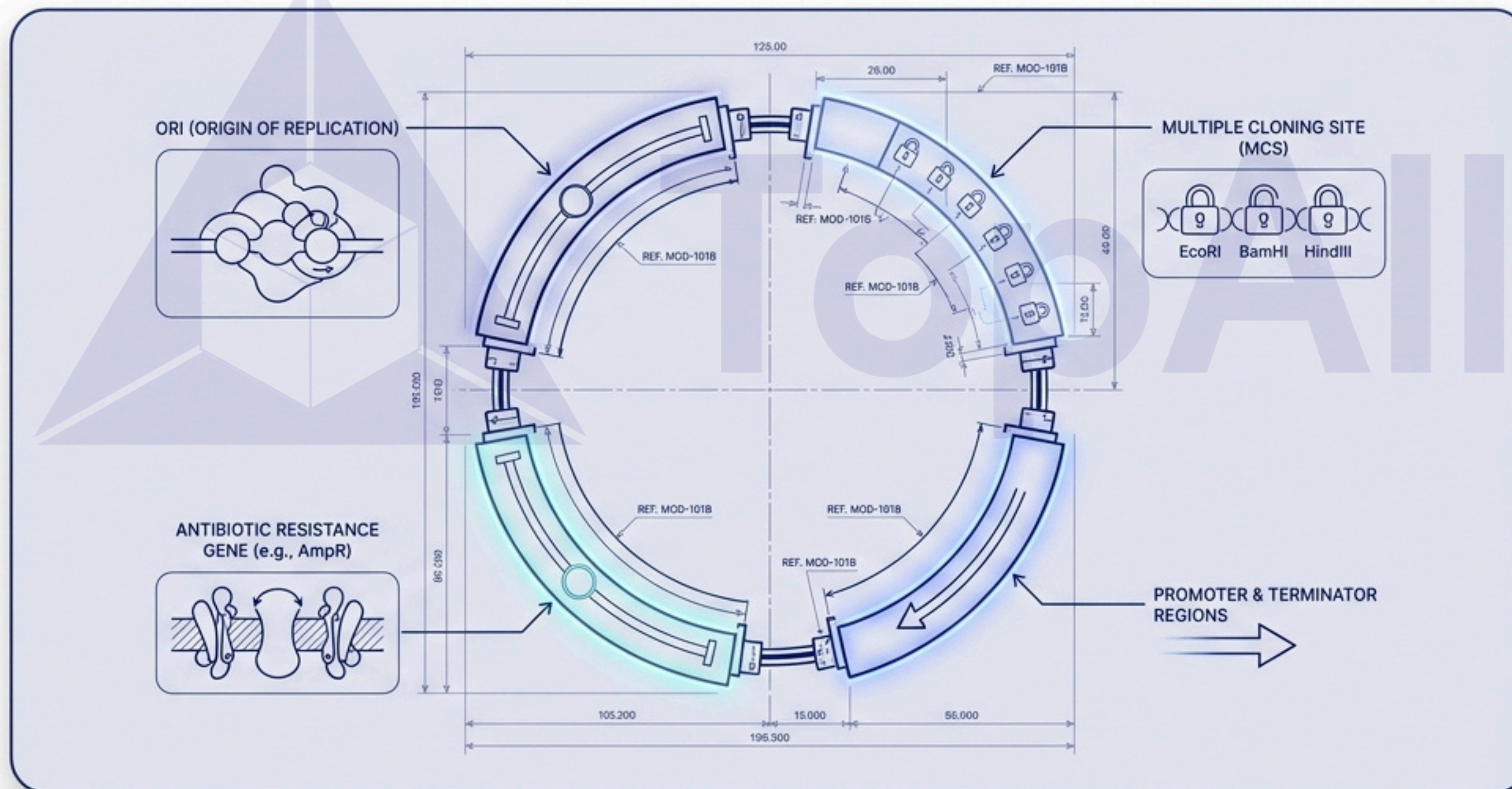


Cloning Vectors

The Anatomy of a Genetic Machine



The Delivery Vehicles



Plasmids

Nature:	Autonomously replicating circular extra-chromosomal DNA.
Behavior:	Replicate independent of chromosomal DNA control.
Copy Range:	Varies widely (1-2 copies up to 15-100+ copies per cell).

