Microbial Identification (contd)

Modern Methods

- Although still widely used, traditional identification methods suffer from **two major drawbacks**.

- **Firstly,** they are **only applicable to organisms that can be cultured in vitro**.

- **Secondly**, some strains exhibit **unique biochemical characteristics** that **do not fit the pattern** of any known genus and species.

- Fortunately, many **modern methods** are **not dependent upon live cultures**, and they can often **reveal minute differences** between organisms that escape detection by traditional means.

The **modern** methods include:

1. **PCR-Based Identification**
2. **Microarray-Based Identification**
3. **Immunological Methods --- ELISA**
4. **FISH (fluorescence in situ hybridization)**
5. **Chemical Methods**
6. **Metabolic Profiles**
7. **DNA Sequencing**

PCR-Based Identification

- As the name implies, it is a **chain reaction**, a small fragment of the DNA section of interest needs to be identified which serves as the **template** for producing the primers that initiate the reaction.

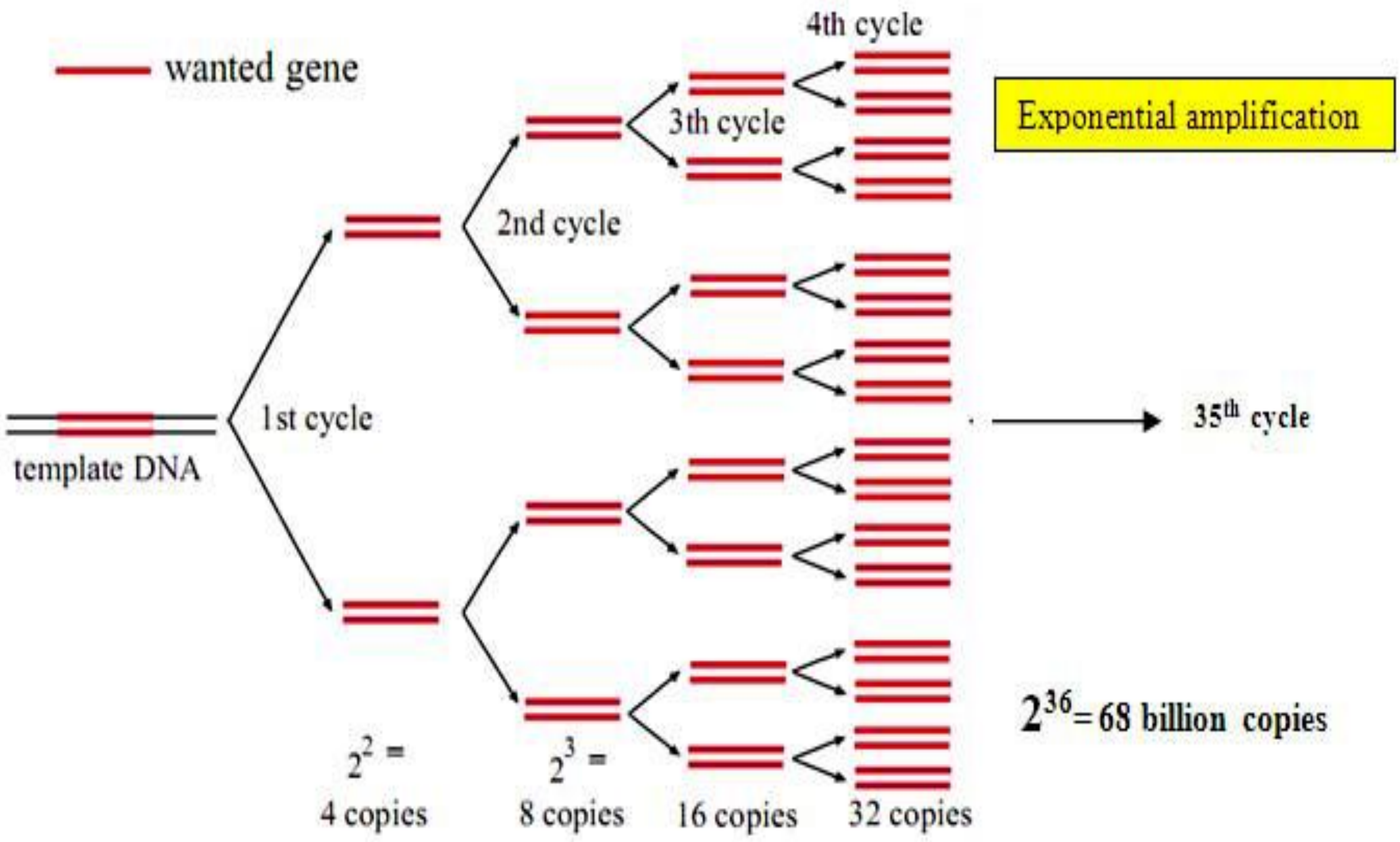
- **One DNA molecule** is used to **produce two copies, then four, then eight and so forth.**

- This **continuous doubling** is accomplished by specific proteins known as **polymerases**, enzymes that are able to **string together individual DNA building blocks** to form long molecular strands.

- To do their job, polymerases require a supply of DNA building blocks, i.e., the nucleotides consisting of the four bases **adenine (A), thymine (T), cytosine (C)** and **guanine (G)**.

- They also need a small fragment of DNA, known as the **primer**, to which they attach the building blocks as well as a longer DNA molecule to serve as a **template** for constructing the new strand.

- If these three ingredients are supplied, the enzymes will construct **exact copies of the templates**.



