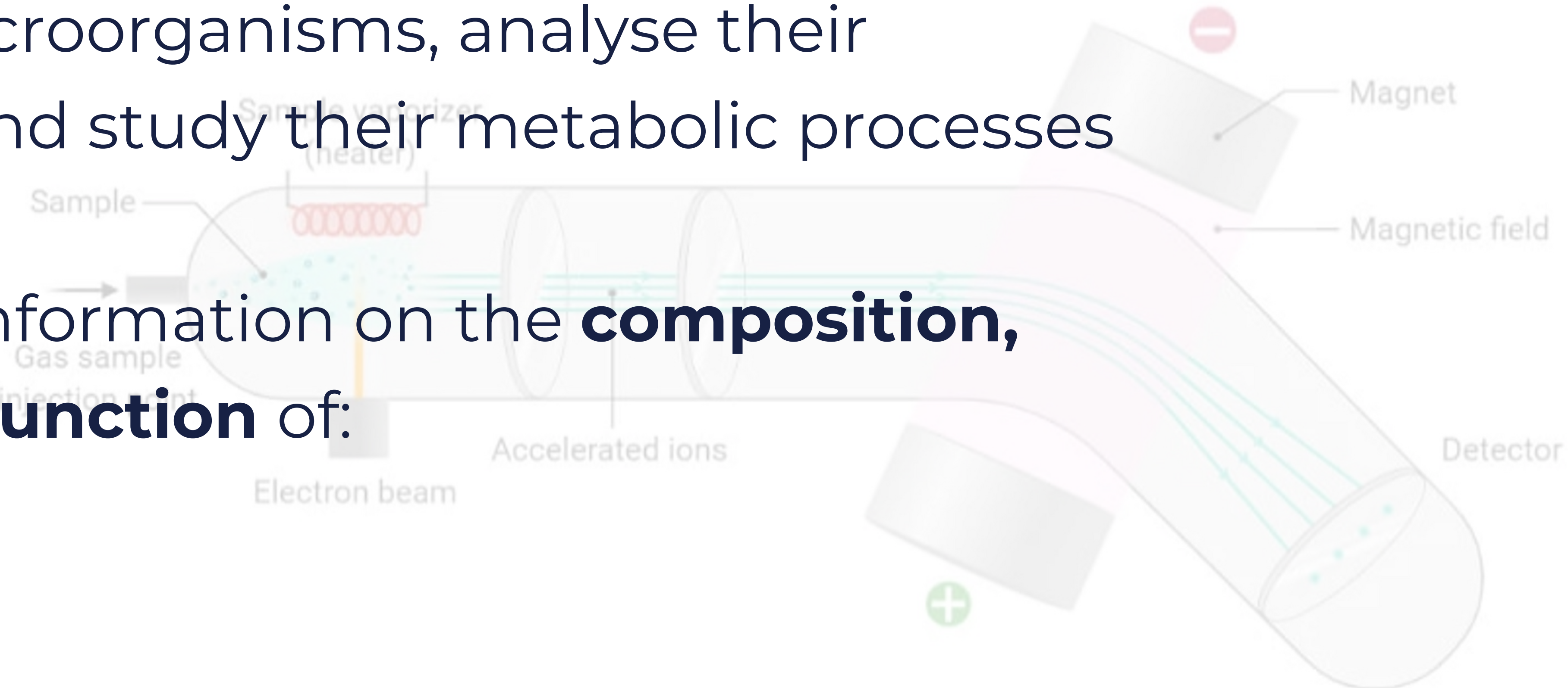
FISH: pros and cons

+	−
Microbial detection and identification	Limitation in the number of target organisms
Simplicity and speed	Possibility of cross-reaction
Spatial information	Restrictions on the structure of the samples
High sensitivity	Limited quantitative analysis

