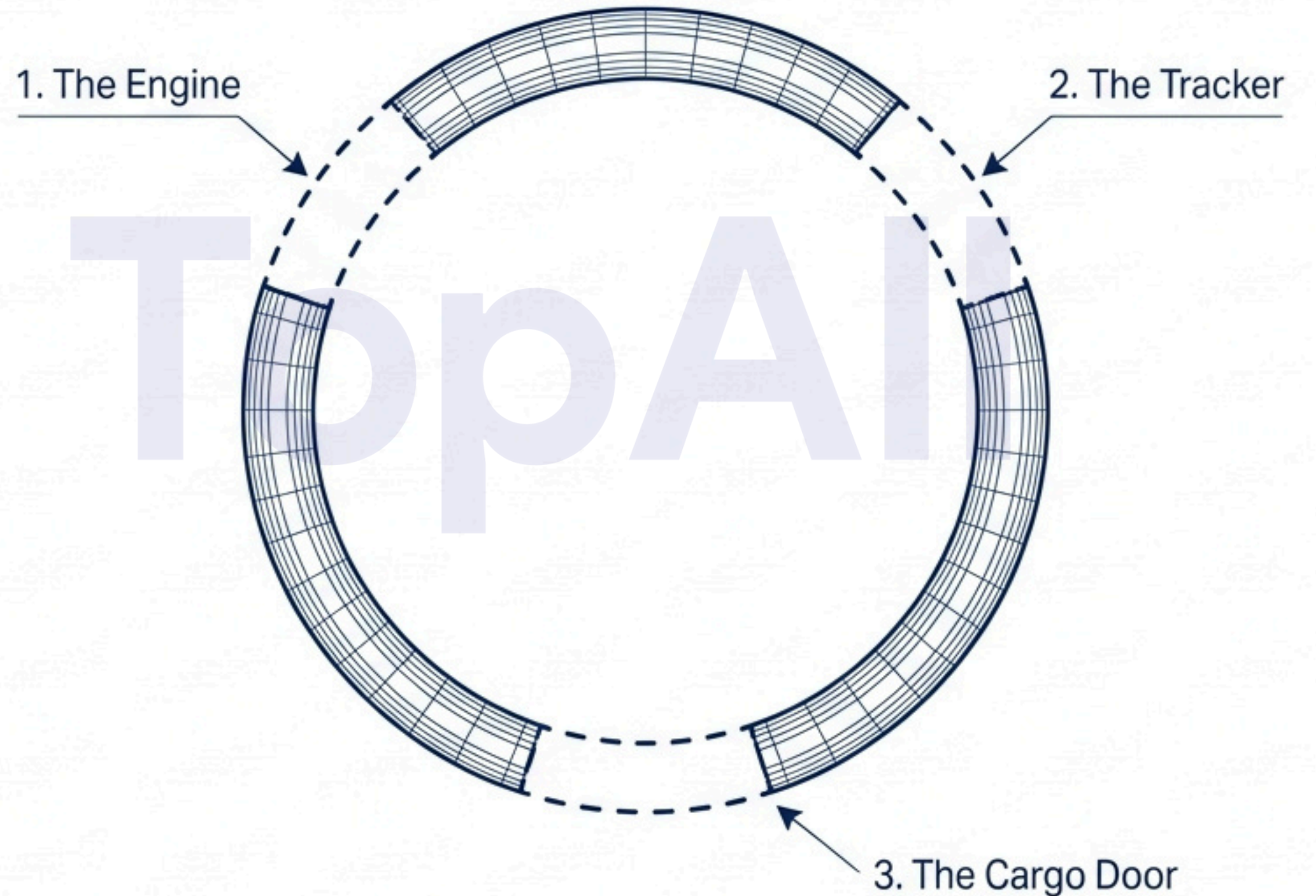


Engineering a Molecular Vehicle: Features of a Good Cloning Vector

A vector is an autonomously replicating DNA molecule used to ferry alien DNA into a host organism. To function effectively, it requires a specific set of engineered features.