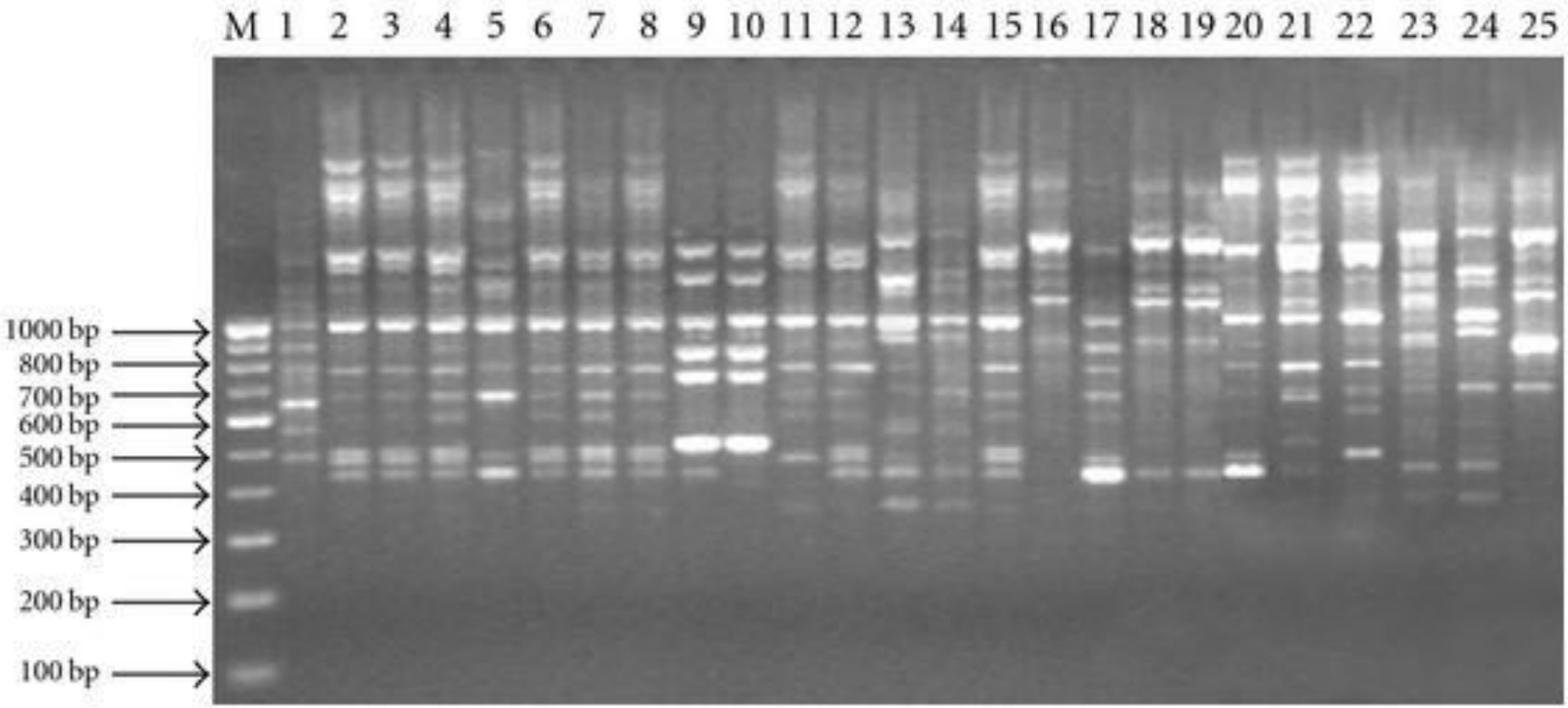
wanted gene
4th cycle
3th cycle
2nd cycle
1st cycle
template DNA
Exponential amplification
35th cycle
2^{36}= 68 billion copies
2^2 =
4 copies
2^3 =
8 copies
16 copies
32 copies

- **PCR, including Real-Time PCR** is probably the **most widely used** molecular technique for microbial identification.

- Using PCR, one can **rapidly detect and identify microbial species directly from clinical samples**, thus speeding up diagnostic procedures.

- Several PCR-based methods exist.

- The **16S rRNA gene** is the gold standard sequence **for bacterial identification by PCR.**


M 1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
1000 bp
800 bp
700 bp
600 bp
500 bp
400 bp
300 bp
200 bp
100 bp

Microarray-Based Identification

- A **DNA microarray** (also commonly known as **DNA chip or biochip**) is a **collection of microscopic DNA spots** attached to a solid surface.

- The basic principle behind microarrays is **hybridization between two DNA strands.**

- **In hybridization, complementary nucleic acid sequences specifically pair with each other by forming hydrogen bonds.**

Hybridization is the process of combining two complementary single-stranded DNA or RNA molecules and allowing them to form a single double-stranded molecule through base pairing.

- A **high number** of complementary base pairs in a nucleotide sequence means **tighter non-covalent bonding** between the two strands.

- After **washing off** non-specific bonding sequences, only **strongly paired strands will remain hybridized**.

- **Fluorescently labeled probes** generate a **signal** when they bind to **target sequence.**

- Total **strength** of the signal **depends** upon the **amount of target sample binding to the probes** present on that spot**.**

- Microarray-based microbial identification relies on the hybridization of **pre-amplified microbial DNA sequences** to **arrayed (arranged) species-specific oligonucleotide probes**.

- Each probe contains a **distinct dye** that fluoresces upon hybridization.