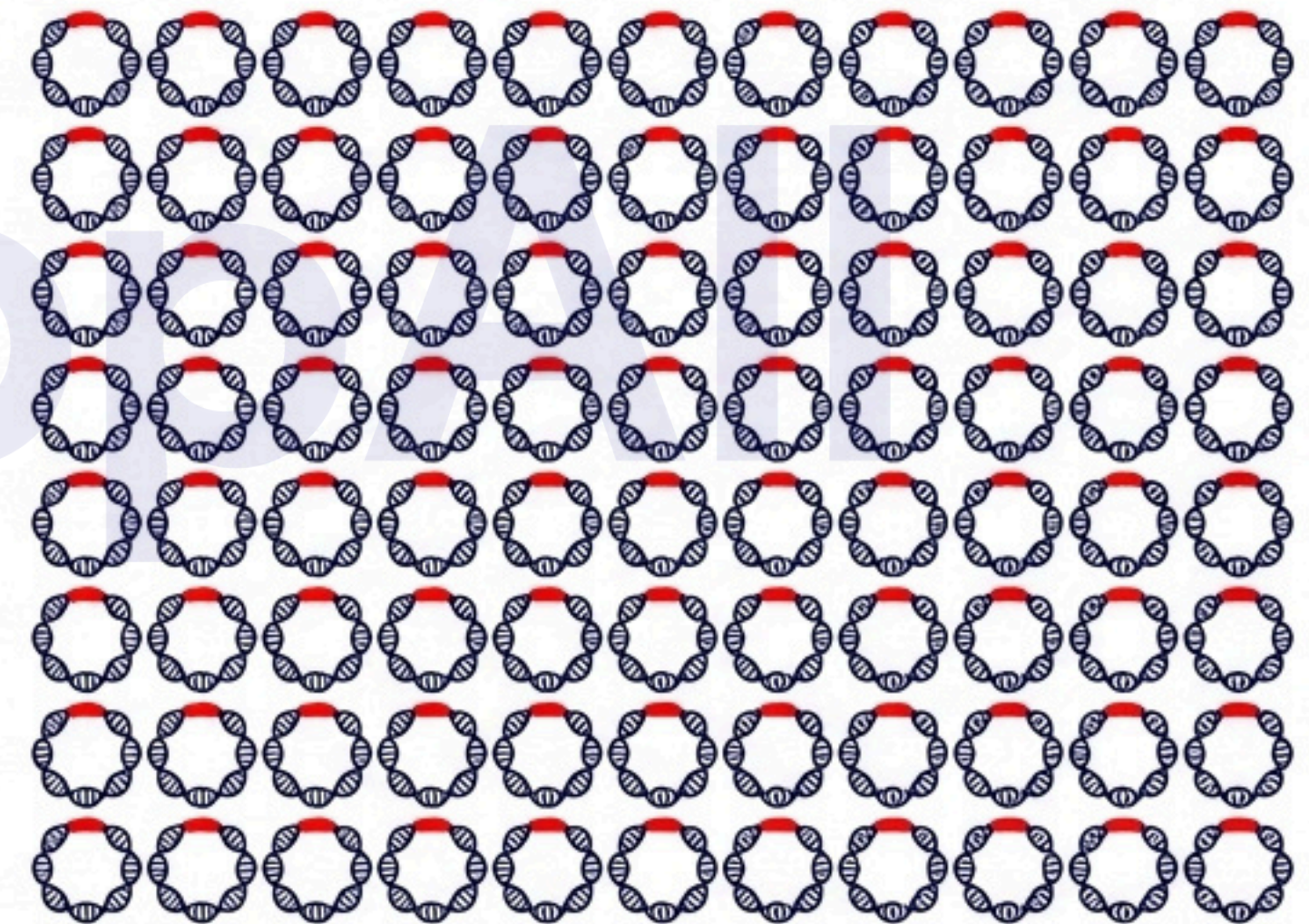