The Engine:

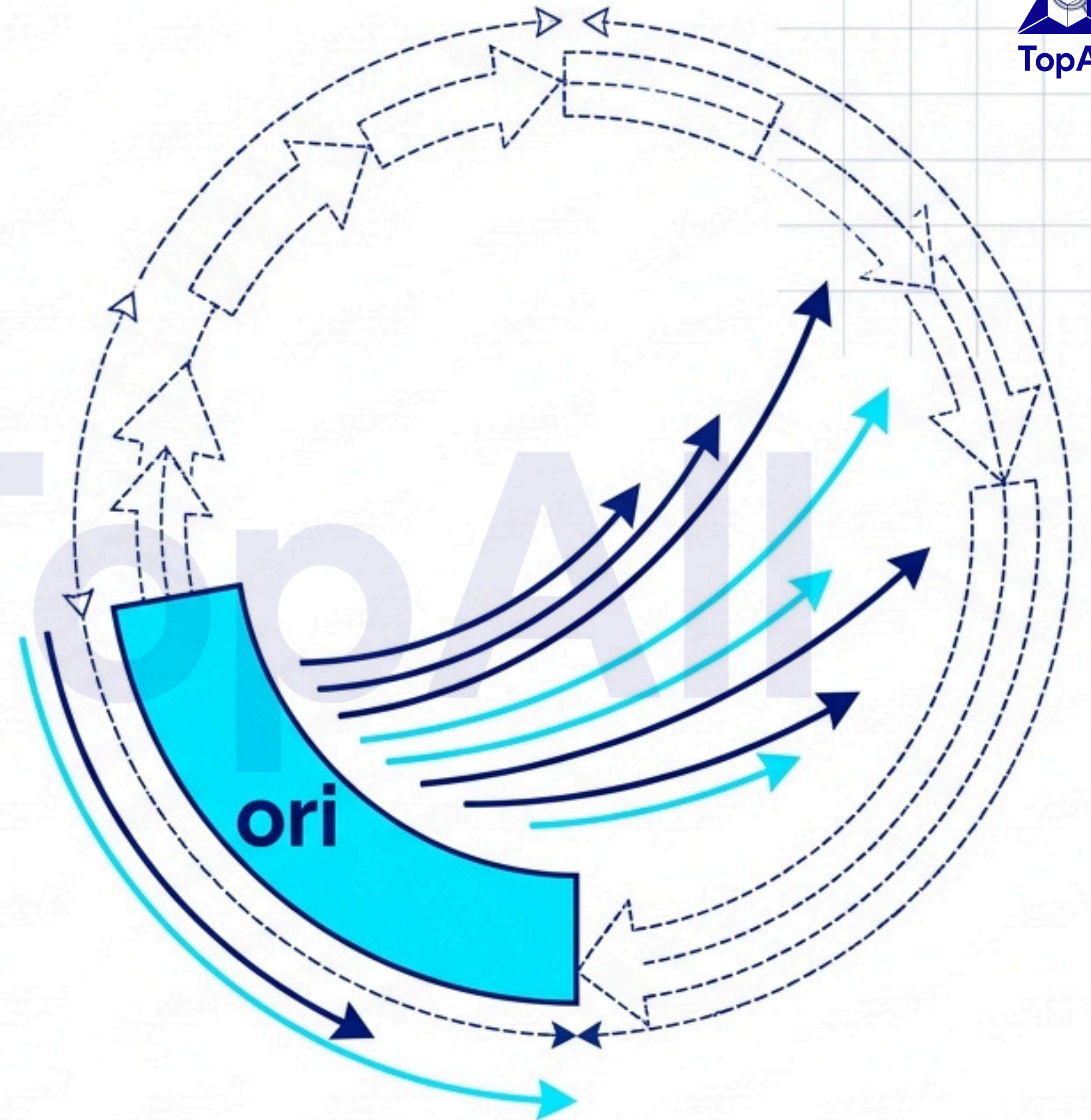
A specific DNA sequence where replication initiates. Any alien piece of DNA linked to this sequence will be forced to replicate within the host cell.