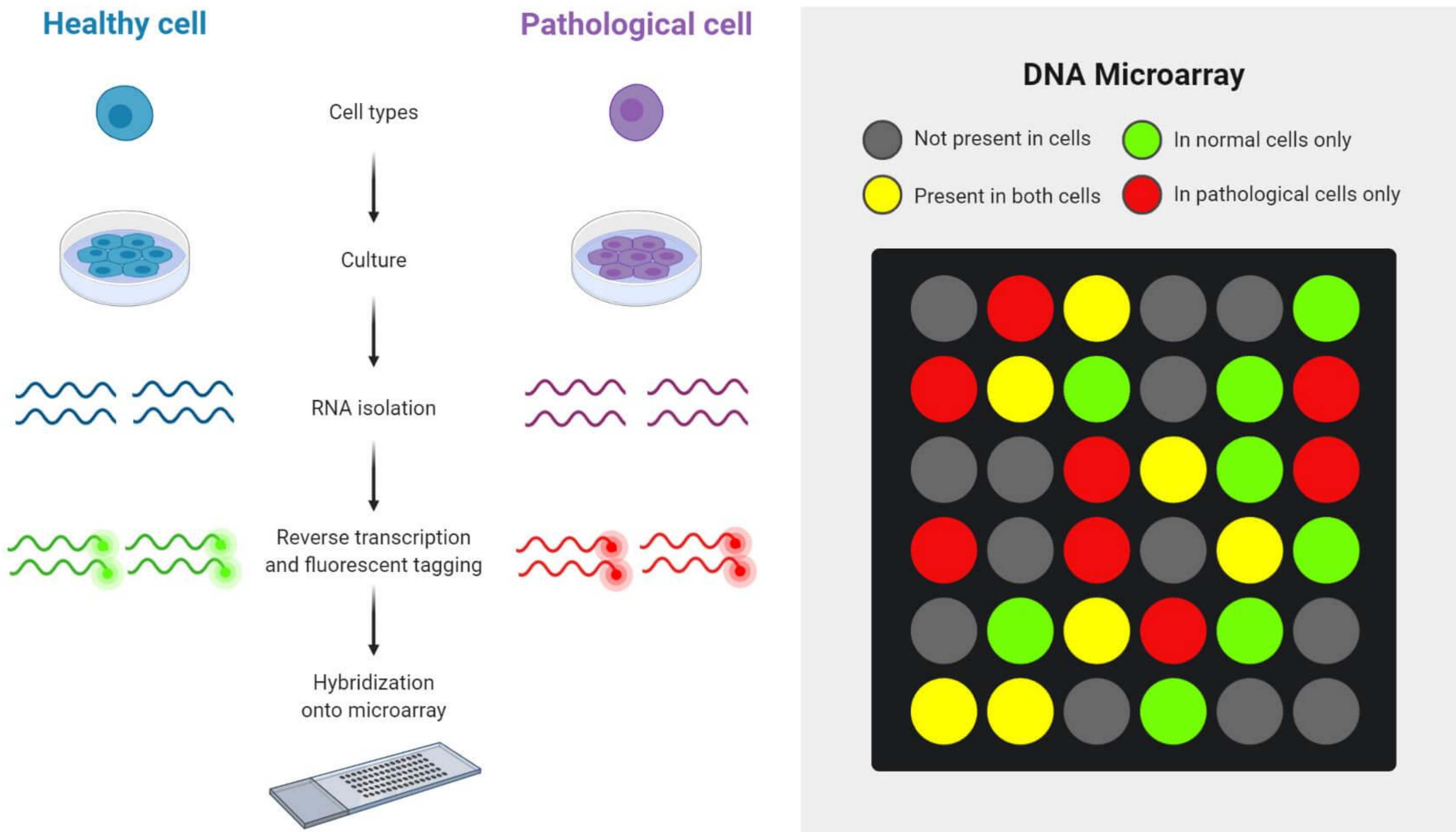


Image By Sagar Aryal, created using biorender.com

- Microarrays are a versatile tool, which facilitate the detection and discrimination of **hundreds** of different microbial samples **on a single slide.**

- Microarray is a **fast technique**, and speed is important, e.g., for diagnosis and timely start of proper antimicrobial therapy.

Immunological Methods

ELISA-based methods

- **ELISA-based methods** can be set up for microbial detection (usually within **diagnostics**) on a species-by-species basis.