Mass spectrometry

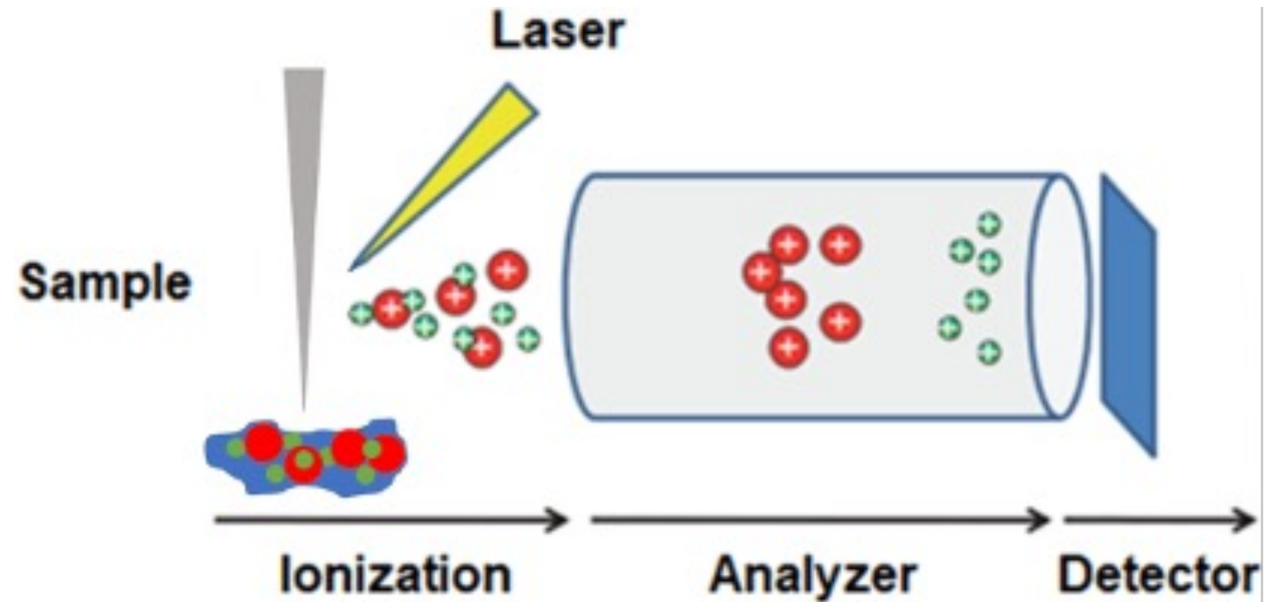
- **Mass spectrometry** in microbiology is used to analyse and identify microorganisms, analyse their biomolecules and study their metabolic processes

- It can provide information on the **composition, structure and function** of:
  - proteins
  - nucleic acids
  - lipids
  - other molecules in microorganisms



How does it work?