Copy Number Control:

The ori sequence dictates how many copies of the plasmid are maintained in the cell.

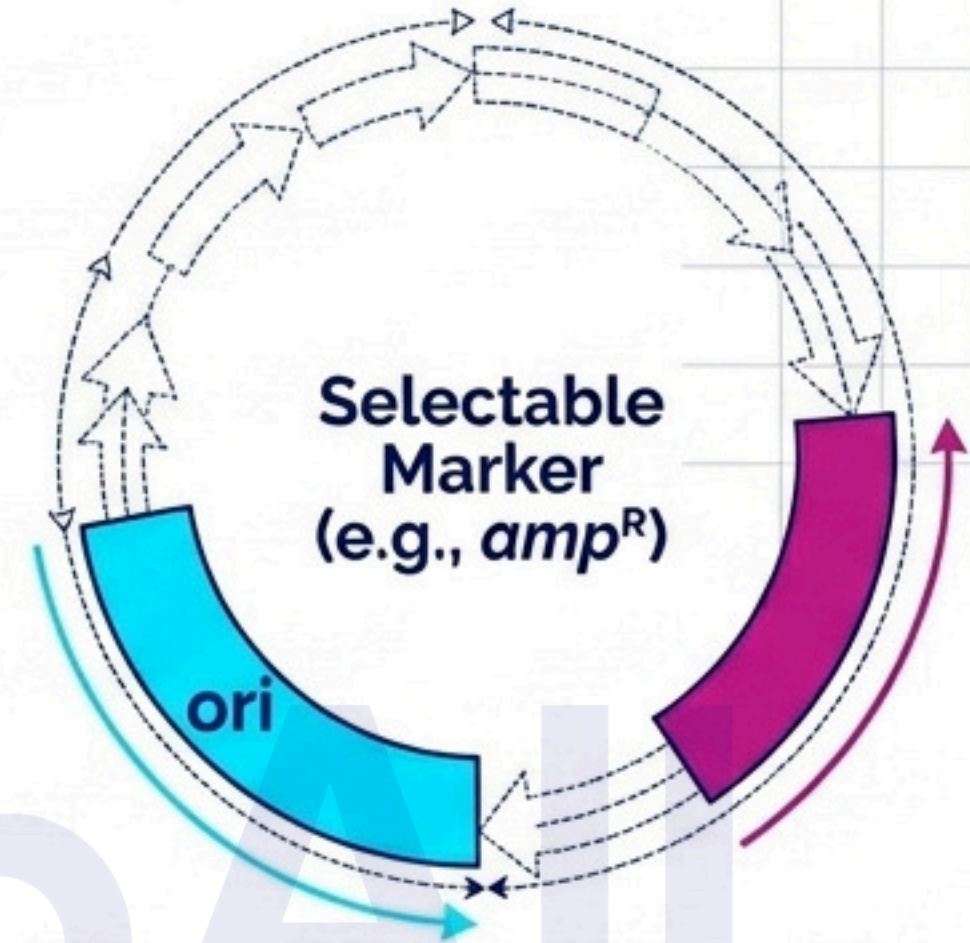
Key Insight:

To recover high yields of target DNA, the alien DNA must be cloned into a vector with an ori that supports a high copy number.