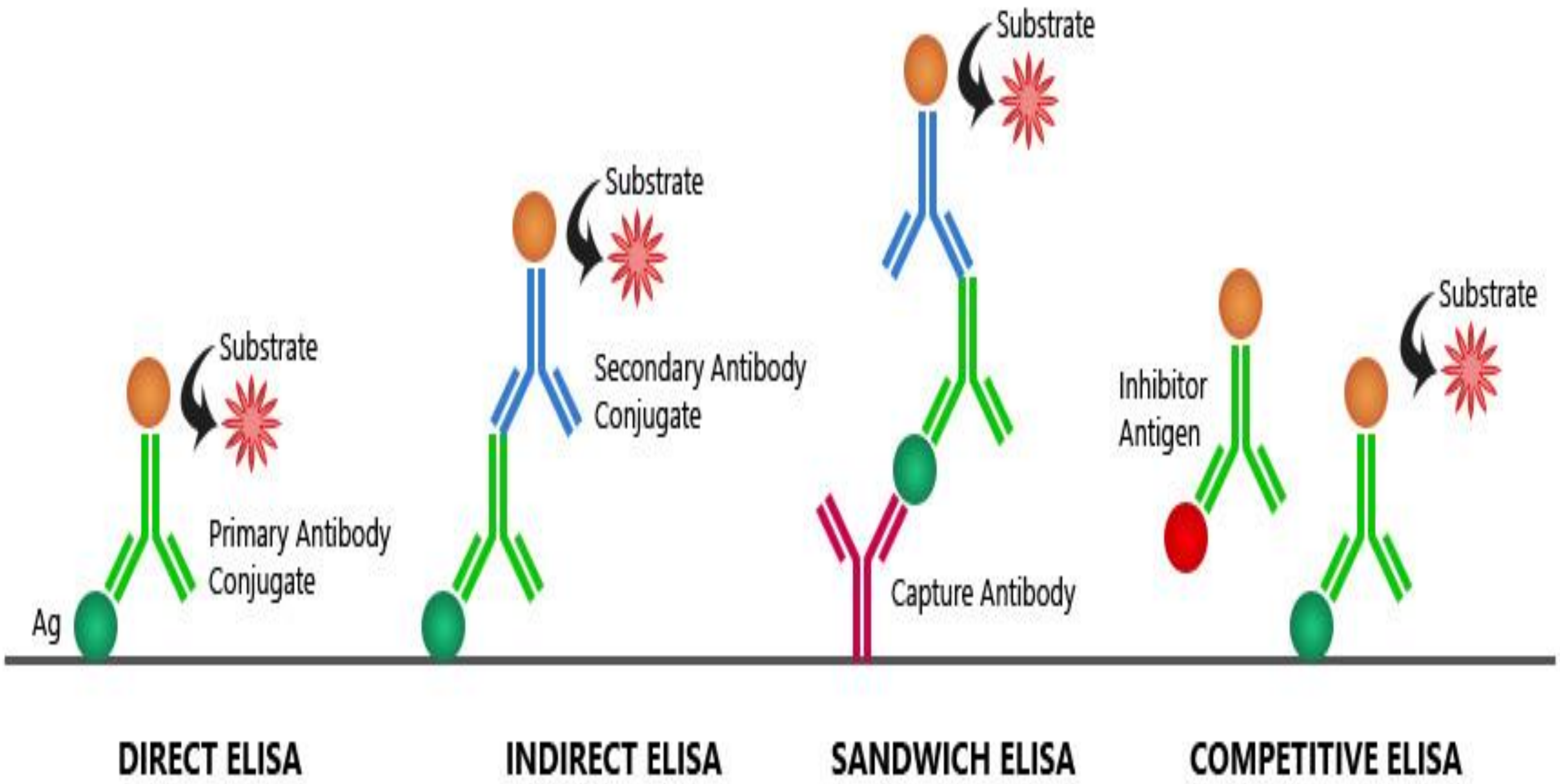
- In ELISA, various antigen-antibody combinations are used, always including an **enzyme-labeled antigen** or **antibody**, and enzyme activity is measured **colorimetrically**.

- The enzyme activity is measured using a **substrate that changes color** when modified by the enzyme.

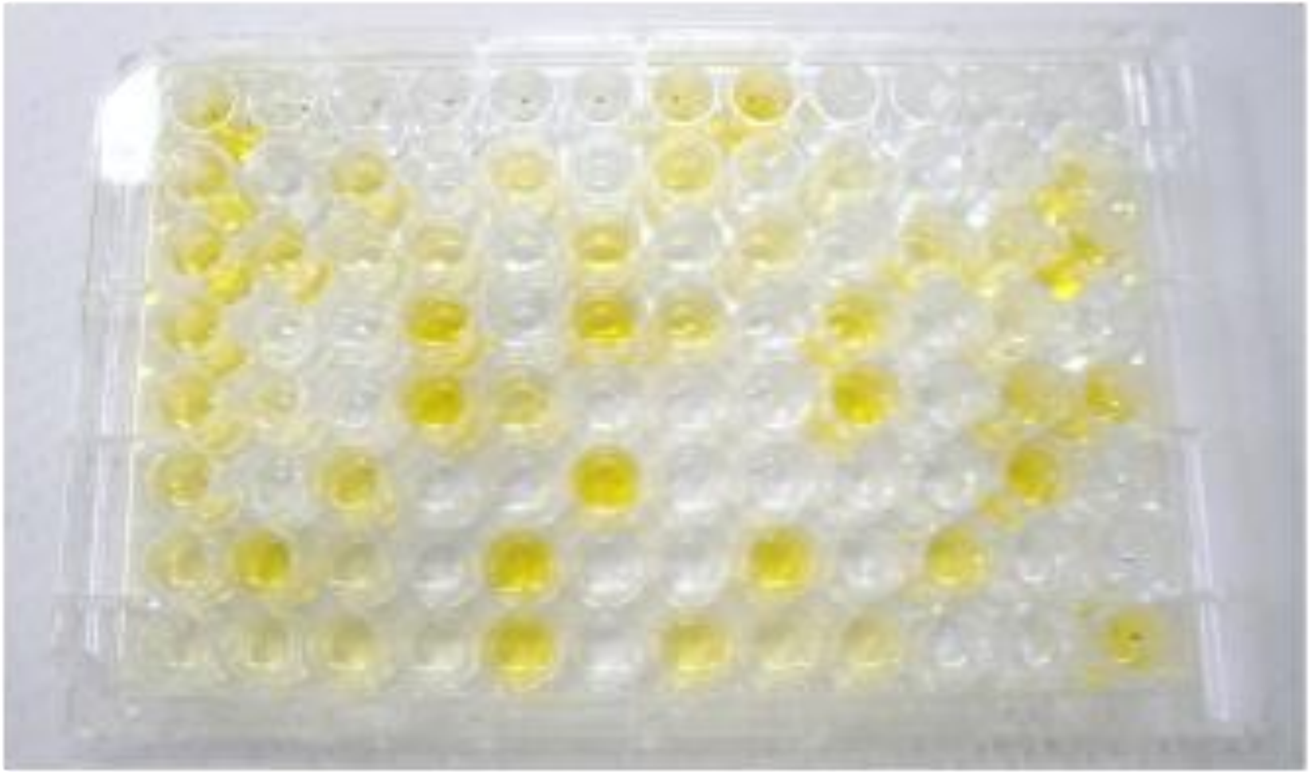
- **Light absorption** of the product formed after substrate addition is measured and **converted to numeric values**.

