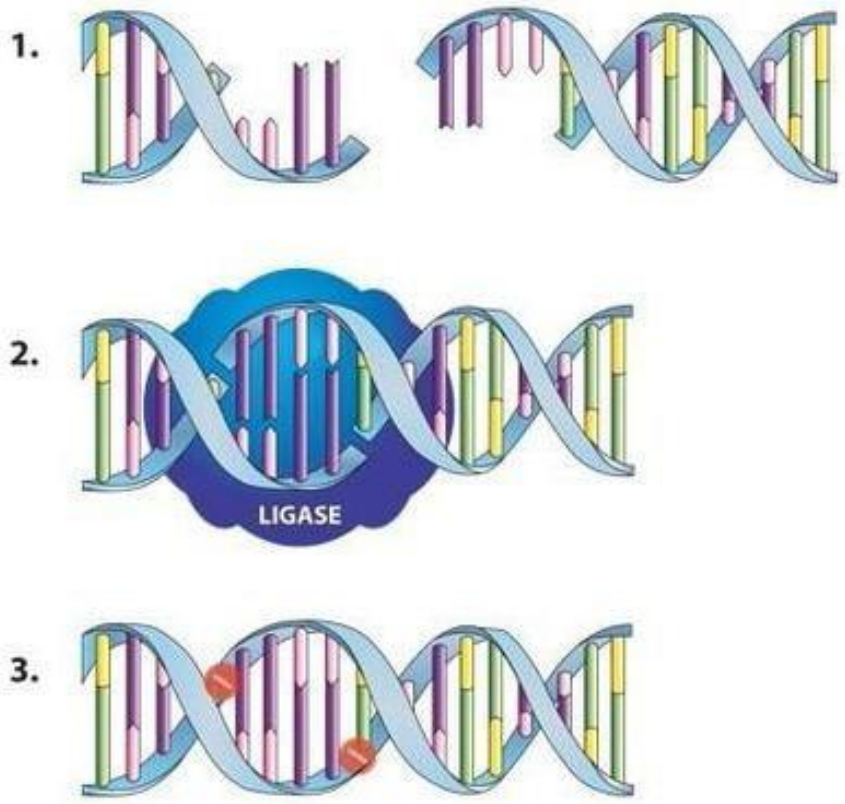
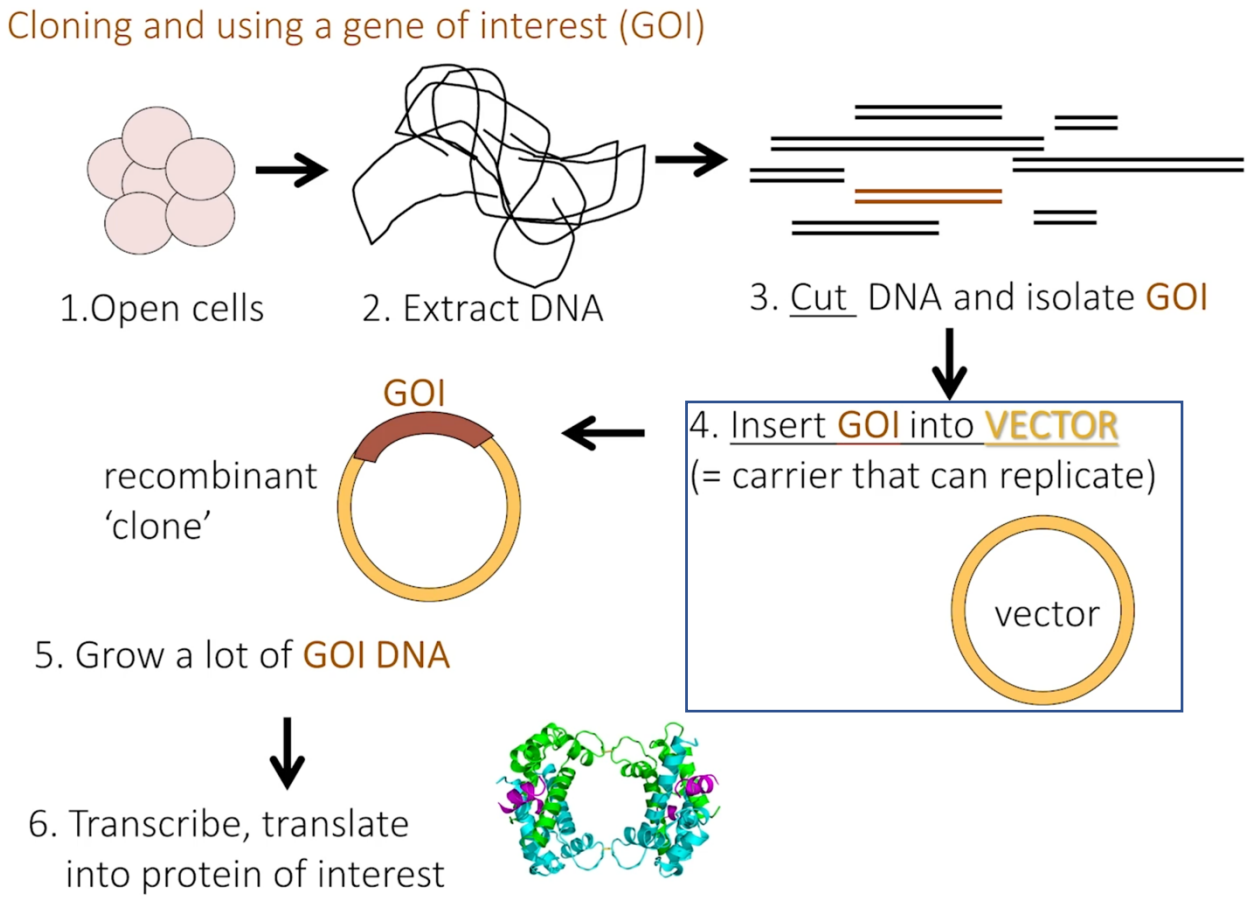
Lesson 18 – Genetic engineering: Vectors and ligation enzymes



