

CLONING VECTORS: DNA DELIVERY VEHICLES

The molecular blueprints of
recombinant DNA technology.

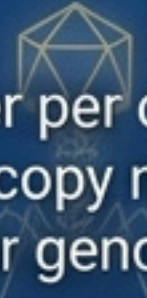
The Core Trait: Plasmids and bacteriophages replicate independently of the control of chromosomal DNA.



Chromosomal DNA



Vectors

Copy Number (Plasmids)	Copy Number (Phages)	The Engineering Goal
Ranges from 1-2 copies up to 15-100+ copies per cell. 	High number per cell results in very high copy numbers of their genome. 	Linking an alien piece of DNA with a vector multiplies its numbers equal to the vector's copy number. 



Feature 1: Origin of Replication (ori)

The Starting Line

The specific sequence from where DNA replication initiates. Any alien DNA linked to this sequence is forced to replicate within the host cell.

The Controller

The 'ori' sequence is strictly responsible for controlling the copy number of the linked DNA.

NEET Exam Insight

To recover high quantities of target DNA, it MUST be cloned into a vector whose origin supports a high copy number.



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$$E=mc^2$$



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Key Highlights

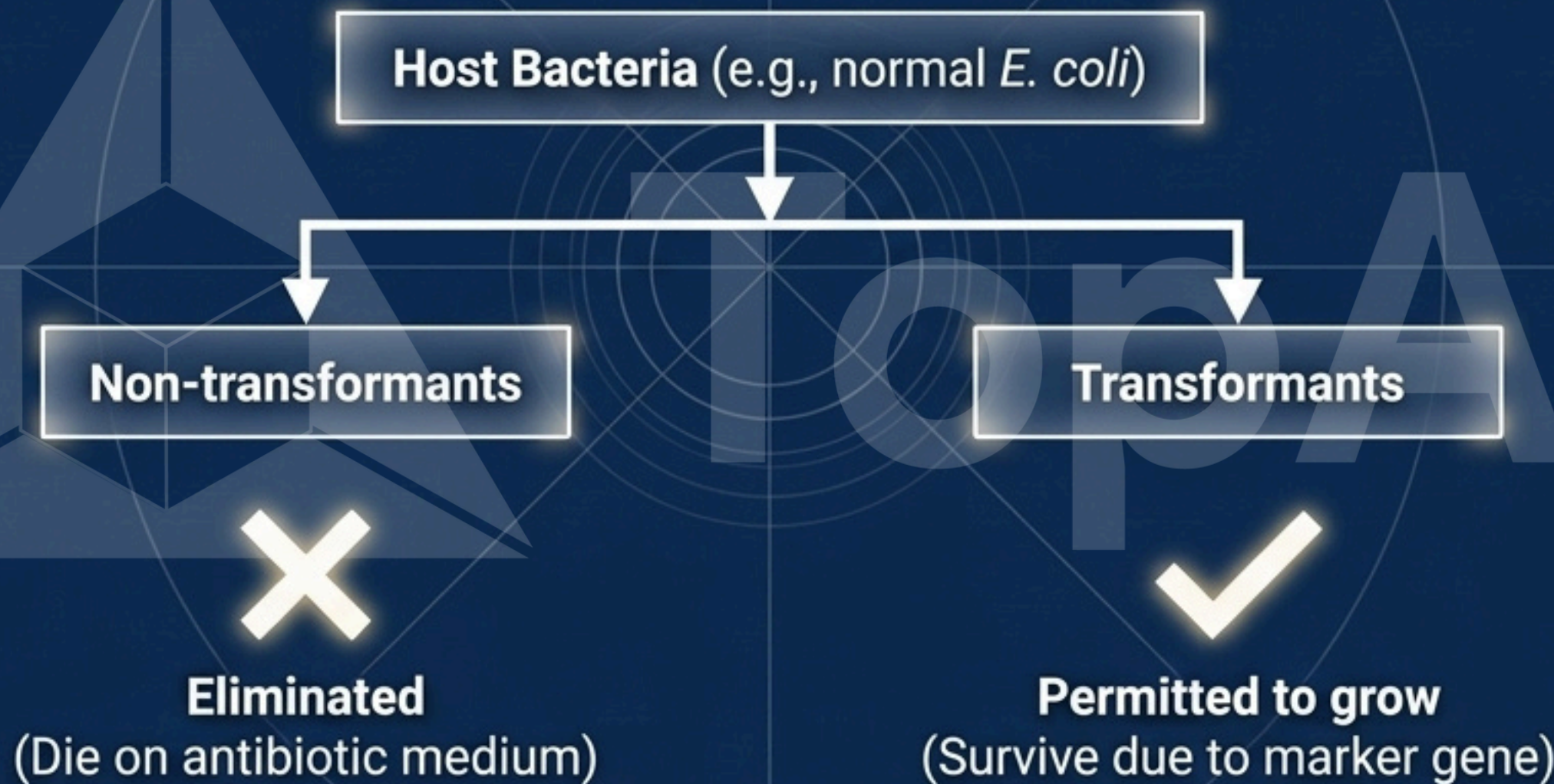
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Feature 2: Selectable Marker