- Depending on the antigen-antibody combination, the assay is called a **direct ELISA, indirect ELISA, sandwich ELISA, competitive ELISA** etc.

- These methods are **highly sensitive and specific** but rely on very specific antibodies and highly discriminating protein(s) within your organism of interest.


Substrate
Substrate
Substrate
Substrate
Secondary Antibody
Conjugate
Inhibitor
Antigen
Primary Antibody
Conjugate
Capture Antibody
Ag
DIRECT ELISA
INDIRECT ELISA
SANDWICH ELISA
COMPETITIVE ELISA

ELISA: An example of an assay using a 96-well plate.



The yellow color indicates that the target protein is present. The higher degree of the color, the higher concentration of the target protein.

FISH (fluorescence in situ hybridization)

- It is used to detect and localize the presence or absence of **specific DNA sequences** in situ (in the original place).

- FISH uses **fluorescent probes** that bind to those targets that show a high degree of **sequence complementarity**.

- FISH can be used to detect **RNA** or **DNA sequences** of interest.

- FISH can also be used to detect and localize **specific RNA targets, including mRNAs,** in cells.

- FISH is based on the **use of probes**.

- The probe must be **large enough to hybridize specifically** with its target**.**

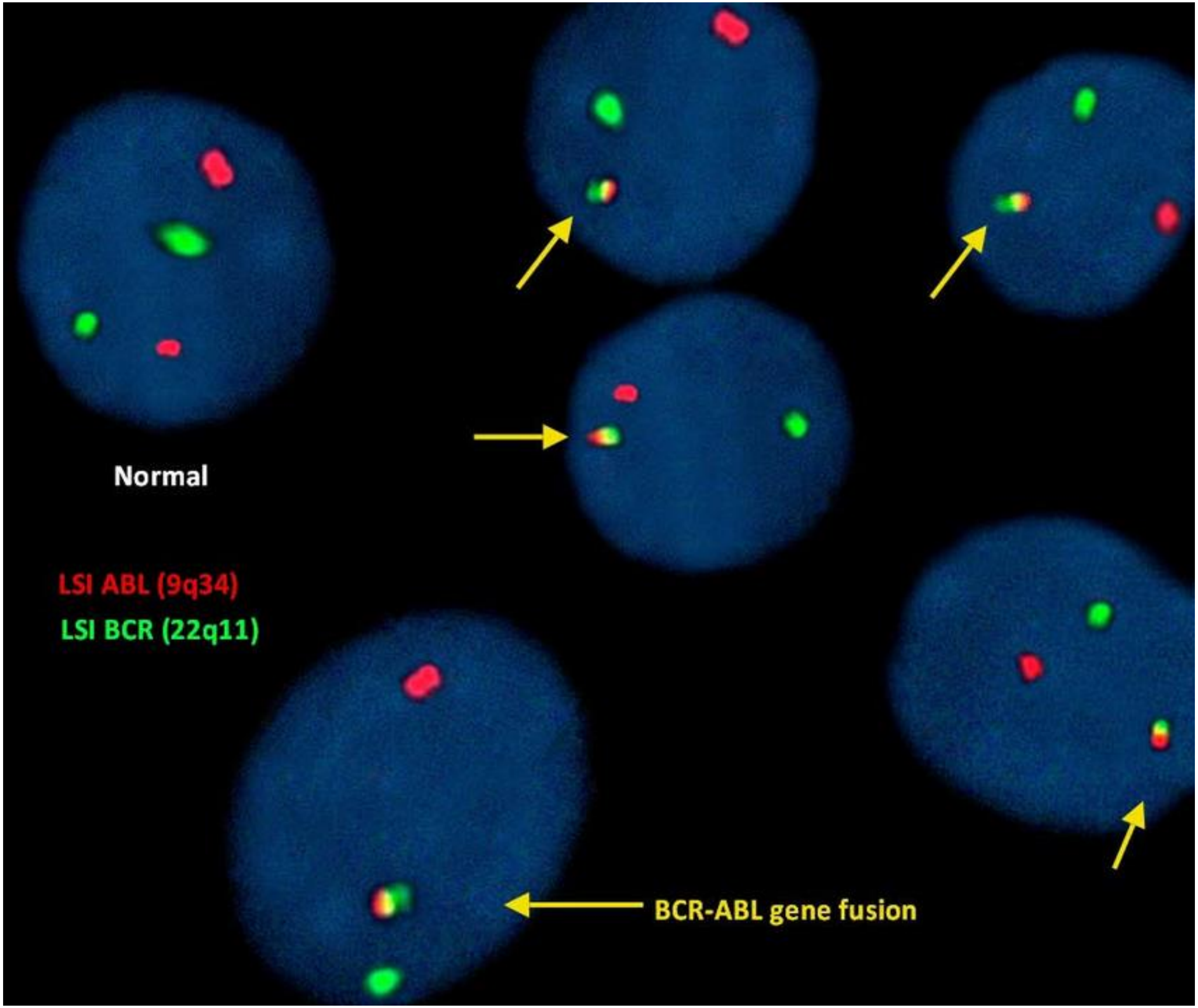
- They are **anti-sense** to the **target mRNA or DNA** of interest, thus they hybridize to targets.

Antisense DNA is the non-coding strand complementary to the coding strand in double-stranded DNA. The antisense strand serves as the template for messenger RNA (mRNA) synthesis.

- The probe are usually tagged **directly with fluorophores.**

- Different types of tags can be used, therefore **different targets can be detected in the same sample** simultaneously (**multi-colour FISH**).

- Probes can vary in length from **20 to 30 nucleotides** to much longer sequences.