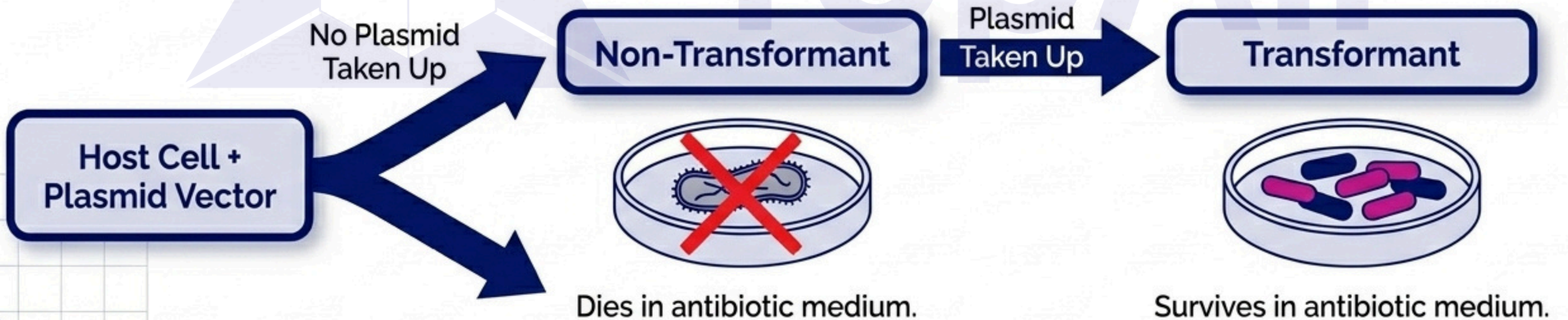


2. Selectable Marker

A gene that allows us to identify and eliminate non-transformants, selectively permitting the growth of transformants. Normal *E. coli* cells lack resistance to antibiotics like ampicillin, tetracycline, or chloramphenicol.



The Sorting Process





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$$E=mc^2$$



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3. Cloning Sites & Single Recognition

To link alien DNA, the vector requires very few—preferably single—recognition sites for commonly used restriction enzymes.

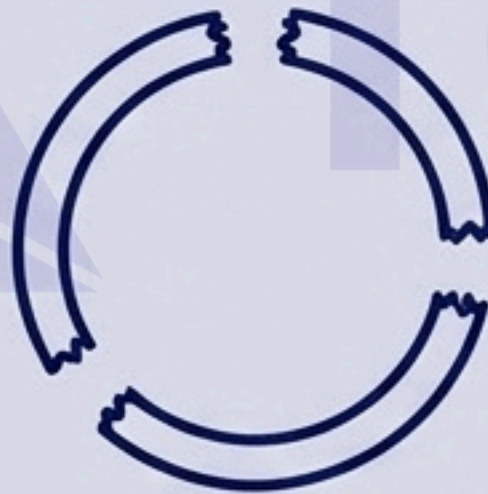


Ideal Design



Single Site → Linearized vector ready for exact cargo for exact cargo insertion.

Complicated Design



Multiple Sites → Vector fragments, complicating gene cloning.



Insertional Inactivation: The Cargo Sensor

State A: Vector without insert



Intact Gene

Antibiotic
Resistance
Active

Cell Survives
(Non-recombinant)

State B: Vector with alien DNA



Insertional
Inactivation

Resistance
Lost

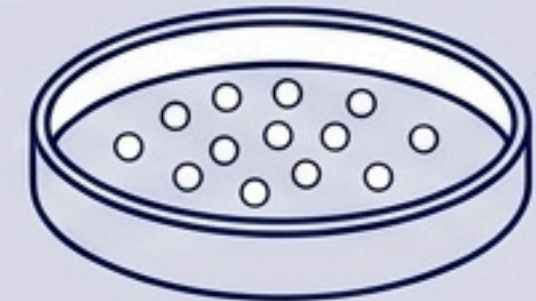
Cell Dies
(Recombinant)

Alternative Chromogenic Marker

Because simultaneous plating on two antibiotics is cumbersome, chromogenic substrates are used. Insertion inactivates the β -galactosidase gene.