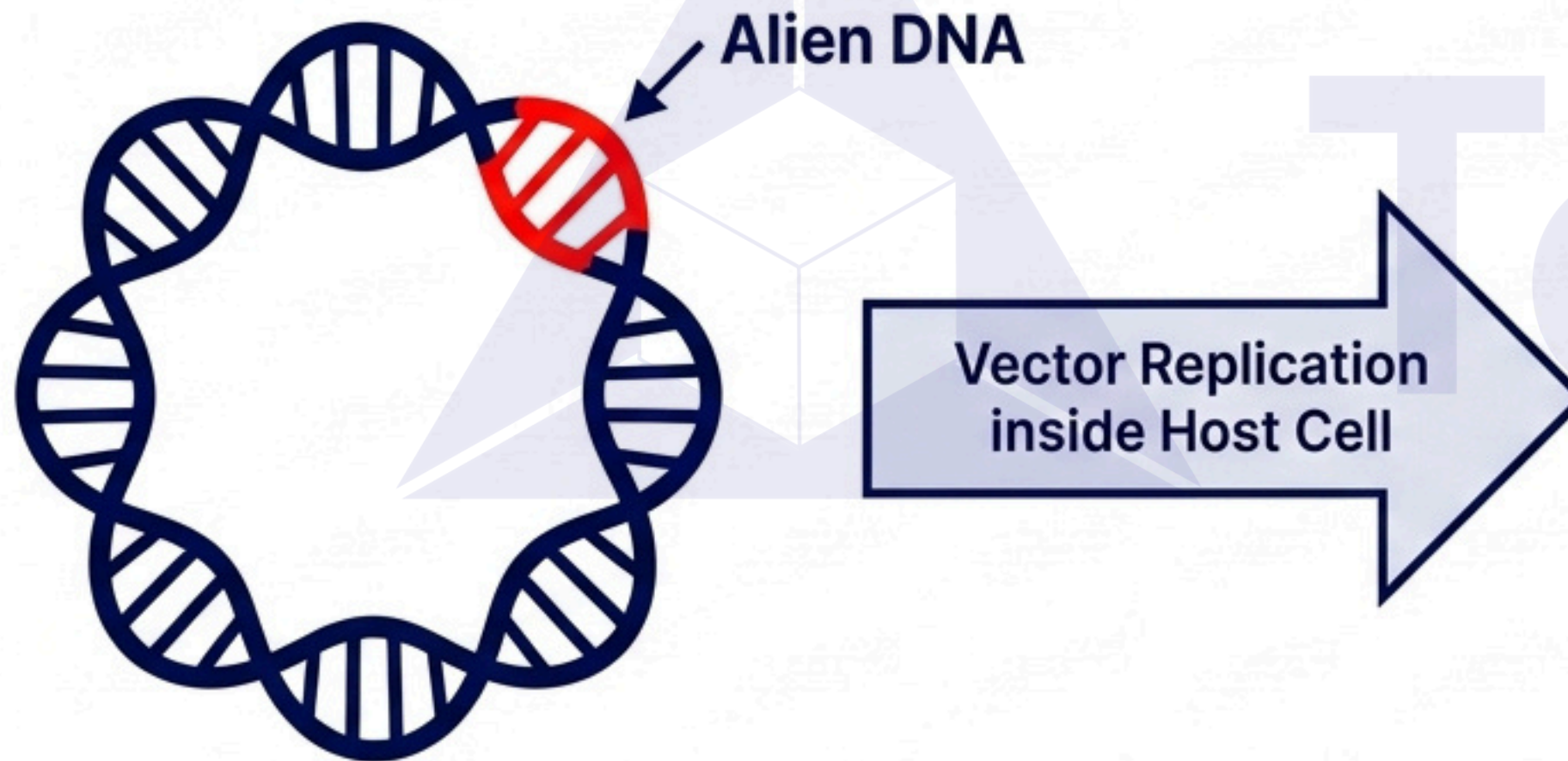