Normal
LSI ABL (9q34)
LSI BCR (22q11)
BCR-ABL gene fusion

Chemical Methods

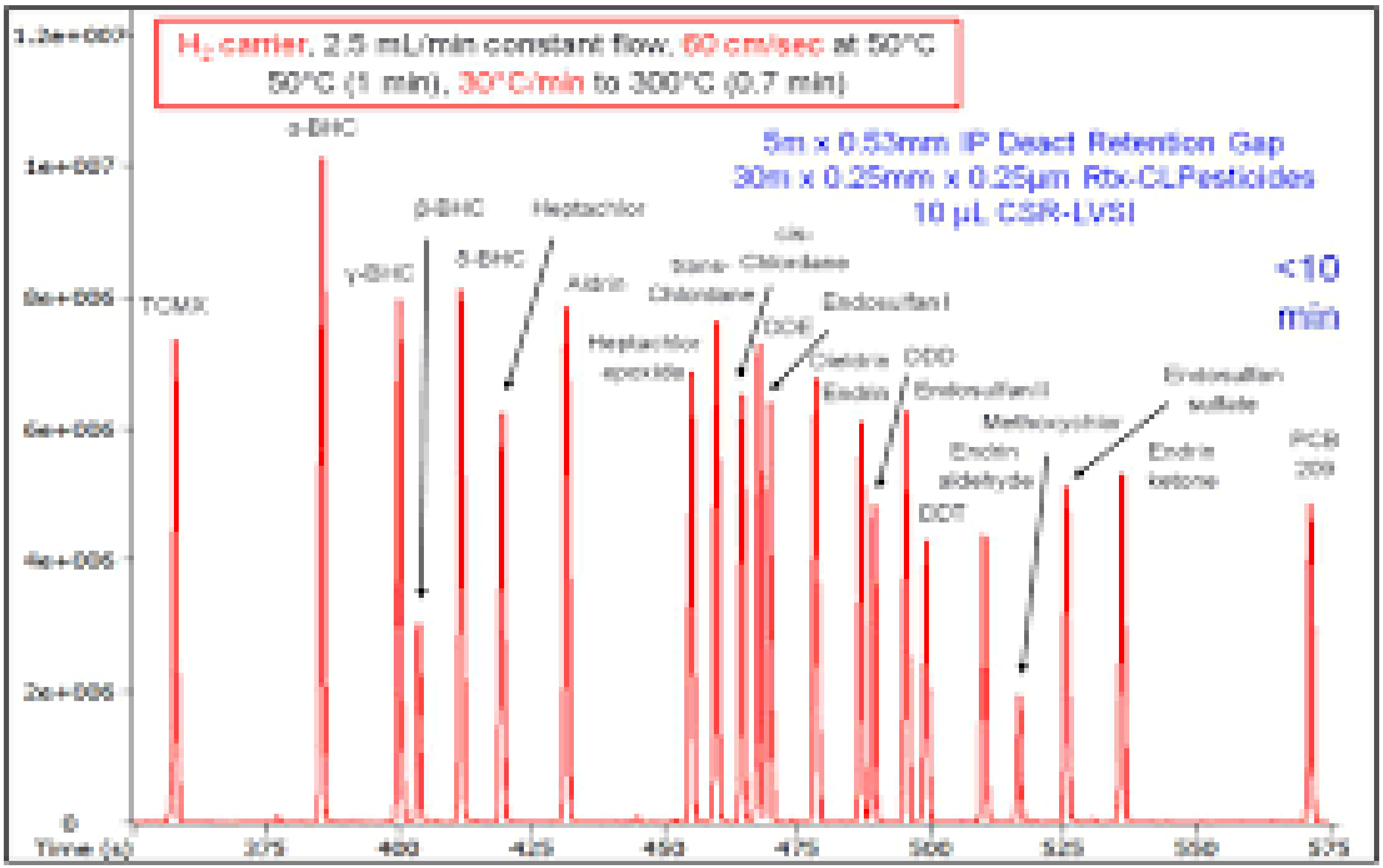
Fatty Acid Profiling

- Fatty acids are essential within bacterial cell membranes, and different bacterial species produce **different combinations of fatty acids.**

- As such, the **profile of fatty acids** obtained from an **unidentified bacterial species** can be used to identify that species by **matching against known fatty acid profiles**.

