

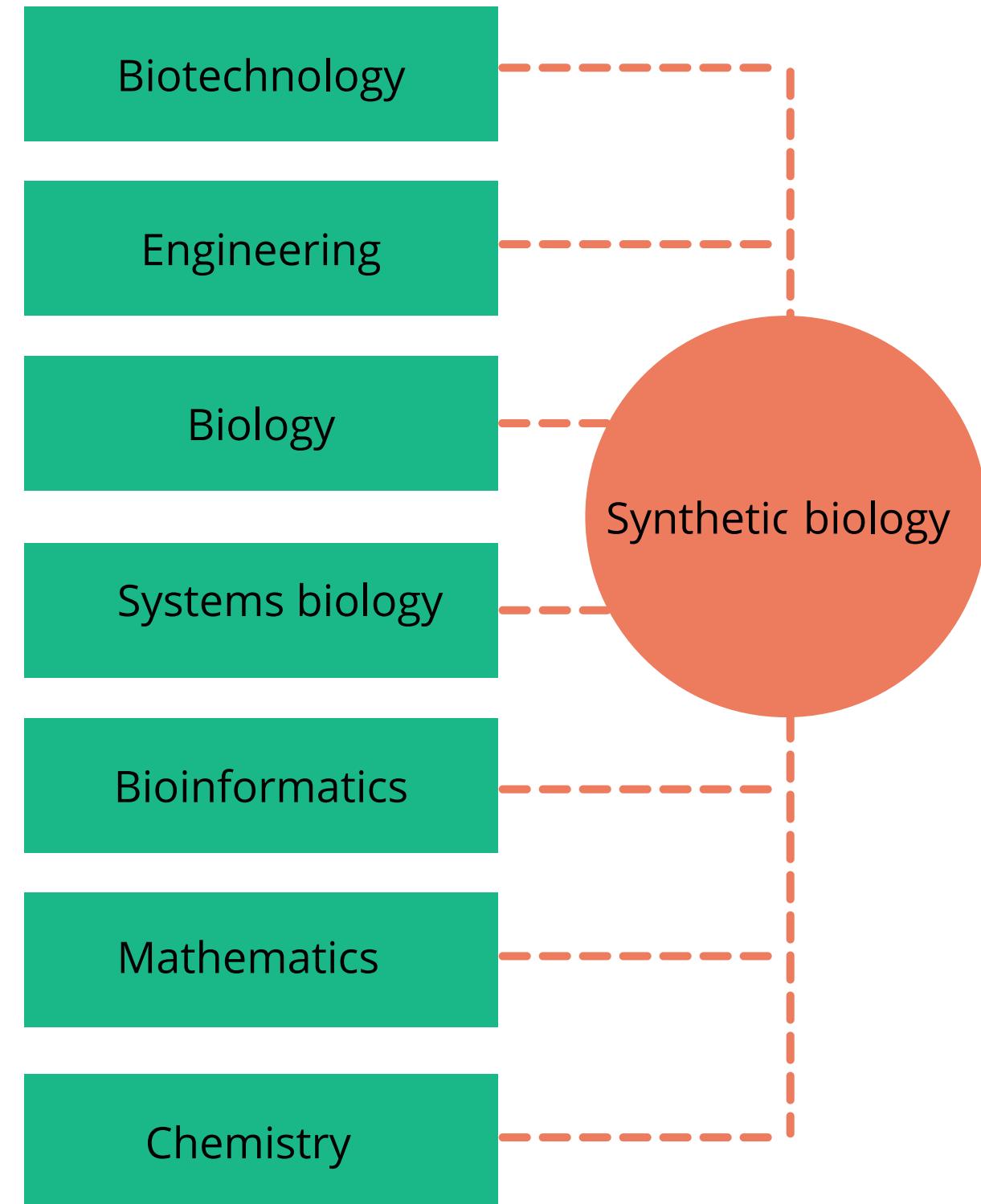
SYN BIO 1.0

an initiative by
the iGEM team of
IISER Thiruvananthapuram



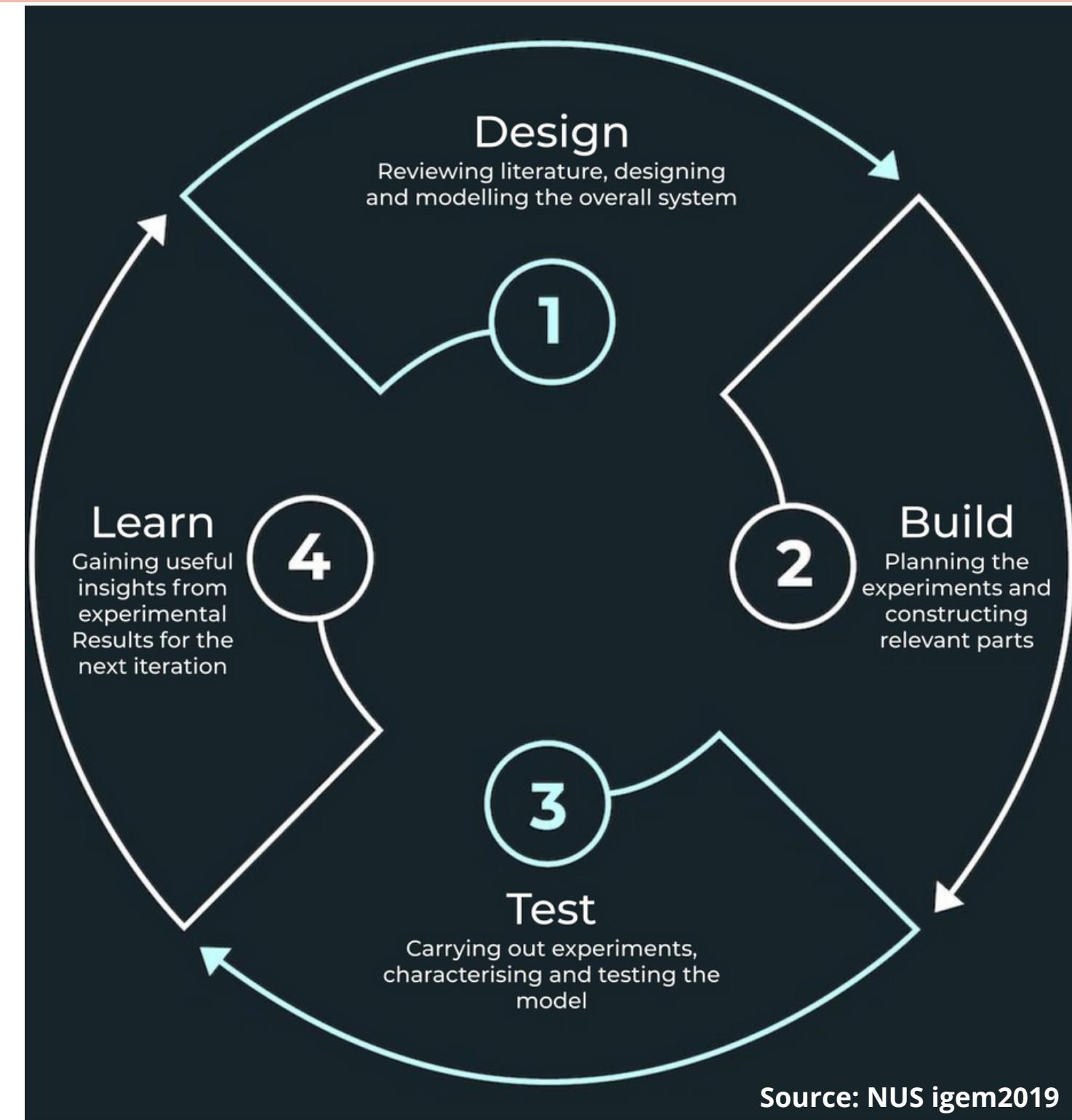
What is Synthetic Biology?

Syn-bio or **synthetic biology** is a relatively new arena of life sciences which deals with **modifying and designing novel biological systems** to resolve the problems which we face .



The Four Milestones in Synbio

1. DESIGN
2. BUILD
3. TEST
4. LEARN



What is iGEM ?



iGEM-International Genetically Engineered Machine, is an annual synthetic biology event where teams across the world come together to design **novel solutions** to address a multitude of problems that the world today faces.

The **iGEM 2021 Team of IISER Thiruvananthapuram** is extremely proud to be the first-ever team from Kerala to take part in this grand event.