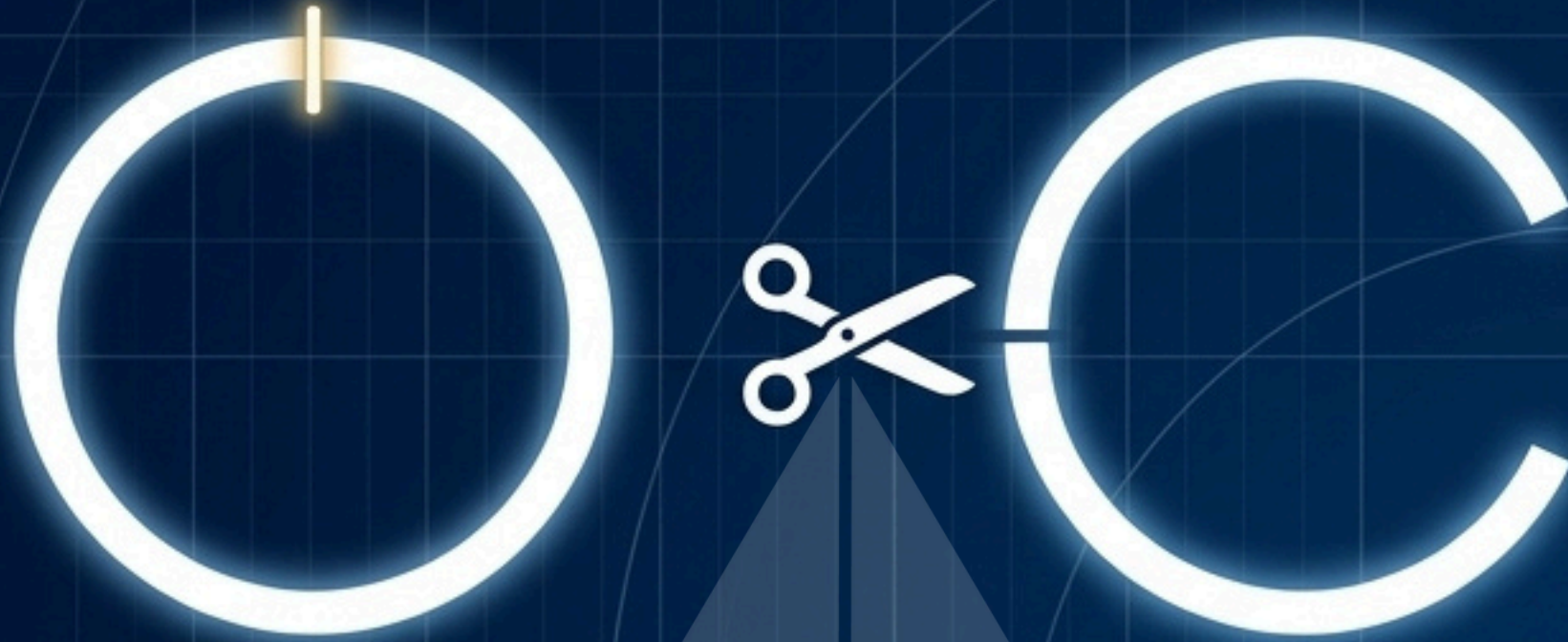
Function: Identifies and eliminates non-transformants while selectively permitting the growth of transformants.

Transformation: The procedure of introducing a piece of DNA into a host bacterium.



Standard Markers (*E. coli*): Genes encoding resistance to antibiotics: Ampicillin, Chloramphenicol, Tetracycline, Kanamycin. (**Note:** Normal *E. coli* cells do not carry resistance against any of these).