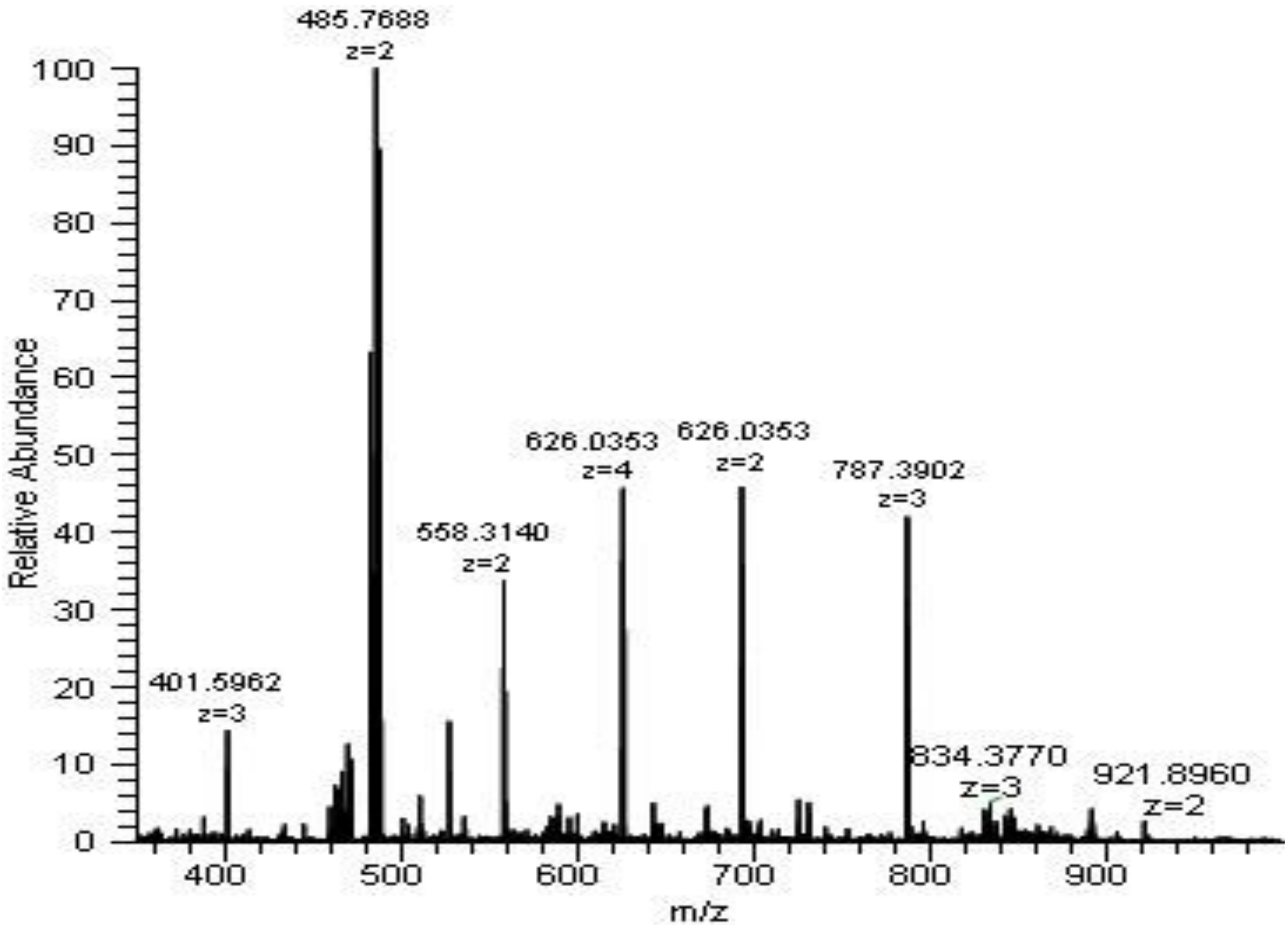
- Fatty acid profiling is typically performed using a **combination of gas chromatography and mass spectrometry.**

Gas chromatography is a common type of chromatography used in analytical chemistry for **separating and analyzing compounds that can be vaporized without decomposition.**



Mass spectrometry (MS) is an analytical technique that **ionizes chemical species and sorts the ions based on their mass-to-charge ratio.**

In simpler terms, a **mass spectrum measures the masses within a sample.**


485.7688
z=2
100
90
80
70
60
50
40
30
20
10
0
Relative Abundance
401.5962
z=3
558.3140
z=2
626.0353
z=4
626.0353
z=2
787.3902
z=3
834.3770
z=3
921.8960
z=2
400
500
600
700
800
900
m/z

Metabolic Profiles

- Besides **primary metabolites (e.g., ATP, ADP)** that are essential for growth, microbes produce a **wide range of secondary metabolites** that are **not essential** for growth.

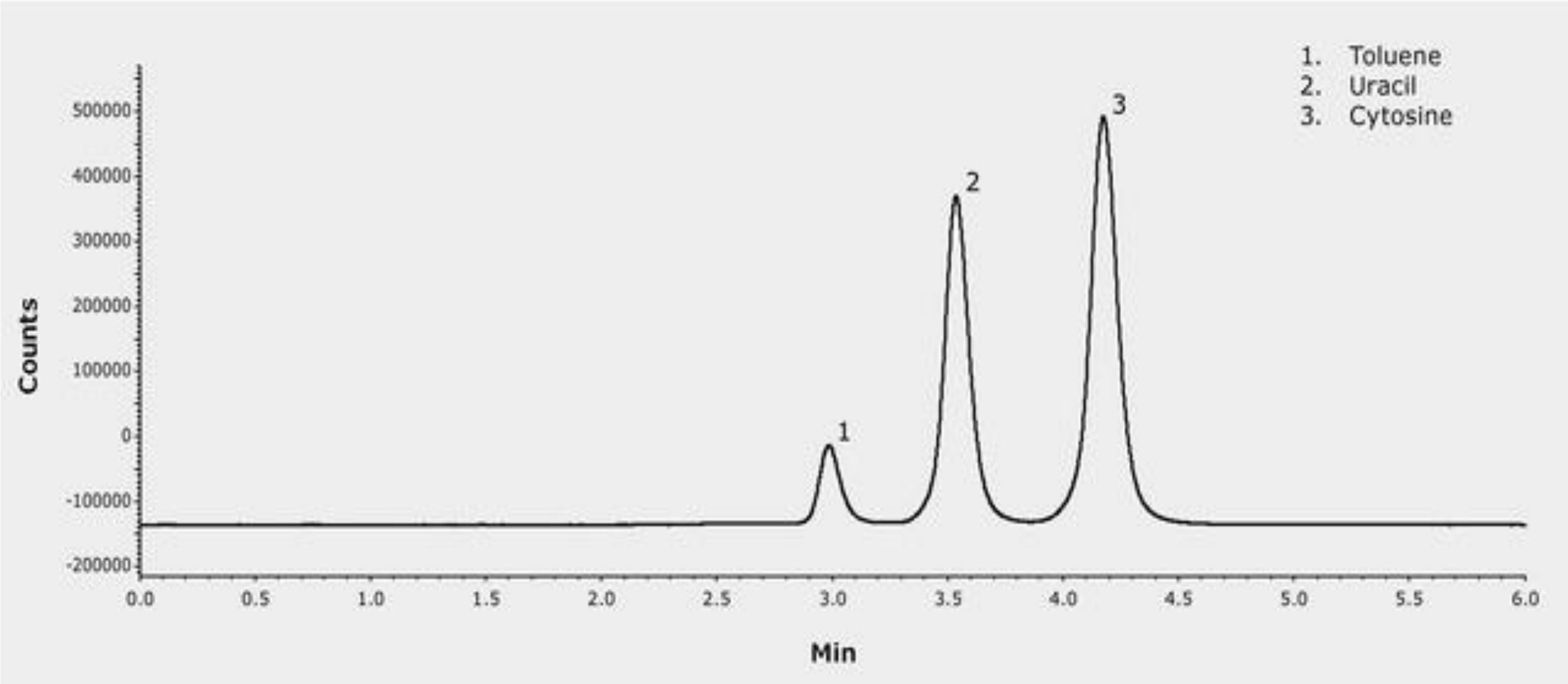
- But they may be advantageous within certain environments, e.g., **outcompeting** other microorganisms for nutrients.