Bacteriophages

Nature:	Viruses that infect bacterial cells.
Behavior:	Exploit the host machinery to replicate their genome.
Copy Range:	Very high copy numbers per cell due to viral replication burst.

The Multiplier Effect: Copy Number

If we link an alien piece of DNA with a vector, we multiply its numbers equal to the copy number of that plasmid or bacteriophage.



Modern engineered vectors can achieve 15–100+ copies per single host cell, turning the host into a microscopic manufacturing plant.



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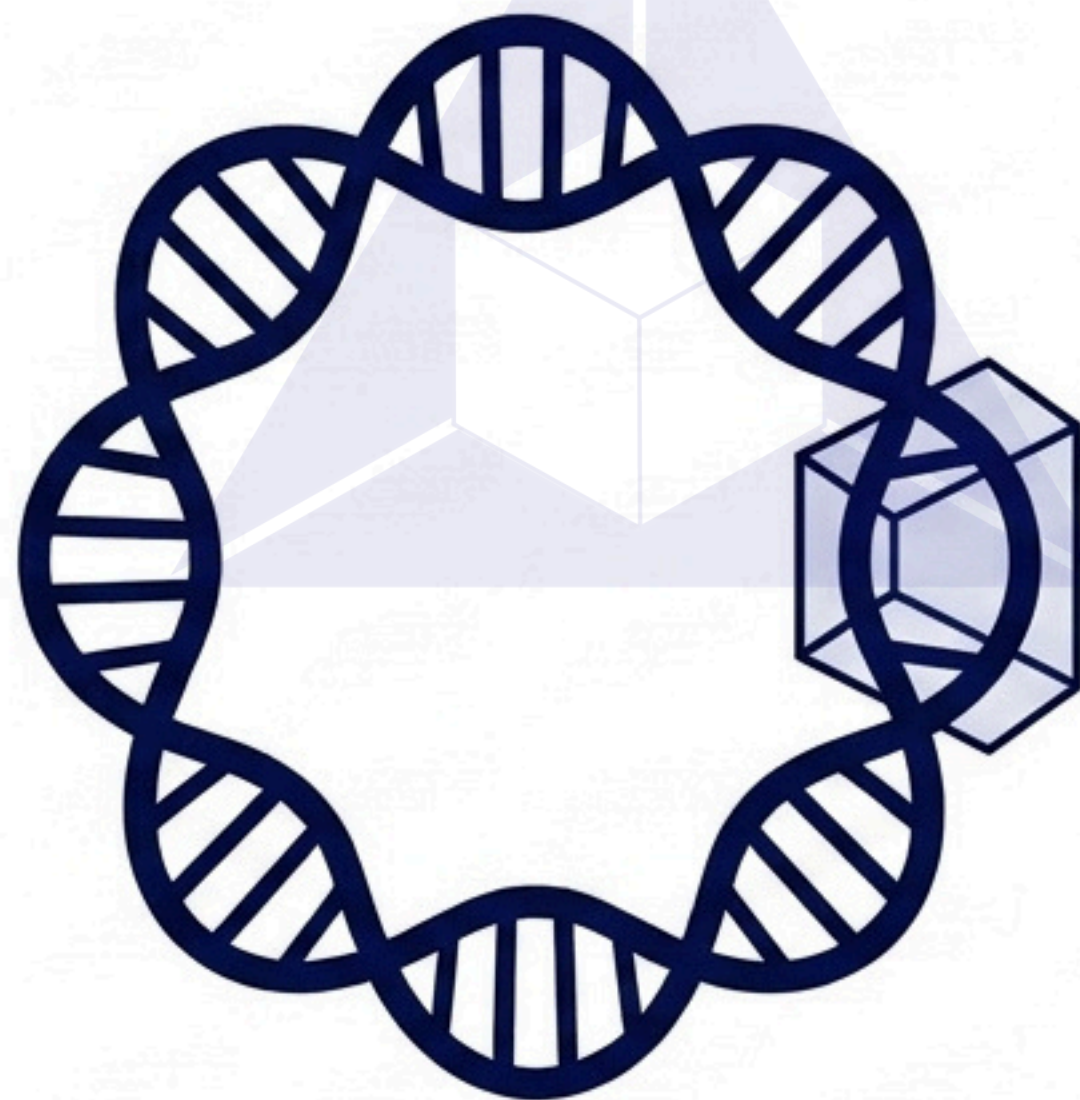
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Feature I: Origin of Replication (ori)

The ori is the specific sequence where DNA replication initiates. Any alien piece of DNA linked to this sequence is forced to replicate within the host.



The Starter Motor

Initiates the replication cycle. Without it, alien DNA cannot multiply.