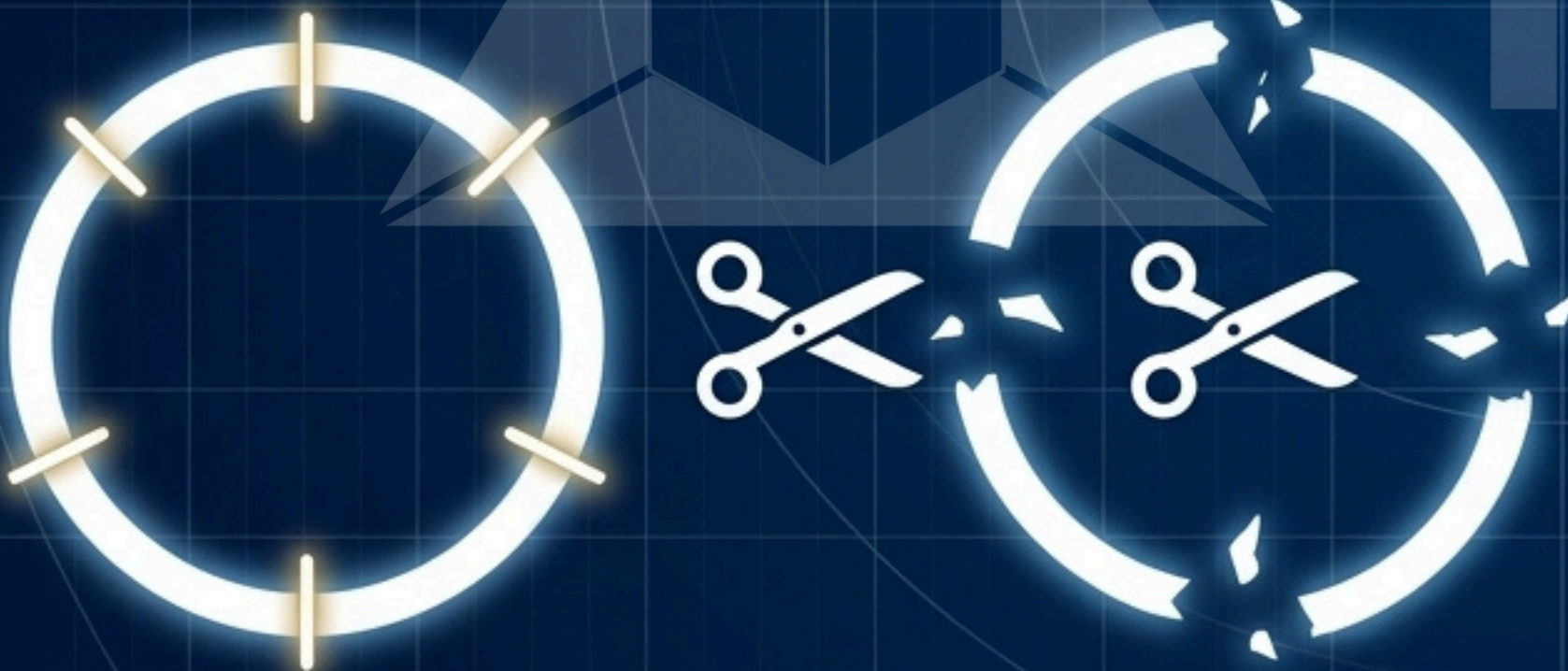
Ideal Design



Feature 3: Cloning Sites

The Rule of One: A vector needs very few, preferably single, recognition sites for commonly used restriction enzymes.