Mass spectrometer



Workflow

Mass spectrometry: pros and cons

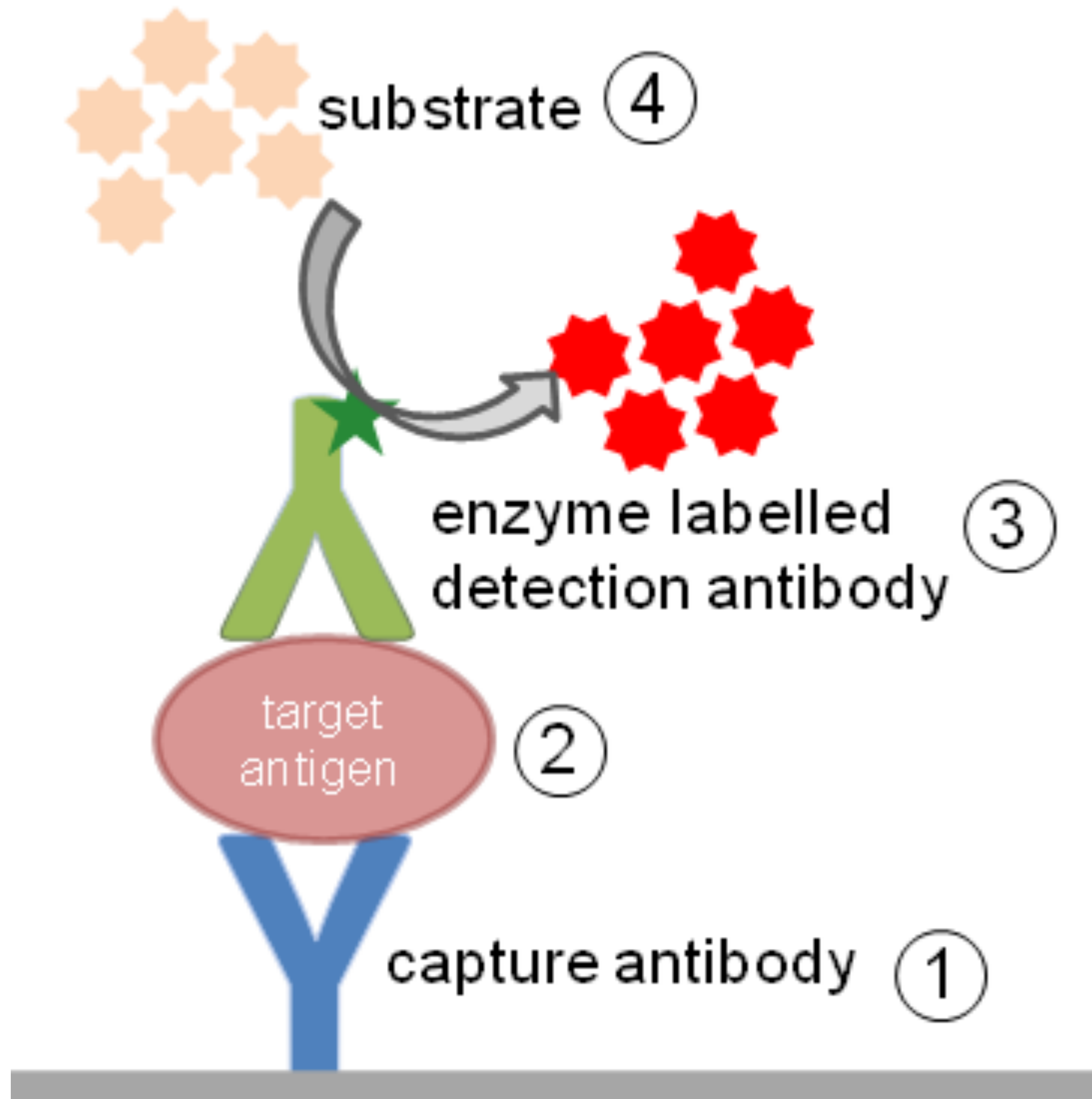
+	−
High sensitivity	Complexity and equipment requirements
High precision and resolution	Sample preparation
Identification and structural analysis	Limitations on sample size
Multiplexing	Interpretation of results

Enzyme-linked immunosorbent assay (ELISA)

- **ELISA –** is an immunological assay commonly used to measure **antibodies, antigens, proteins and glycoproteins** in biological samples

- Each ELISA measures a specific antigen, and kits for a variety of antigens are widely available

How does it work?



There are many other modifications!