The Volume Dial

Controls the copy number of the linked DNA. To recover high yields of target DNA, it must be cloned into a vector whose ori supports a high copy number.

Feature II: Selectable Markers

The Problem: Normal E. coli cells do not carry resistance against common antibiotics. We need to identify which cells successfully took up the vector (Transformation).

The Solution:
The vector carries an antibiotic resistance gene (e.g., ampR).

Transformants

Non-transformants

Antibiotic Medium
(e.g., Ampicillin)

Dies in
antibiotic

Transformants

The Filter Logic:

No Vector = Non-transformant ->
Dies in antibiotic.

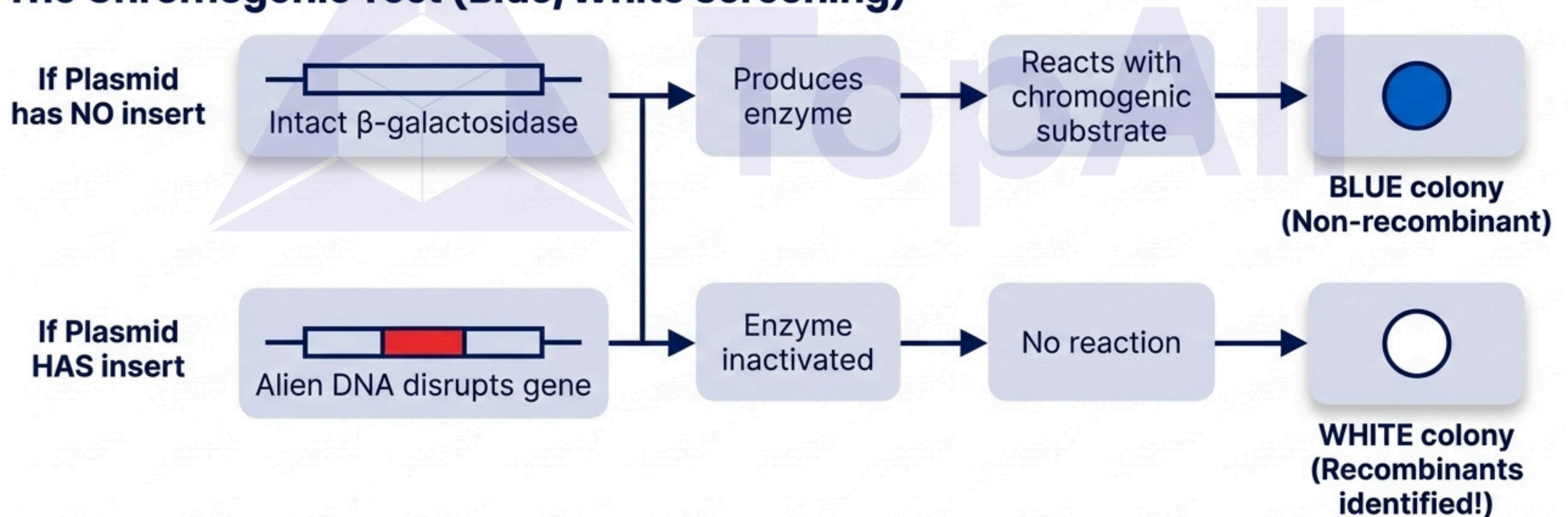
Vector Present = Transformant ->
Survives and forms colonies.

Common Markers: Ampicillin,
Chloramphenicol, Tetracycline,
Kanamycin.