DNA: The molecule of life

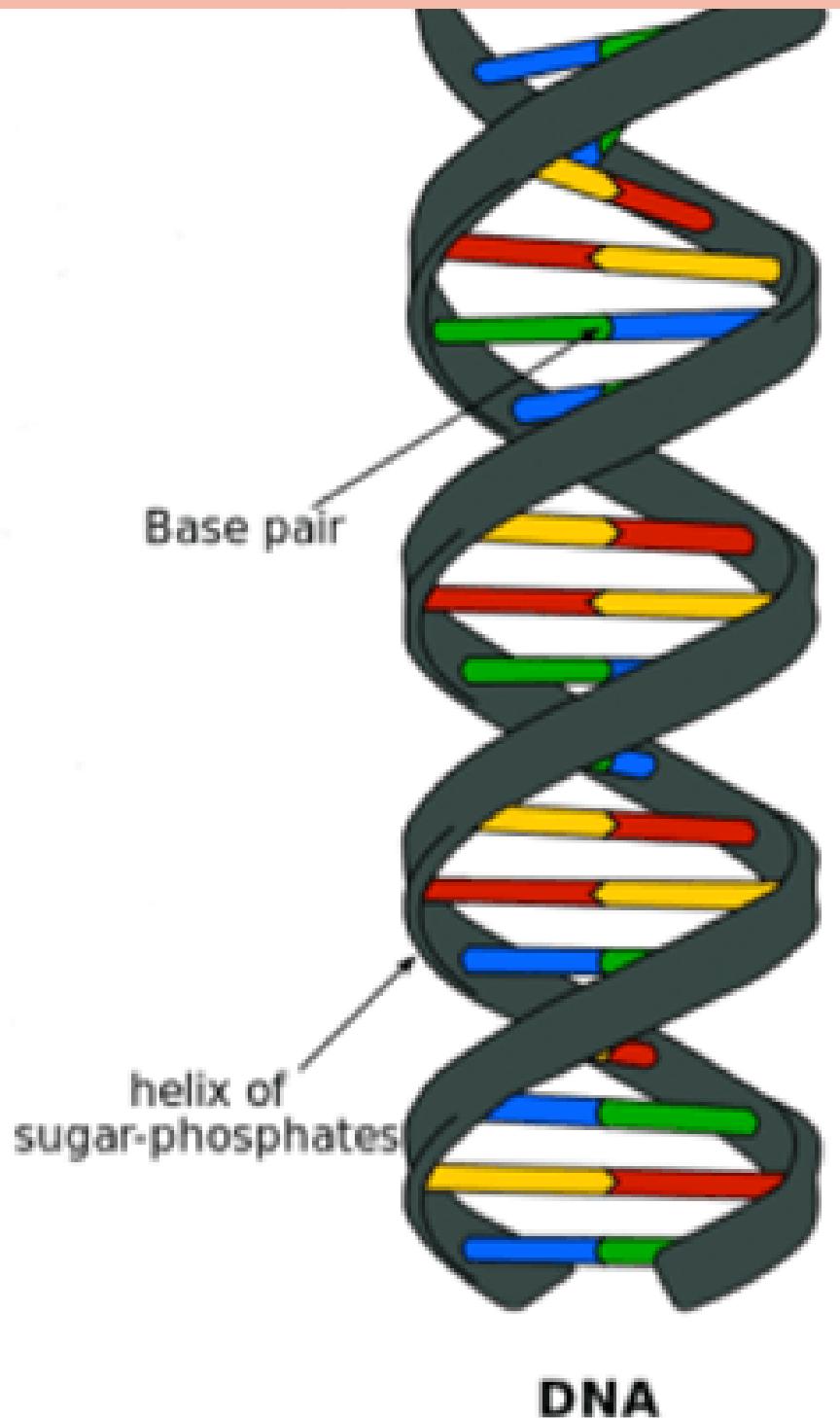
Our **genetic material** that decide all our characteristics.
It is the basic component of our **chromosomes**.

Double helix of sugar-phosphate backbone, nitrogen base pairs, hydrogen bonds.

The four nitrogen bases:

- **Adenine** which pairs with **Thymine**
- **Guanine** which pairs with **Cytosine**

$$A=T, G \equiv C$$



Source: nebula.org/blog/mrna-messenger-rna/

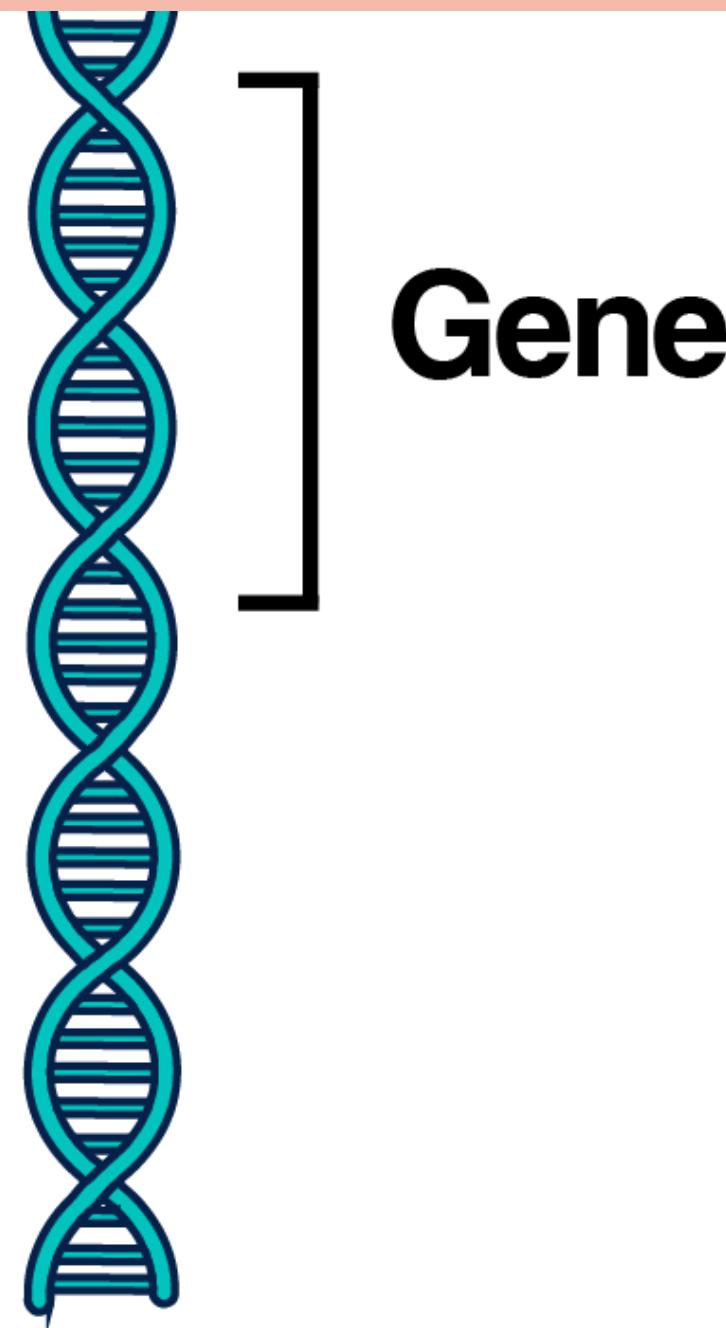
Think further: Imagine I have a DNA fragment with 100 base pairs. 22 of the nucleotides which I have are Guanine. How many Thymine nucleotides are there in my DNA?

Gene

Segment of DNA which are physical and functional **unit of heredity**

The genes are **expressed as proteins** through transcription and translation

Proteins create visible phenotype and also determine how efficiently our body works



DNA

Source: www.dnaexpress.it/glossario/gene/

Think further: We know that our DNA is double-stranded. Since gene is basically a small piece of DNA will both strands of the small piece undergo transcription to form RNA or is it just one strand?