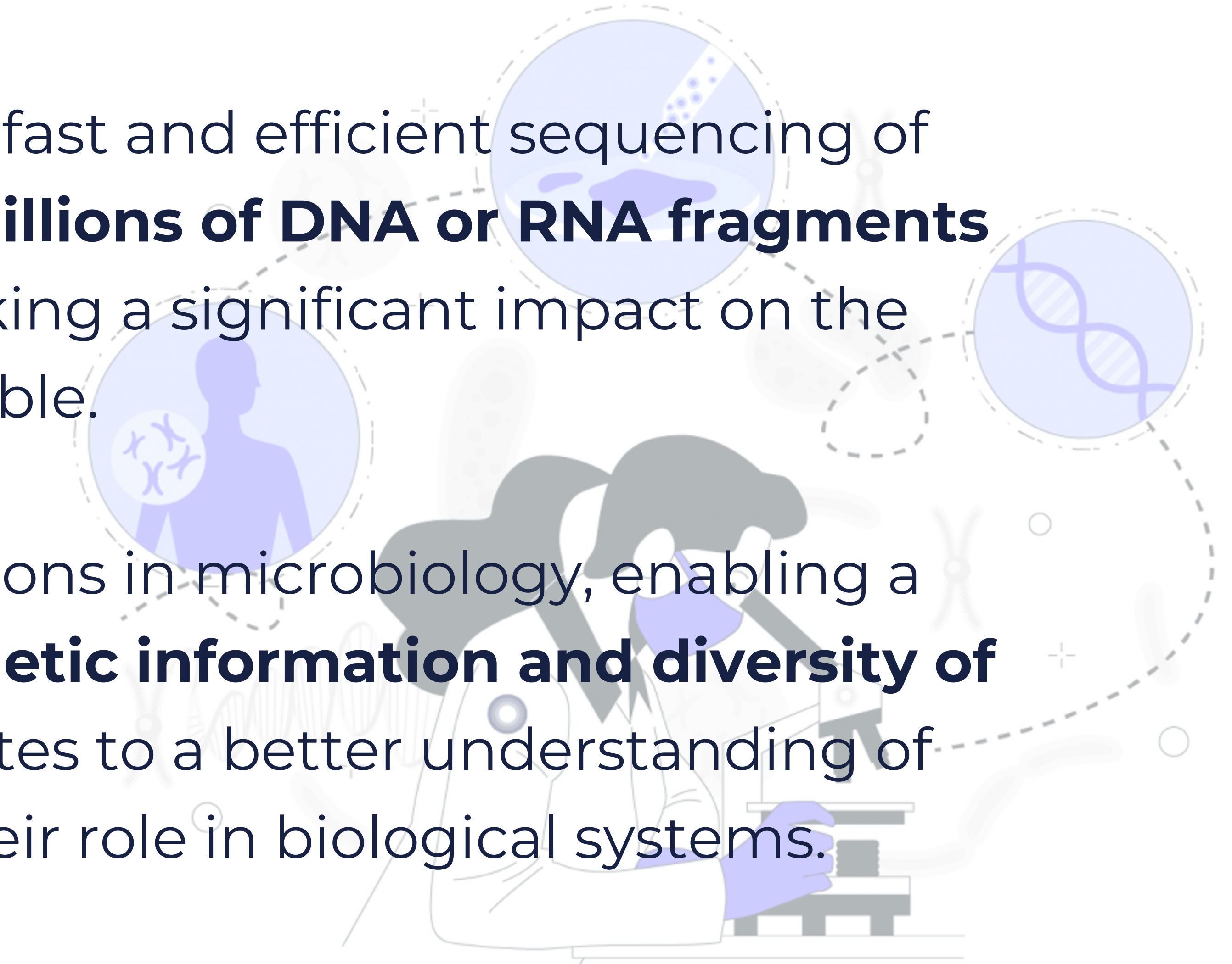
ELISA: pros and cons

+	−
High sensitivity and specificity	Possibility of cross-reaction
Wide range of applications	Limited diversity of antigens
Simplicity and speed	Possibility of false results
Quantitative analysis	Need for specialised equipment and reagents

Next Generation Sequencing (NGS)

- **NGS** is a technology that enables fast and efficient sequencing of DNA or RNA samples. **It allows millions of DNA or RNA fragments to be sequenced in parallel**, making a significant impact on the speed and scope of analysis possible.

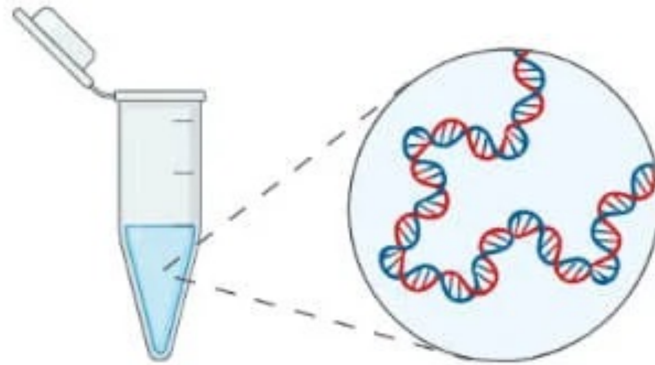
- **NGS** has a wide range of applications in microbiology, enabling a better **understanding of the genetic information and diversity of microorganisms**, which contributes to a better understanding of microbiological processes and their role in biological systems.