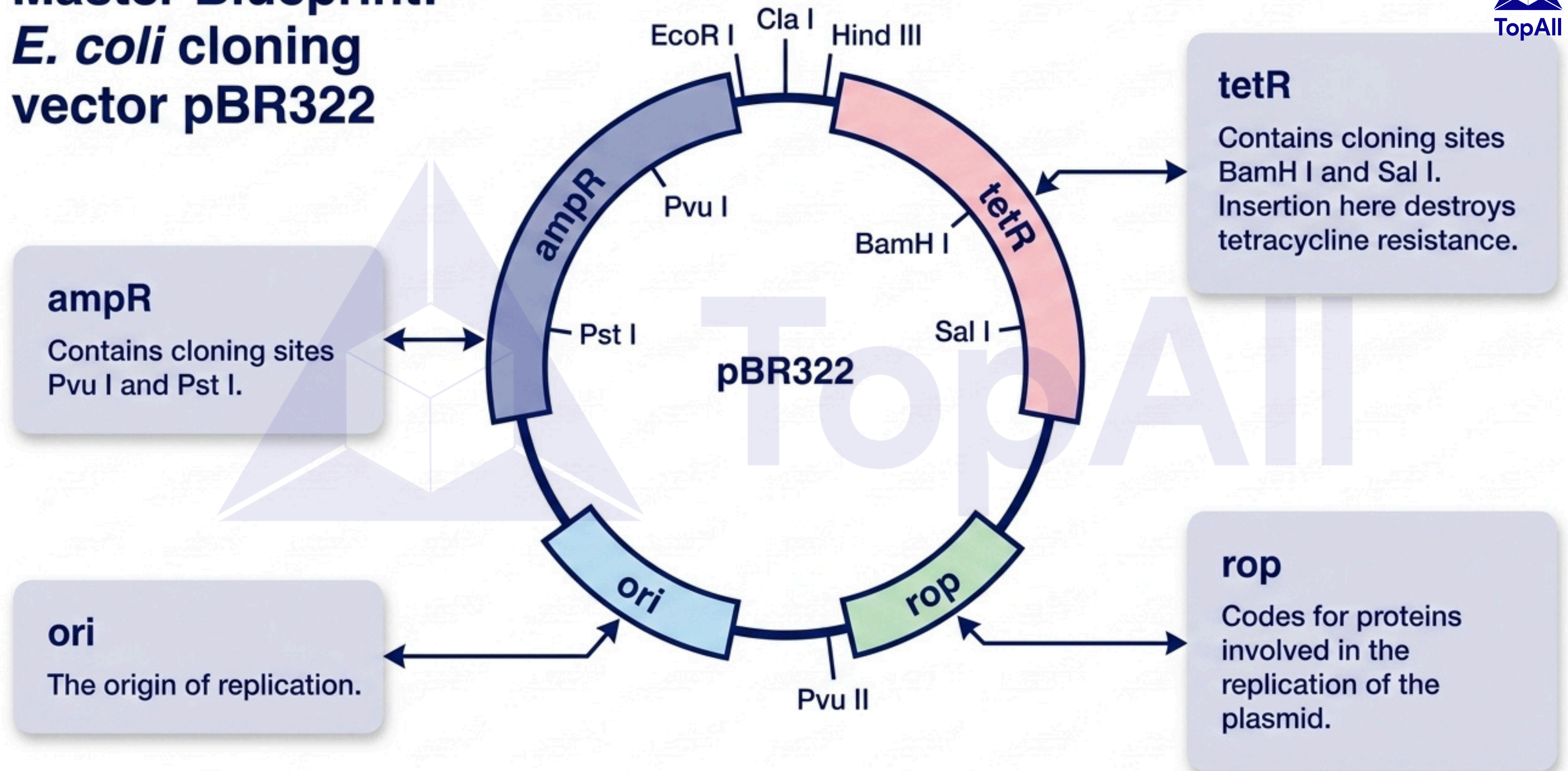
Feature II: Cloning Sites & Insertional Inactivation

Vectors need single recognition sites for restriction enzymes to link alien DNA. Inserting alien DNA inside a marker gene inactivates it—proving the insertion worked.

The Chromogenic Test (Blue/White Screening)



Master Blueprint: *E. coli* cloning vector pBR322



NEET Key Takeaway: Vector Dynamics



The pBR322 Map Trap:

BamH I and Sal I are located inside the tetR gene. Pvu I and PstI are inside the ampR gene.



Blue/White Screening:

White colonies = Recombinants (gene is disrupted).
Blue colonies = Non-recombinants (gene is intact).



Insertional Inactivation Reality Check:

Inserting alien DNA at BamH I means the recombinant will grow on ampicillin, but die on tetracycline.



Function of 'rop':

It codes for proteins involved in the replication of the plasmid (frequently tested vocabulary).



Transformation Prerequisite:

DNA is hydrophilic; cells must be made 'competent' (via divalent cations like Calcium + heat shock) to take up plasmids.



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