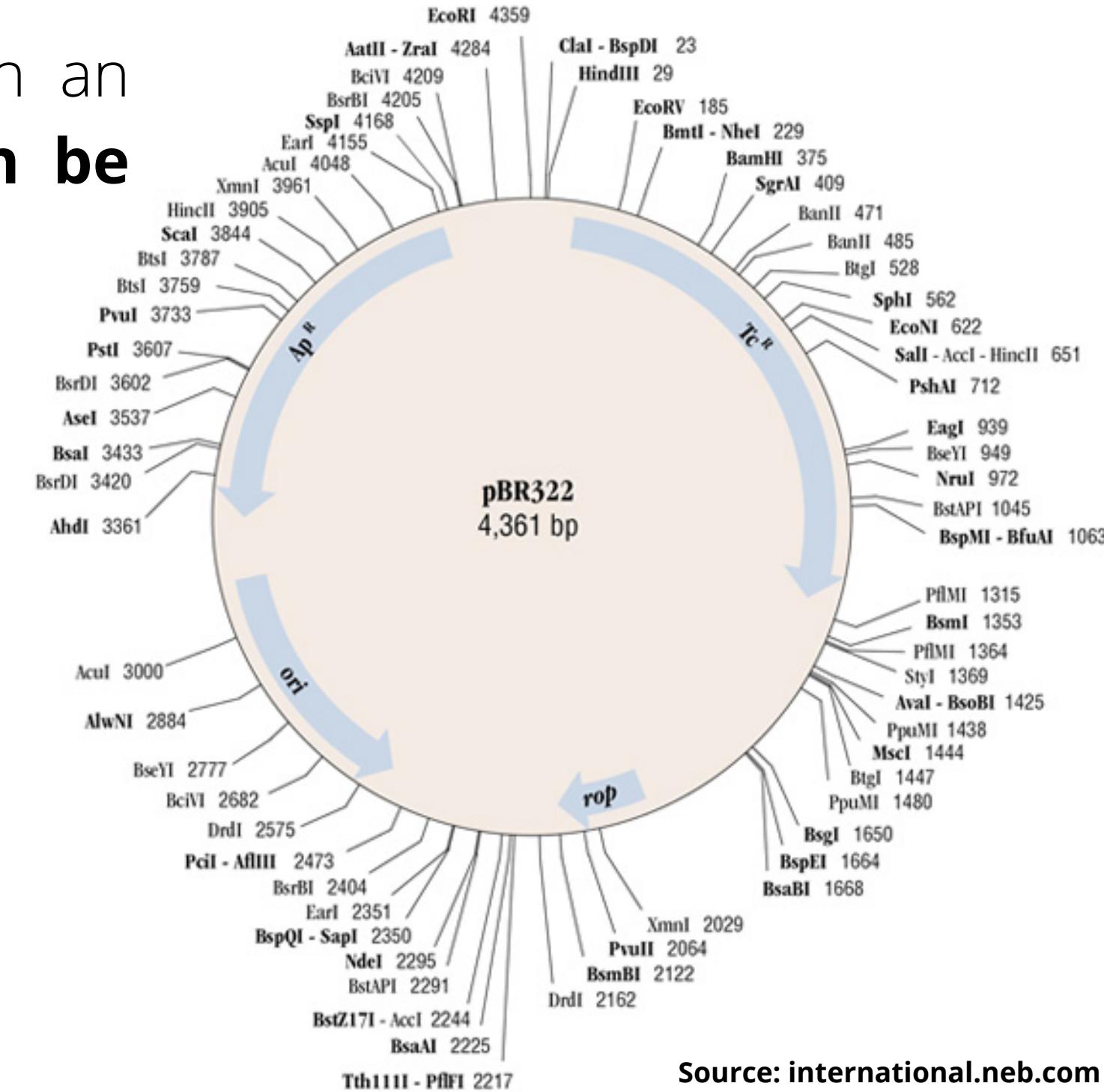
Cloning Vector

A **small piece of DNA** that can be stably maintained in an organism, and into which a **foreign DNA fragment can be inserted** for cloning purposes.

Can be plasmid, bacteriophages

Features required:

1. **Origin of replication (ori)**
2. **Selectable Markers**
3. **Cloning Sites**



Source: international.neb.com

Think further: What happens if the cloning vector do not posses the Ori ?

Competent Host

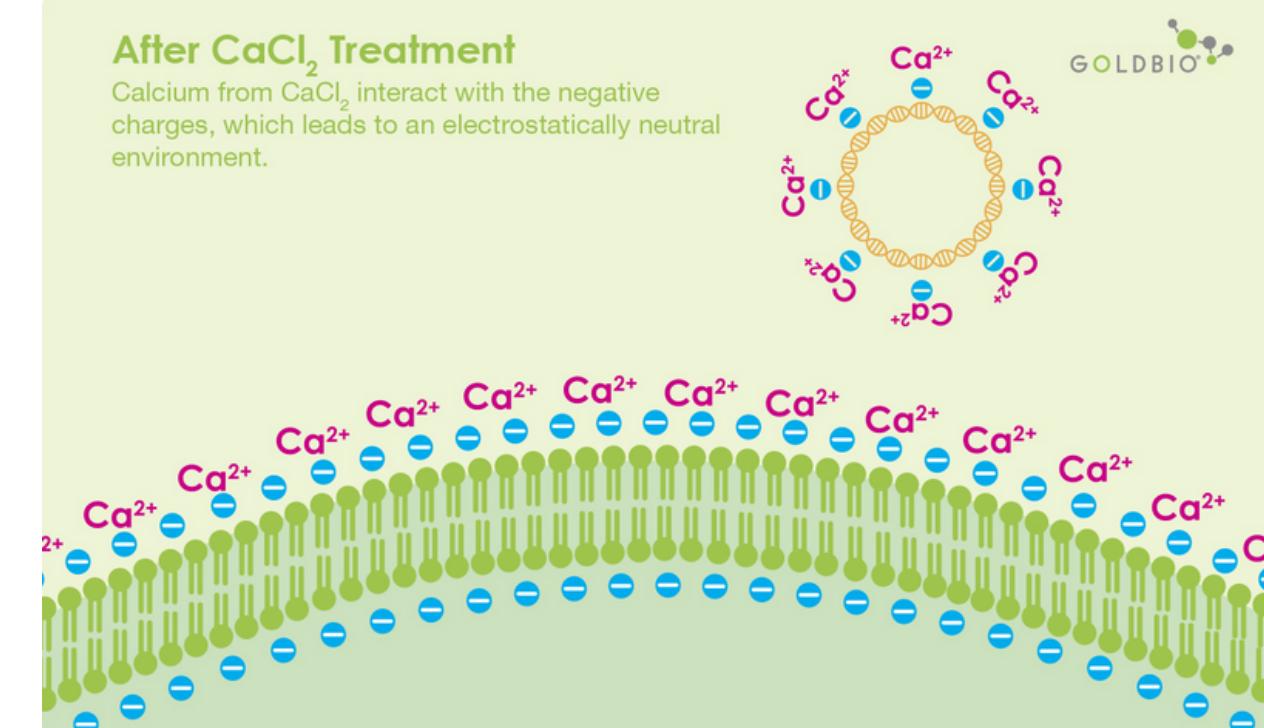
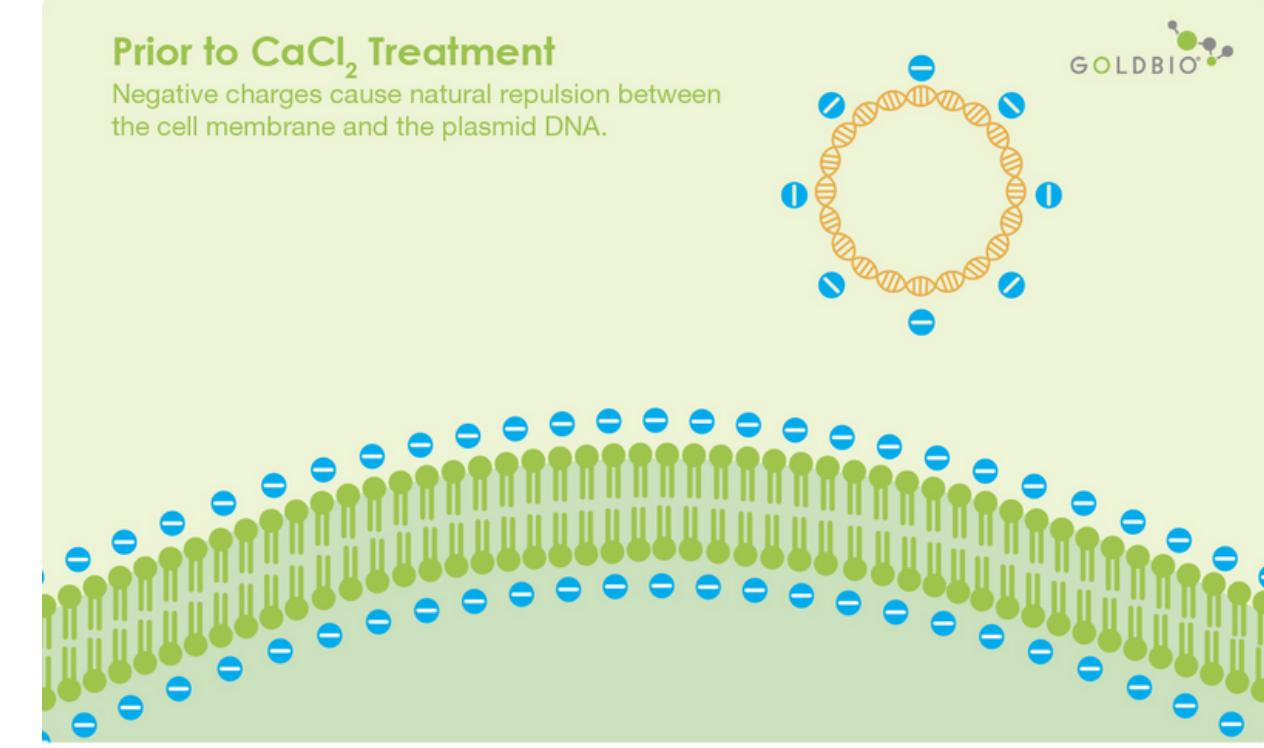
Cells which are capable of **taking up alien genetic material** from surroundings.

The absorbed genetic material (DNA) is **incorporated into the host genome** or is **expressed independently** in the host.

Two methods to make cell competent:

1. **Calcium Chloride** method
2. **Electroporation**

Think further: Why do we have to make the host competent? Why doesn't the host take up DNA spontaneously?



Source: www.goldbio.com

Our workbench



Biosafety Cabinet



Restriction Enzymes

To insert our gene of interest we have to **cut the DNA at specific regions** using an **enzyme** which behaves like a pair of **scissors** called **restriction enzymes**.