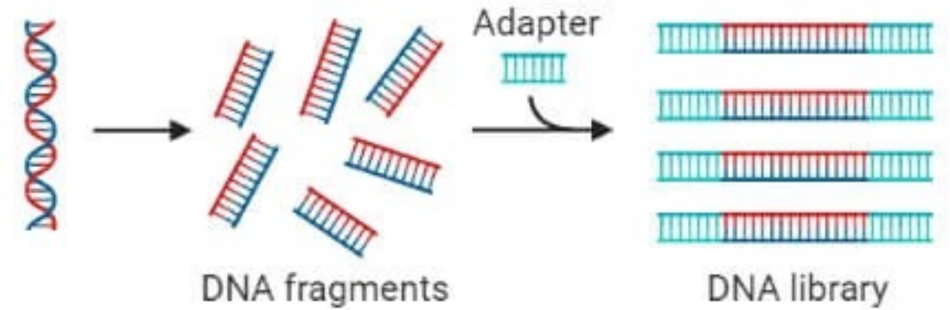
How does it work?

Next Generation Sequencing Workflow

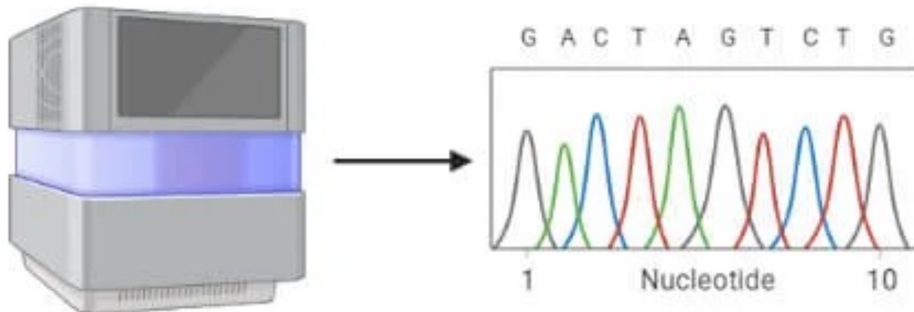
Step 1:
DNA extraction



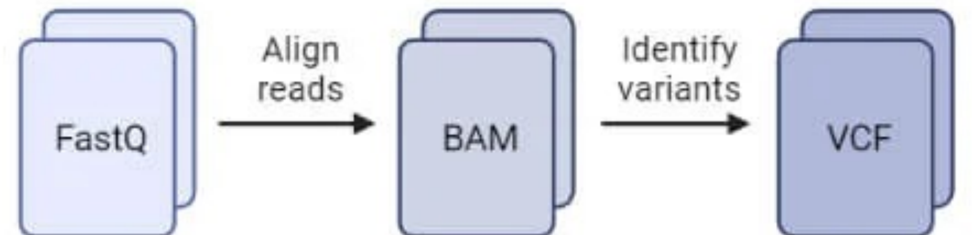
Step 2:
Library preparation



Step 3:
Sequencing

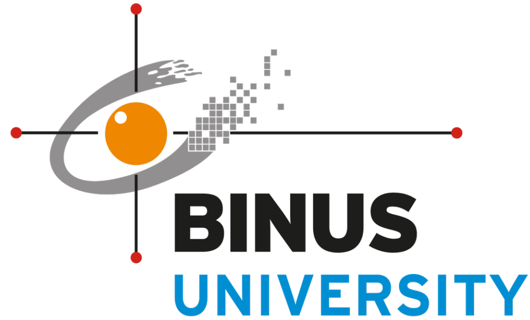


Step 4:
Analysis



NGS: pros and cons

+	−
High speed and high capacity	Errors and inaccuracies
High resolution capability	High costs
Discovery of new microbial species and genes	**Headache with analysing the data…**



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