- **Secondary metabolites** include **antibiotics, immunosuppressive compounds, pigments, antioxidants,** and others.

- These metabolites are a **huge source of existing and newly emerging drugs.**

- Different species often produce **unique secondary metabolite profiles**, and these profiles allow us to identify known microbial species.

- Metabolic profiling is usually carried out using **HPLC and mass spectrometry.**

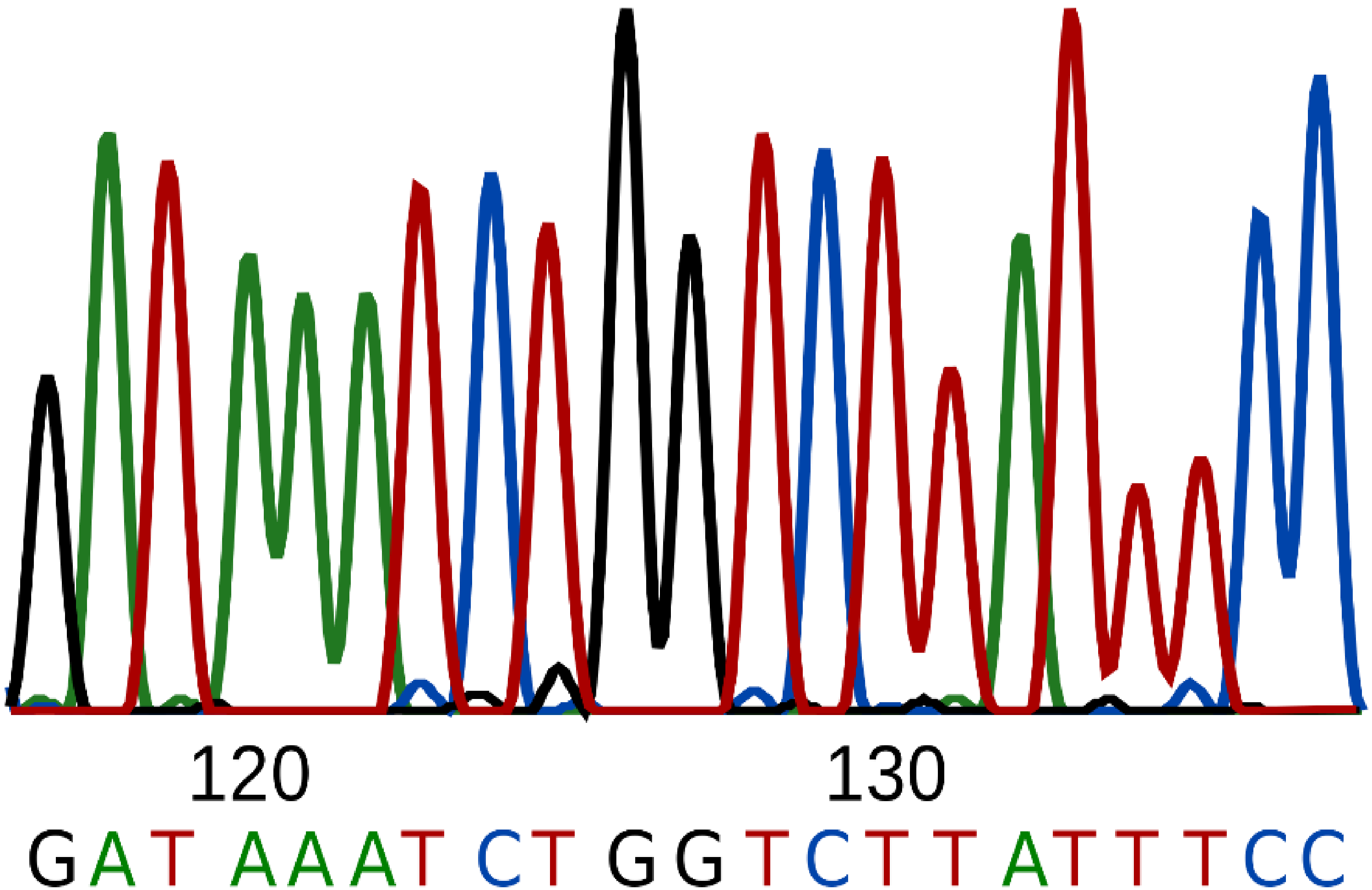


DNA Sequencing

- DNA sequencing is the process of **determining the order of nucleotides in DNA.**

- It includes any method or technology that is used to determine the order of the **four bases**: adenine, guanine, cytosine, and thymine.

- The advent of **rapid DNA sequencing methods** has greatly accelerated biological and medical research and discovery.


120
130
G A T A A A T C T G G T C T T A T T T C C


Thank You!