Naturally **found in bacteria**.

HindII is the first isolated and characterised restriction enzyme.

Think further: Why did bacteria evolve to have restriction enzymes?

Working of Restriction Enzymes

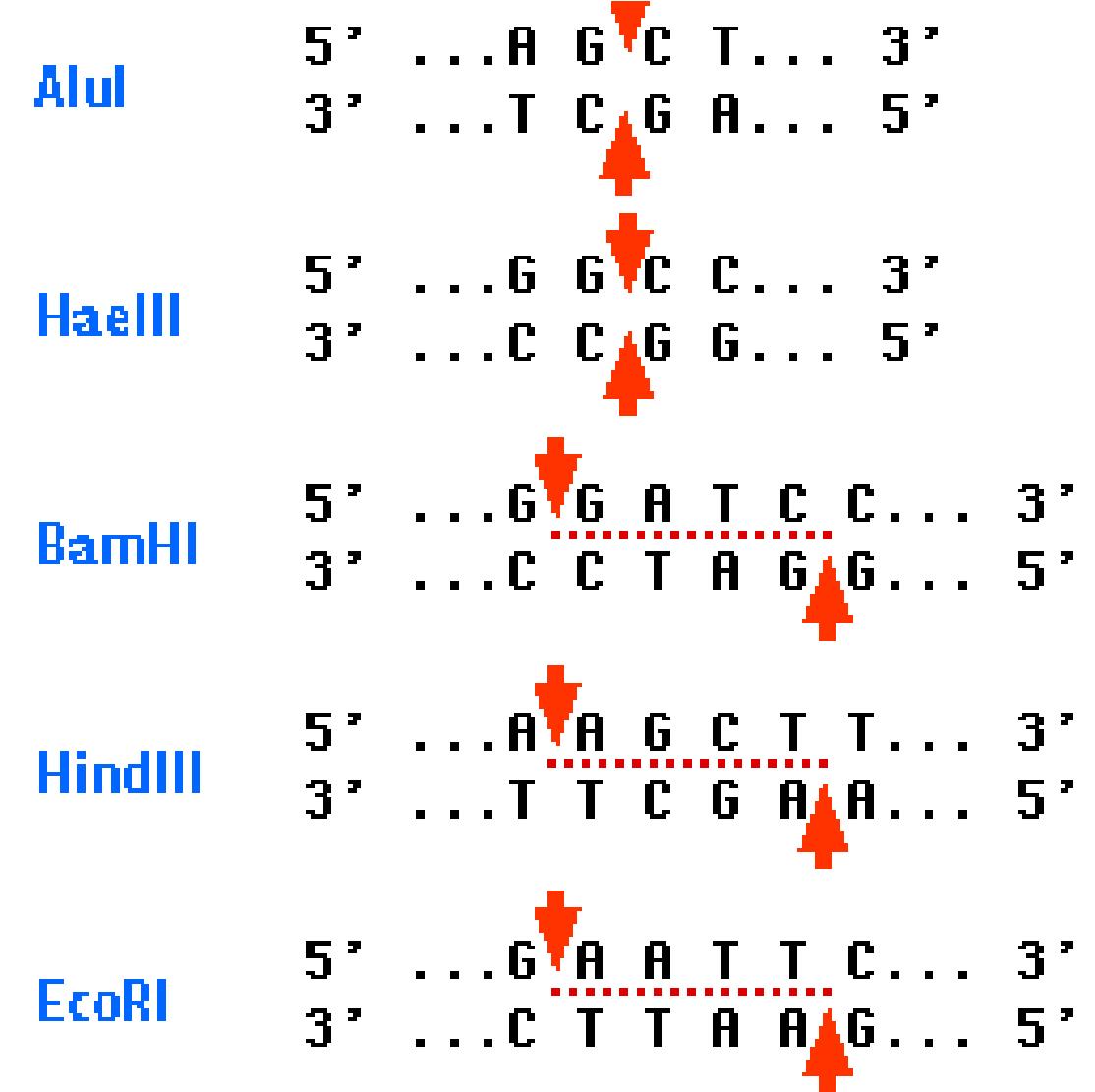
Recognise **palindromic sequences** to cleave.

Eg : 5' - TGCA -3'

3' - ACGT -5'

Palindromic sequences reads same on both sides in same orientation.

Cut at both strands **at same location**; between same nucleotides.

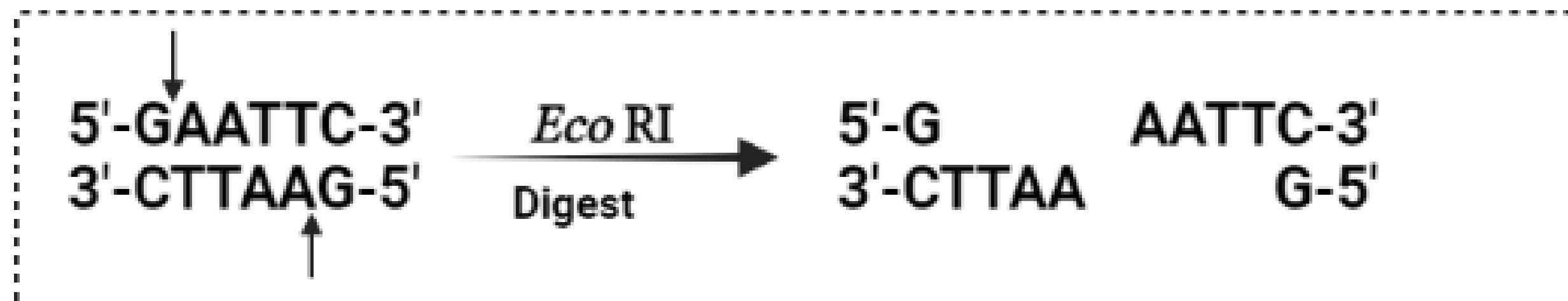


Source: bio.libretexts.org

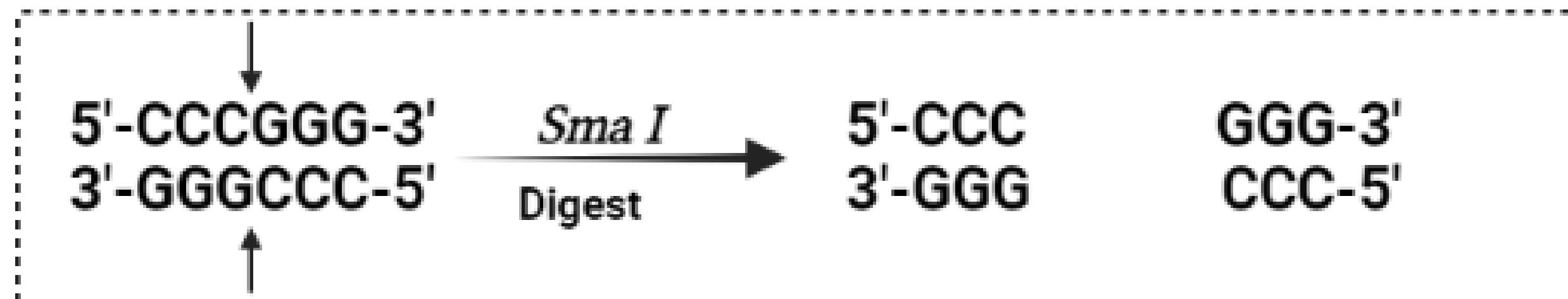
Think further: Find the restriction site where a hypothetical restriction enzyme would probably cleave the given DNA strand 5'- ATGCAAGCGCTCCCT -3' [look for a 6 nucleotide sequence]

Types of cleavage:

1. Sticky end formation



2. Blunt end formation

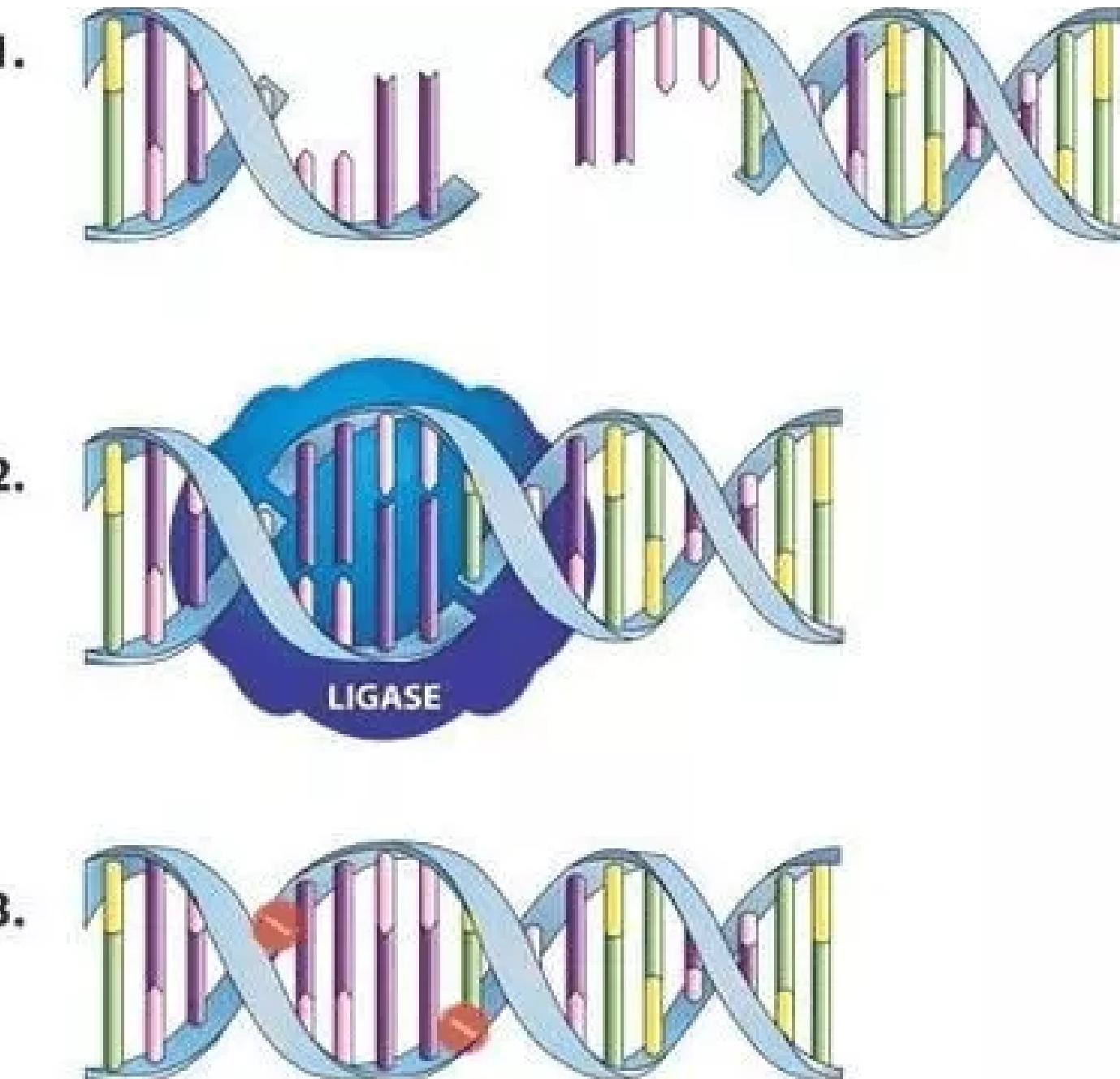


Ligases

Enzymes that are capable of catalyzing the reaction of **joining two large molecules** by establishing a new chemical bond.

DNA ligases are the ligases that **join or bind the two DNA fragments** by forming a **phosphodiester bond**.

DNA Ligase is needed for **DNA replication** as well as the **DNA repairing process**.



Source: Sciencelearn.org.nz.

Isolation of Genetic Material

The **gene of our desire** is to be extracted from an organism.

To extract the DNA and **separate** it from all other macromolecules we use **lysozyme** (in bacteria), **chitinase** (in fungi), **cellulase** (in plants). RNA is removed using **ribonuclease** and the proteins are removed using **proteases**.

On addition of **chilled ethanol**, purified **DNA precipitates** out.

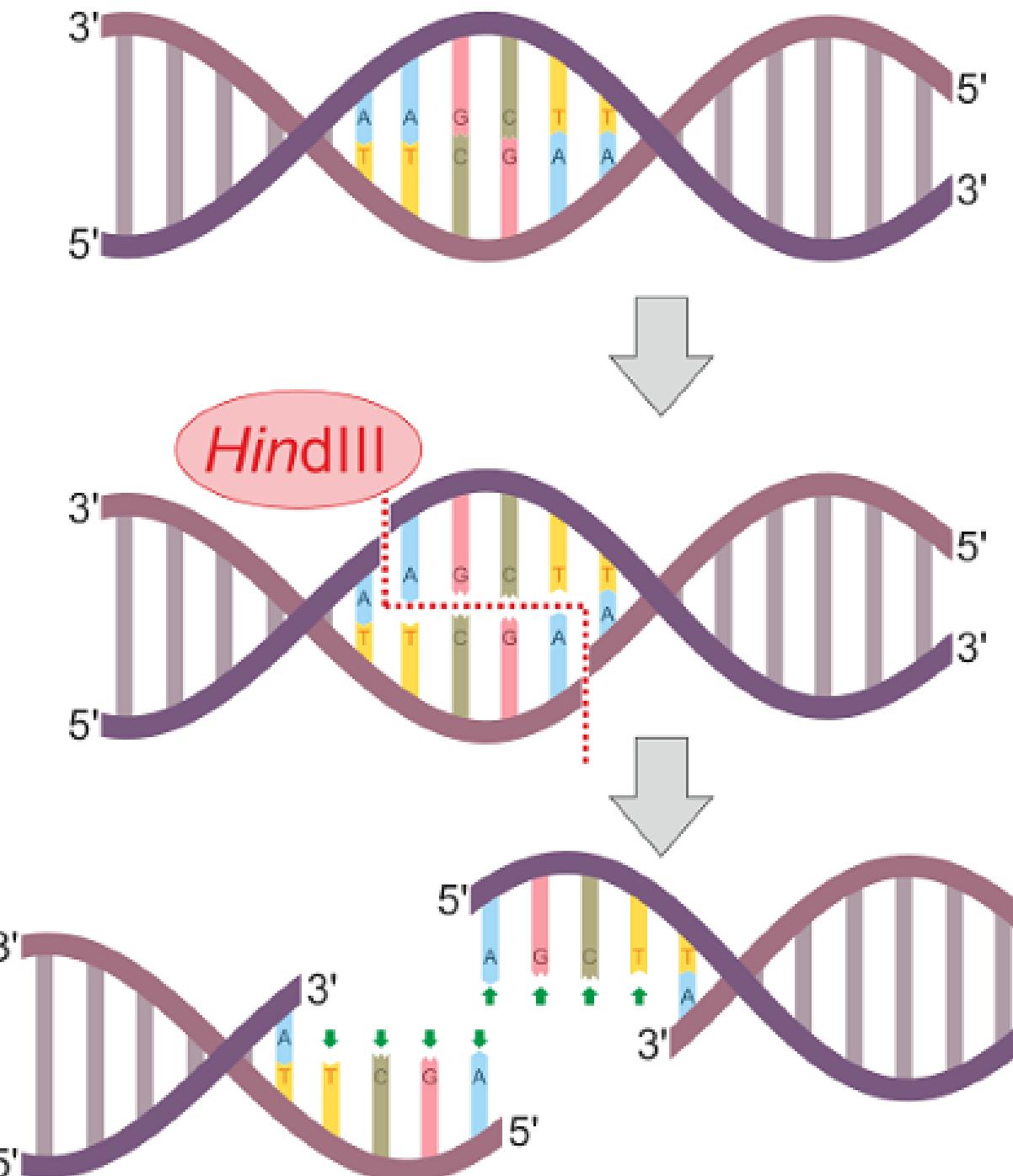
Think further: What will be the consequence if I add cellulase instead of chitinase to isolate the genetic material from fungi?

Restriction Digestion

A process in which **DNA is cut at specific sites**, dictated by the surrounding DNA sequence.

Components- the DNA template, the restriction enzyme of choice, a buffer and sometimes **BSA protein**.

The reaction is incubated at a specific temperature required for **optimal activity** of the restriction enzyme and is terminated by heat.