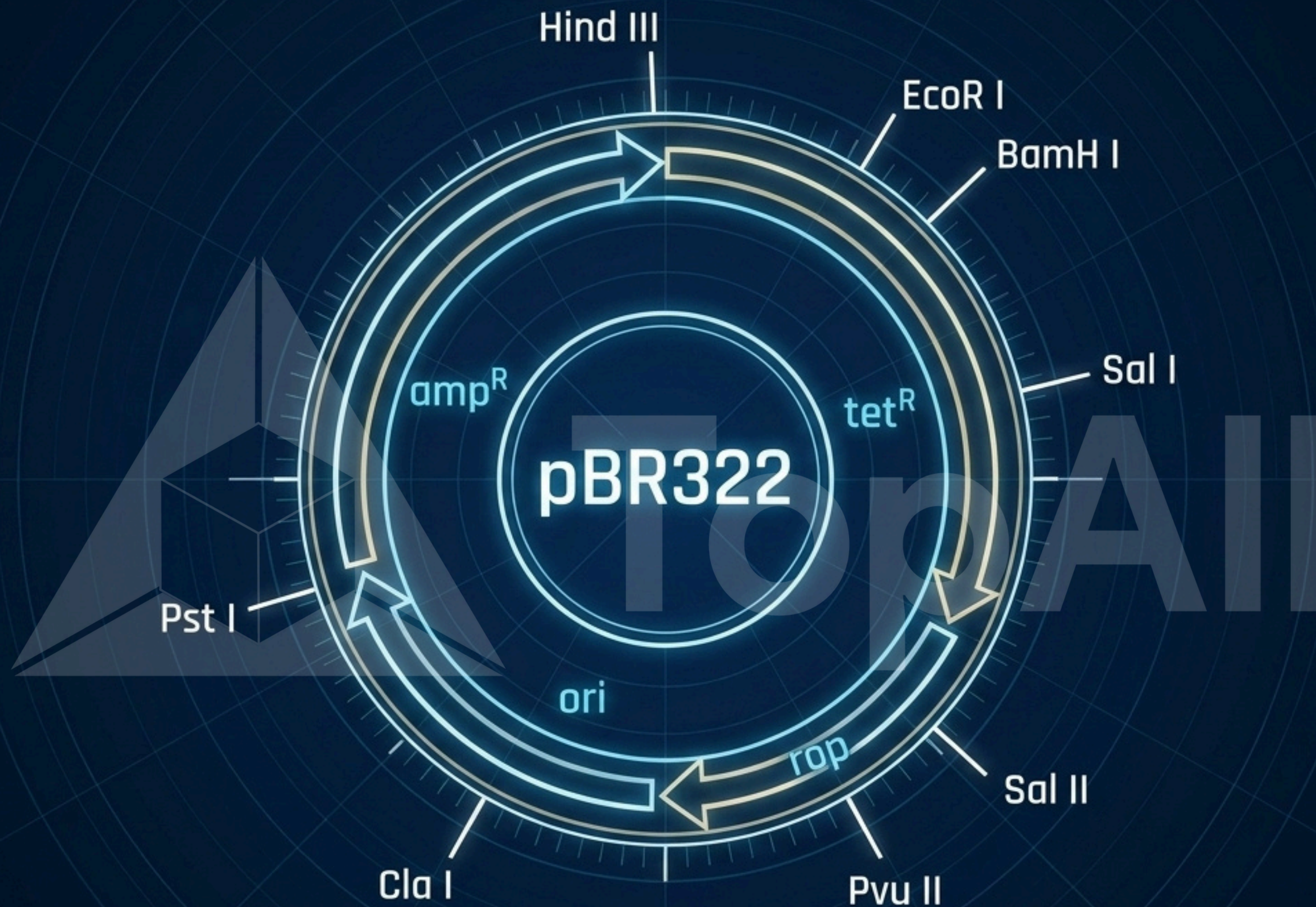
Flawed Design



The Risk: The presence of more than one recognition site within the vector will generate several fragments, severely complicating gene cloning.

The Insertion Point

Ligation of alien DNA is typically carried out at a restriction site present within one of the the antibiotic resistance genes.



Card 1: Ampicillin Resistance (amp^R)

Contains recognition sites for Pvu I and Pst I.

Card 2: Tetracycline Resistance (tet^R)

Contains recognition sites for BamH I and Sal I.

Ligating foreign DNA here causes insertional inactivation of tetracycline resistance.

Card 3: Replication Control (rop)

Codes for proteins involved in the replication of the plasmid.

Contains the Pvu II recognition site.

Card 4: Other Restriction Sites

EcoR I, Cla I, and Hind III sit outside the major resistance gene loci.



NEET Summary: The Vector Framework



INSERT

Cloning Sites

Vector Feature: Single recognition site.

Application: Use restriction enzymes (e.g., BamH I) to cut and ligate foreign DNA without fragmenting the vector.

SELECT

Selectable Marker

Vector Feature: Antibiotic Resistance or β -galactosidase gene.

Application: Insertional inactivation. (e.g., Chromogenic substrate: non-recombinants turn blue; recombinants are white/colorless due to inactivated β -galactosidase).

MULTIPLY

Origin of Replication

Vector Feature: ori sequence.

Application: Hijacks the host machinery to duplicate the linked DNA to the specific copy number of the vector.





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