No insert = Blue colonies



Insert present = White colonies
(Recombinant)

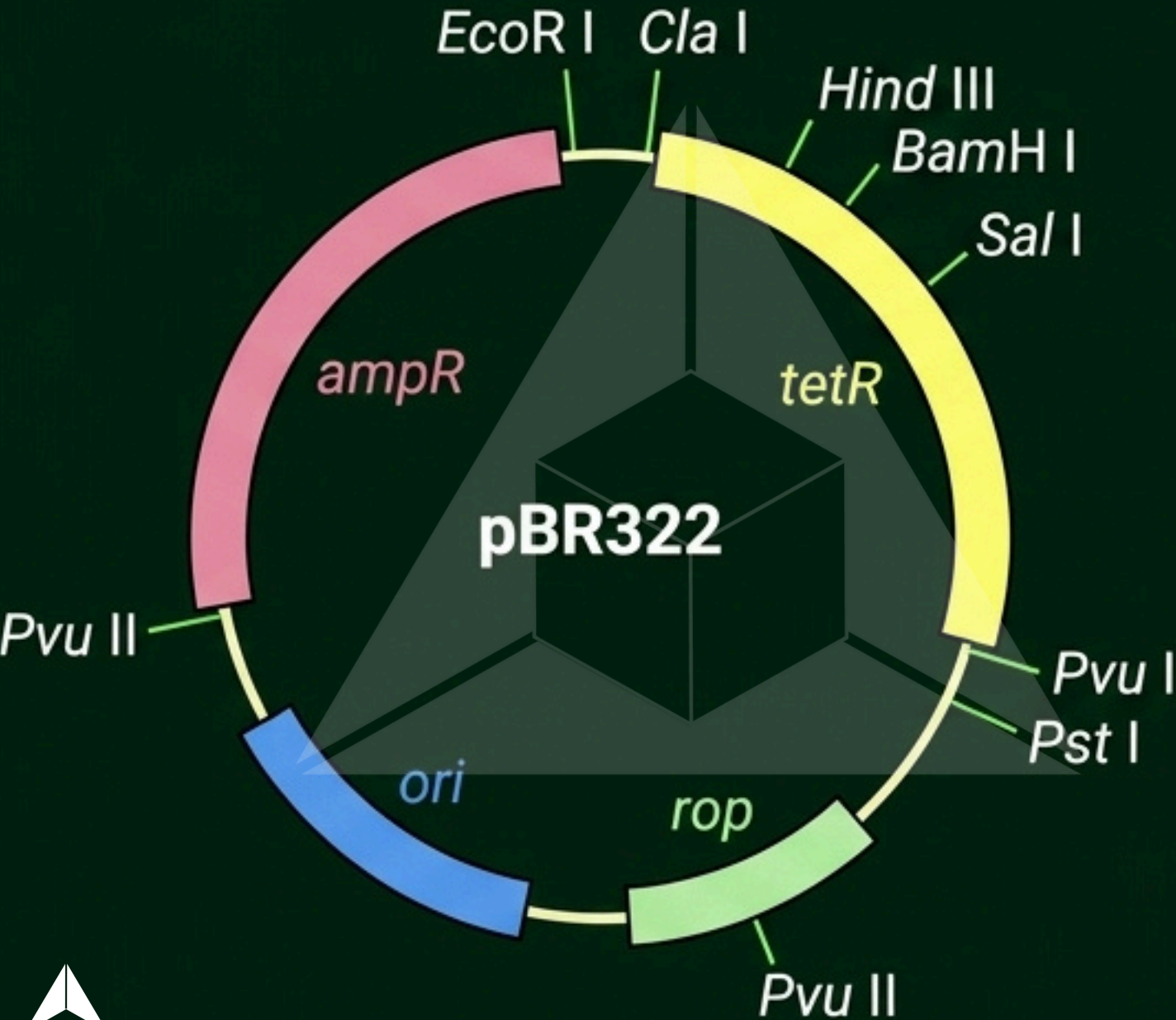
NEET Key Takeaway: The pBR322 Masterpiece

Diagnostic Matrix

	Ampicillin Medium	Tetracycline Medium
Non-transformant	Dies	Dies
Transformant (Non-recombinant)	Survives	Survives
Transformant (Recombinant at <i>BamH</i> I)	Survives	Dies

High-Yield Bullets

- Ligation at *BamH* I site disrupts the *tetR* gene.
- *rop* codes for proteins involved in plasmid replication.
- One marker selects transformants (*ampR*), the other selects recombinants (*tetR*).





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