Source: biologydictionary.net

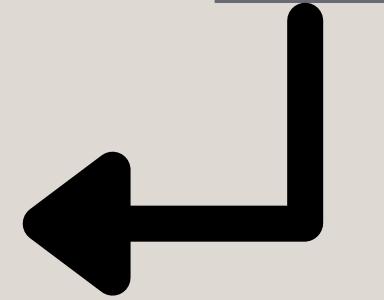


Autoclave →

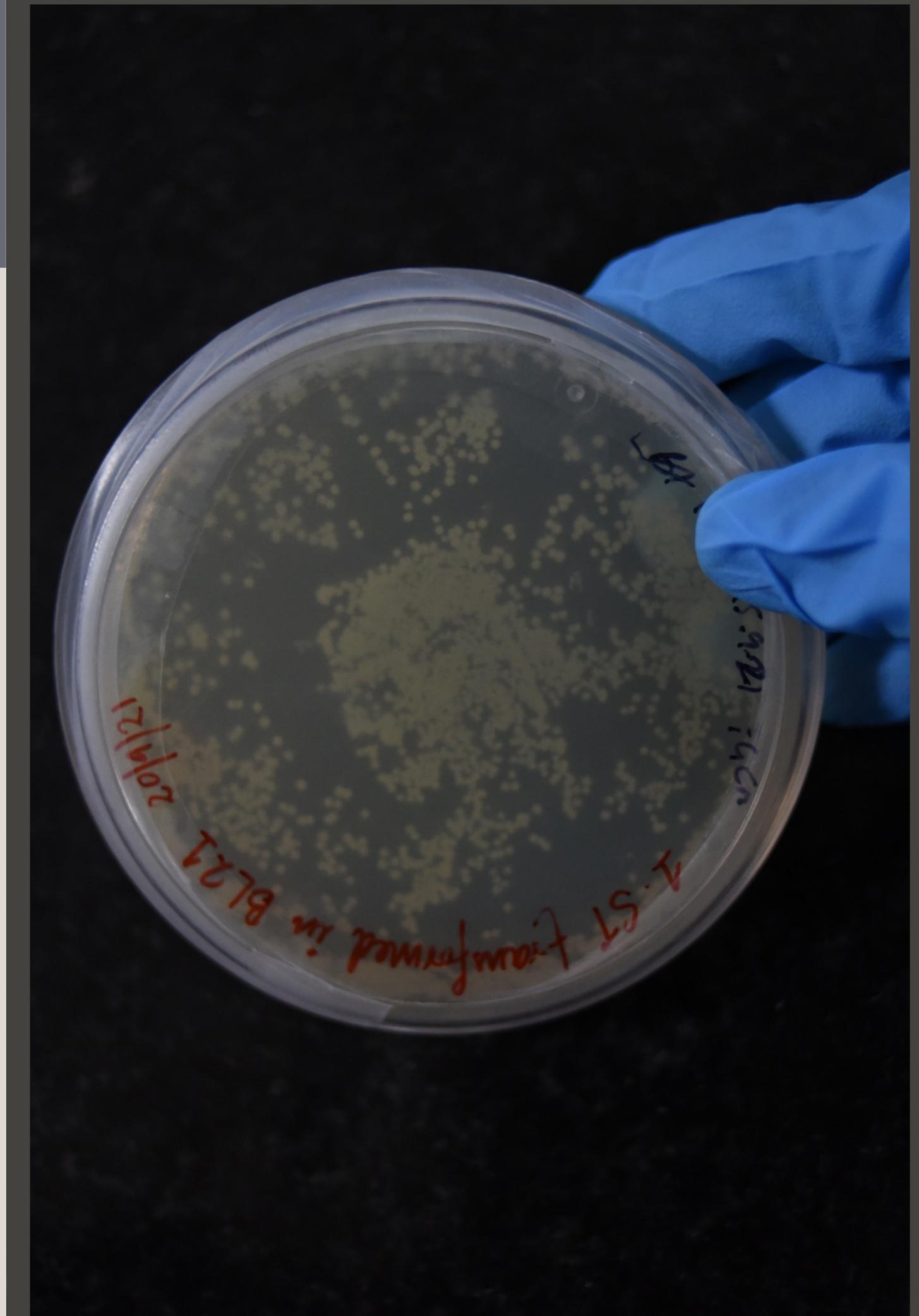
← Centrifuge



Incubator-
Shaker



Bacterial
colony



PCR (Polymerase Chain Reaction)

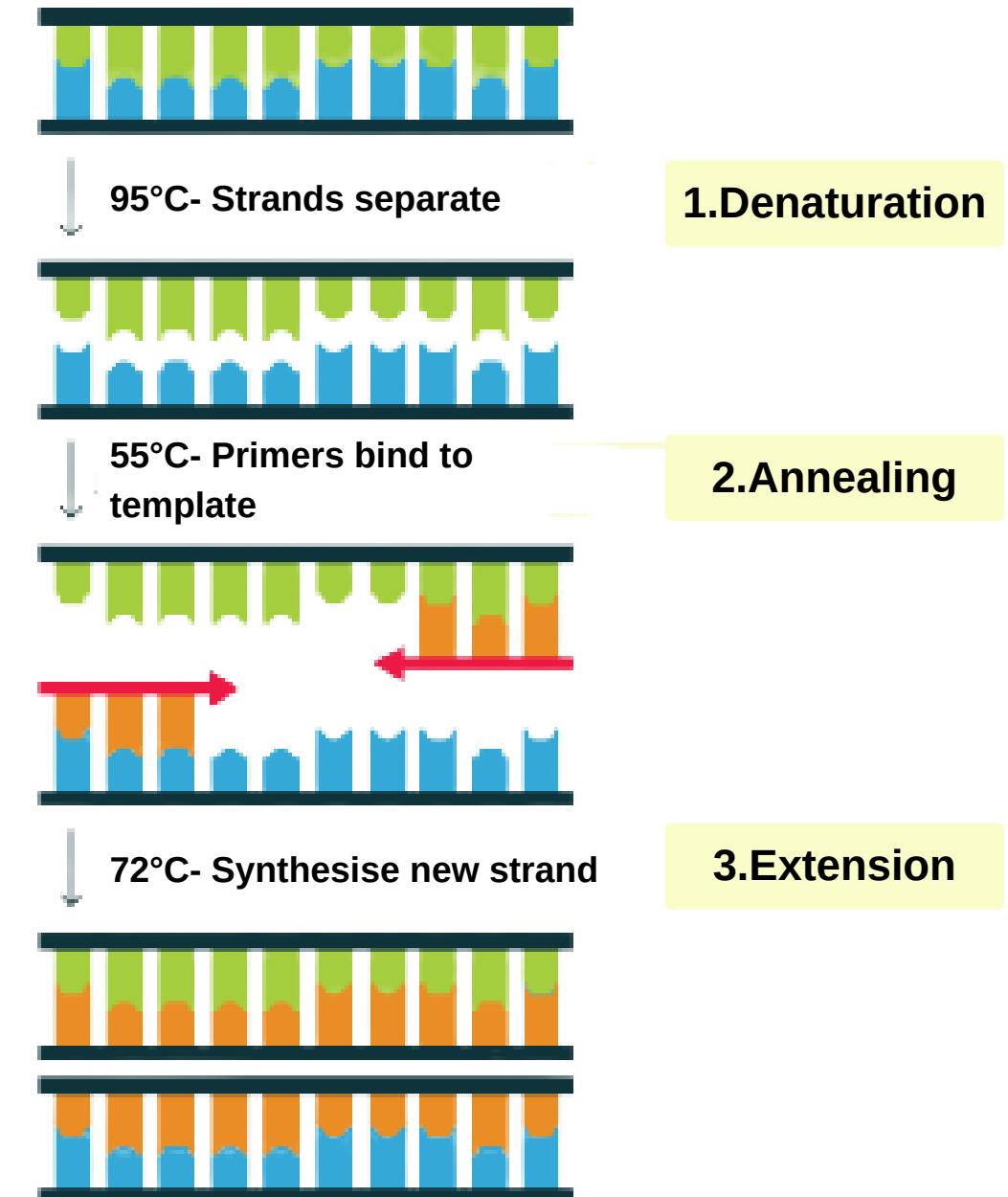
Technique used to **amplify small segments of DNA**.

A single cycle of PCR constitutes **3 steps**:

1. Denaturation
2. Annealing
3. Extension

The high initial temperature favours **denaturation** of hydrogen bonds between the two strands of DNA. This is followed by **binding of primers** (small fragments of DNA) to the template strands. The enzymes added in the reaction mixture will further cause **polymerisation to form the new DNA strand**.

PCR Process (One Cycle)



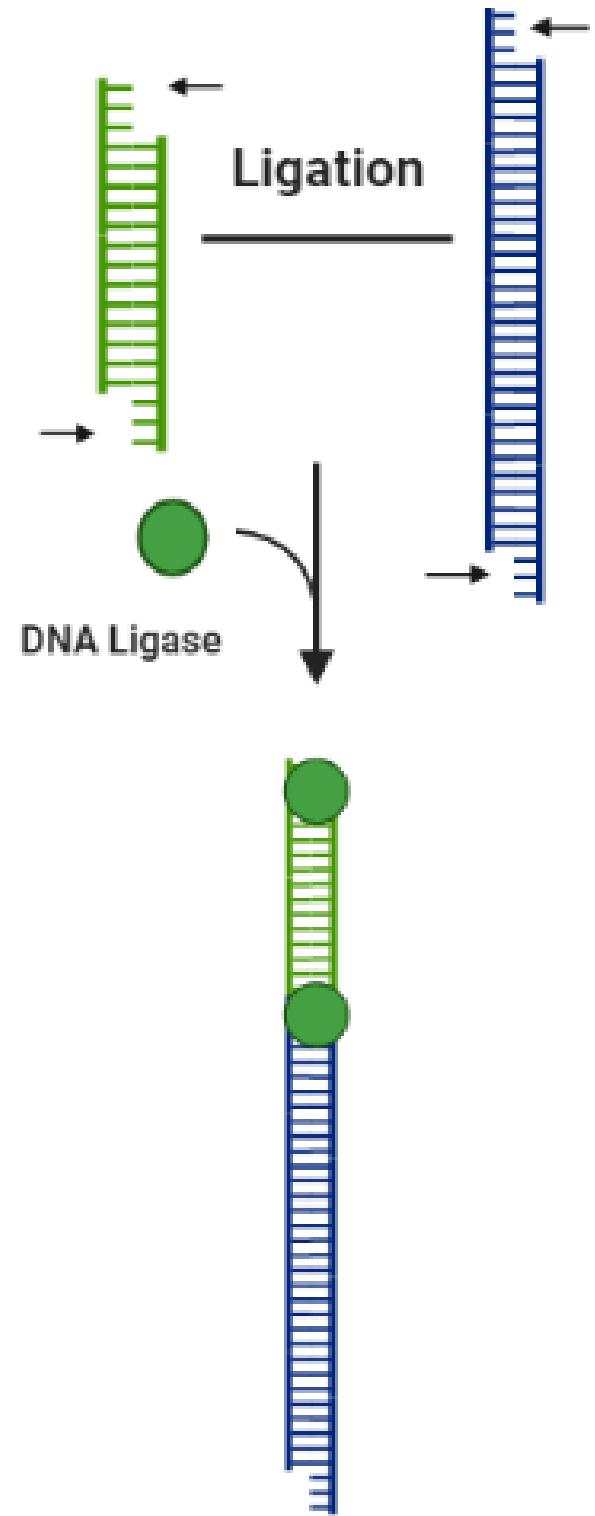
Source: bosterbio.com

Think further: What is the minimum number of PCR cycles required to obtain the desired length of DNA -the target gene sequence without any adjacent DNA contamination?

Ligation

Joining of two nucleic acid fragments through the action of an enzyme

Two DNA fragments can be joined together with the help of enzyme DNA ligase



The ends of DNA fragments are joined together by the **formation of phosphodiester bonds** between the 3'-hydroxyl of one DNA terminus with the 5'-phosphoryl of another.

Think further: What ensures that ligation takes place between only the desired fragments and not between any two random DNA fragments?

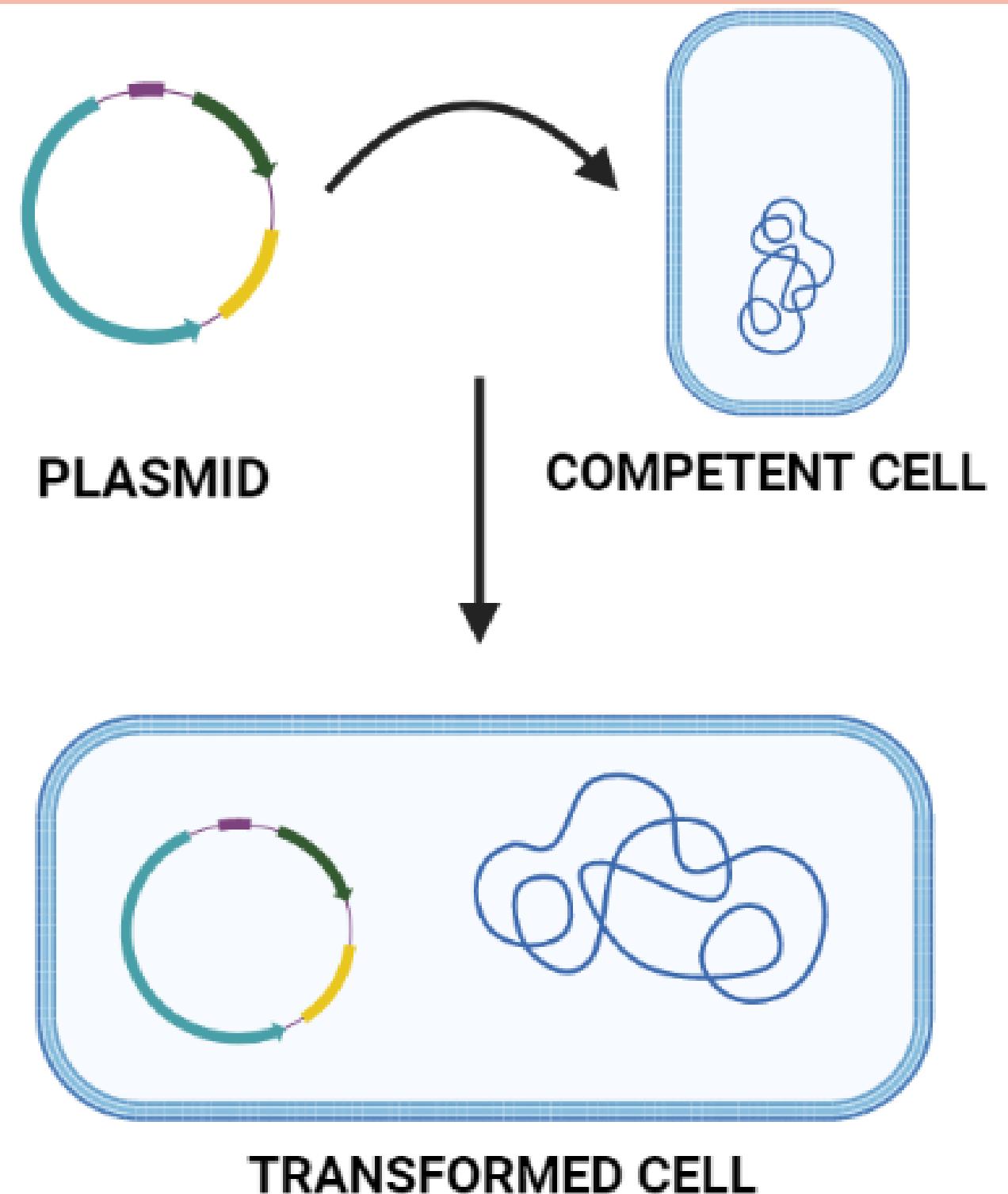
Transformation

The process by which a **cell take up some foreign genetic material** (naked DNA) from the environment.

Cell has to be **competent** enough to take in the genetic material.

Most popular methods of bacterial transformation:

1. **Heat shock** of chemically prepared competent cells
2. **Electroporation** of electrocompetent cells
3. **Microinjection**



Think further: How do we know if a bacterium or cell has taken up our desired gene?

Protein Expression

Traditional strategies for recombinant protein expression involve **transfected cells with a DNA vector** that contains the template and then culturing the cells so that they **transcribe and translate** the **desired protein**.

Typically, the cells are then lysed to **extract the expressed protein** for subsequent purification.

Several protein expression systems used in the synthesis of recombinant protein production.

Expression system	Most common application	Advantages	Challenges
Mammalian	<ul style="list-style-type: none"> Functional assays Structural analysis Antibody production Expression of complex proteins Protein interactions Virus production 	<ul style="list-style-type: none"> Highest-level protein processing Can produce proteins either transiently, or by stable expression Robust optimized transient systems for rapid, ultrahigh-yield protein production 	<ul style="list-style-type: none"> Gram-per-liter yields only possible in suspension cultures More demanding culture conditions
Insect	<ul style="list-style-type: none"> Functional assays Structural analysis Expression of intracellular proteins Expression of protein complexes Virus production 	<ul style="list-style-type: none"> Similar to mammalian protein processing Can be used in static or suspension culture 	<ul style="list-style-type: none"> More demanding culture conditions than prokaryotic systems Production of recombinant baculovirus vectors is time consuming
Yeast	<ul style="list-style-type: none"> Structural analysis Antibody generation Functional analysis Protein interactions 	<ul style="list-style-type: none"> Eukaryotic protein processing Scalable up to fermentation (grams per liter) Simple media requirements 	<ul style="list-style-type: none"> Fermentation required for very high yields Growth conditions may require optimization
Bacterial	<ul style="list-style-type: none"> Structural analysis Antibody generation Functional assays Protein interactions 	<ul style="list-style-type: none"> Scalable Low cost Simple culture conditions 	<ul style="list-style-type: none"> Protein solubility May require protein-specific optimization May be difficult to express some mammalian proteins
Algal	<ul style="list-style-type: none"> Studying photosynthesis, plant biology, lipid metabolism Genetic engineering Biofuel production 	<ul style="list-style-type: none"> Genetic modification and expression systems for photosynthetic microalgae Superb experimental control for biofuel, nutraceuticals, and specialty chemical production Optimized system for robust selection and expression 	<ul style="list-style-type: none"> Nascent technology Less developed compared to other host platforms
Cell-free	<ul style="list-style-type: none"> Toxic proteins Incorporation of unnatural label or amino acids Functional assays Protein interactions Translational inhibitor 	<ul style="list-style-type: none"> Open system; able to add unnatural components Fast expression Simple format 	<ul style="list-style-type: none"> Scaling above multigram quantities may not be costly

Source: www.thermofisher.com

Do it Yourself!

The plasmid "Z" has the property of **Kanamycin** and **Chloramphenicol** resistance. The desired gene of interest is inserted into the site, which confers the plasmid **Chloramphenicol** resistance. The bacteria is transformed with this plasmid. Match the following figures under the following conditions.

- If the bacteria are grown in a medium that contains **Chloramphenicol**.
- If the bacteria are grown in a medium that contains **Kanamycin**.
- If bacteria are grown in a medium that contains both **Kanamycin** and **Chloramphenicol**.

Figure 1

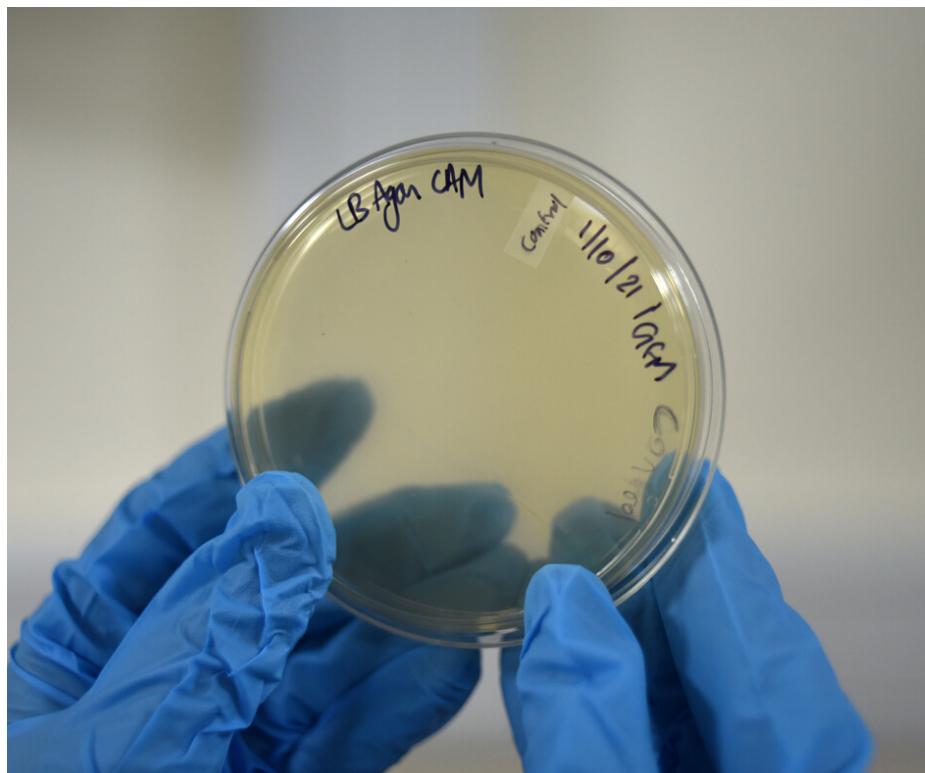
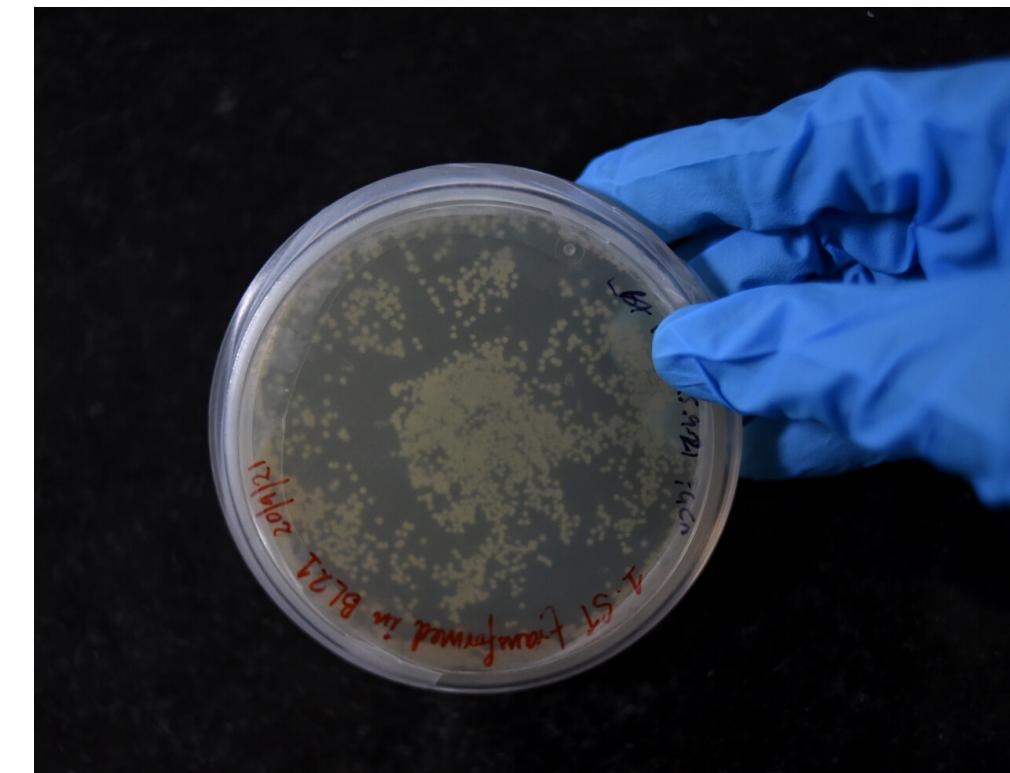
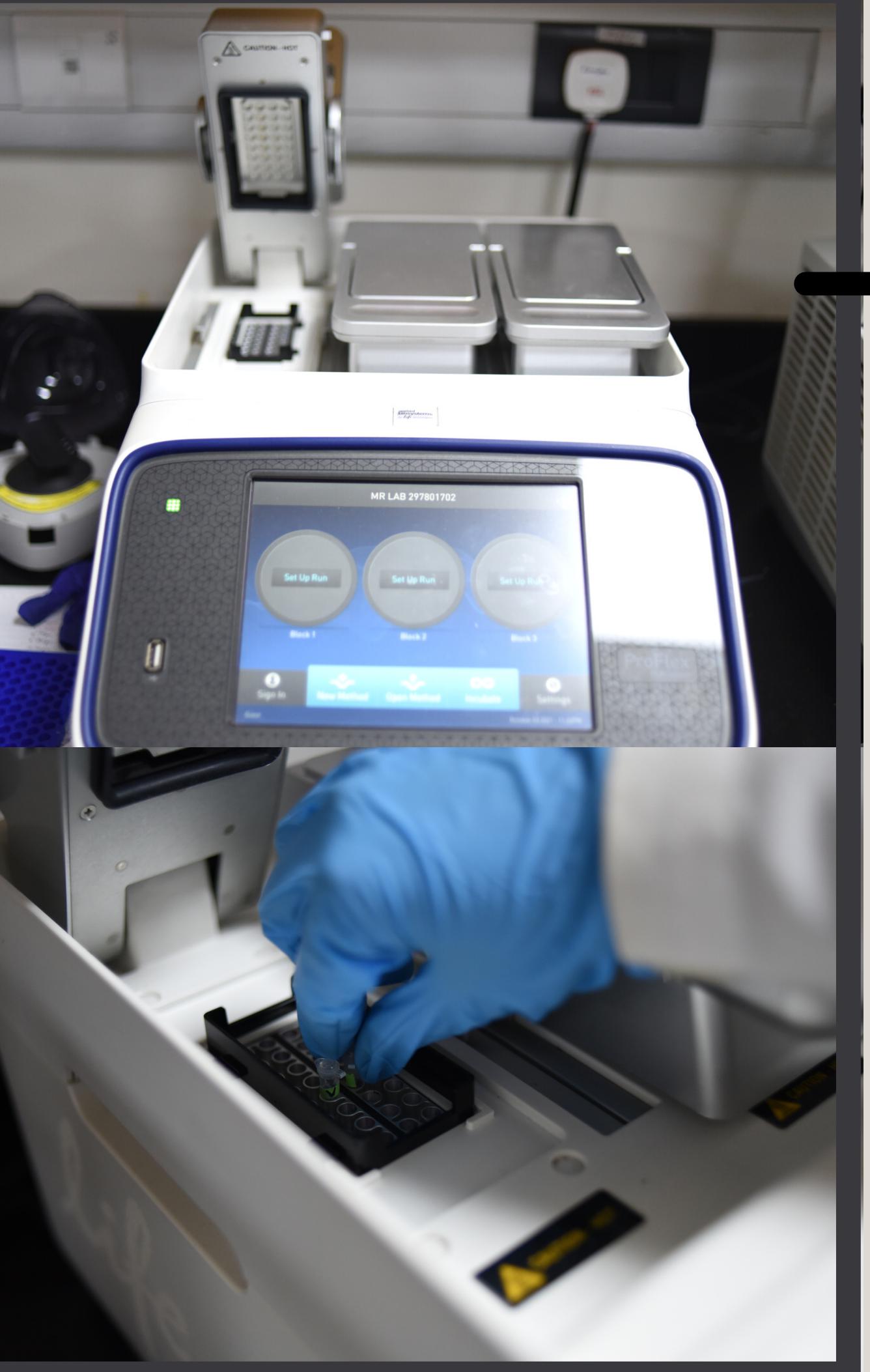


Figure 2



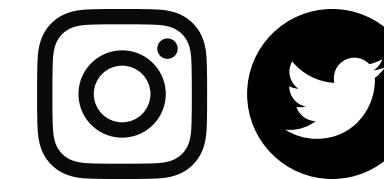


PCR Machine



Water Bath

Thank You!



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