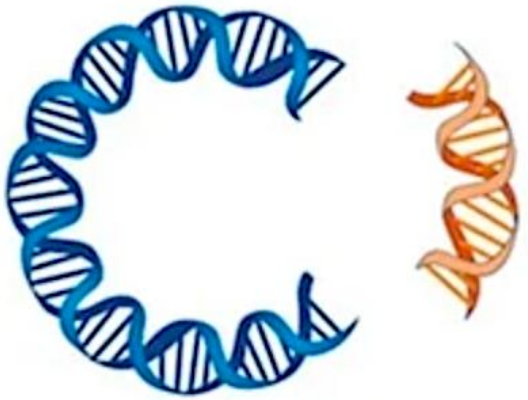
Vectors and ligation enzymes

- How do you isolate your GOI?
  - Advanced topic
- How do you insert your GOI into a DNA carrier molecule (vector)
  - Allows to replicate (clone) your GOI
- Vectors and ligation enzymes (aka ligases)



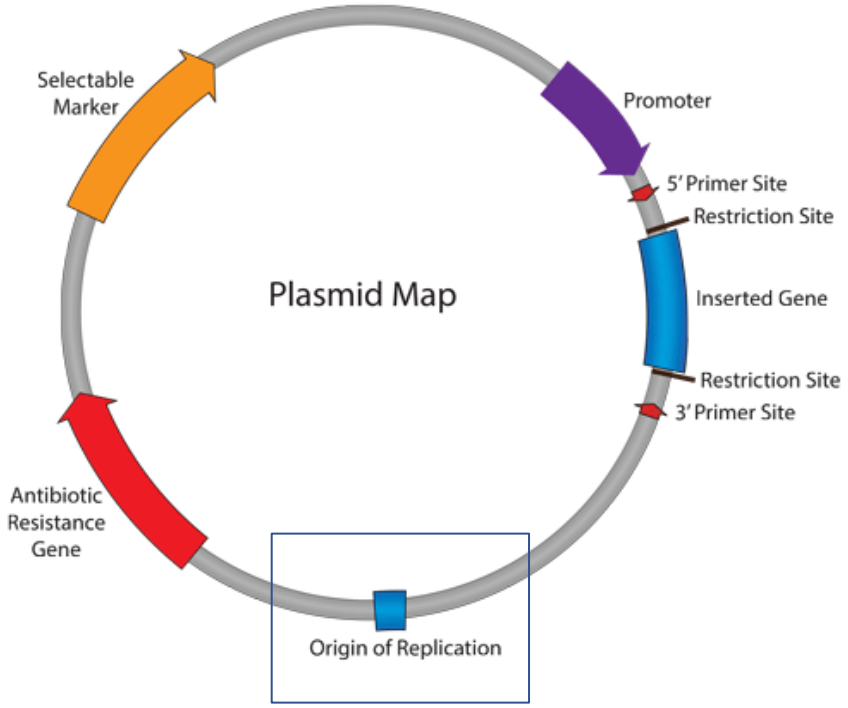
Vectors

- Bacterial cells often possess molecules of **closed (circular) DNA** known as **plasmids**
- Plasmids are non-essential self-replicating double-stranded DNA molecules which are important for the prokaryotic gene pool
  - Have a huge size distribution (*e.g.*, 2kb-200kb, kb = kilobases)
  - Are important for their ability to confer antibiotic resistance to bacteria
- Plasmids can only exist and replicate within a cell where they uses host cell machinery
- Natural plasmids must be genetically modified before being used as a vector for cloning
  - The ideal cloning plasmids should contain one site for your GOI insert
  - They are engineered so that the target restriction fragments, cut by specific restriction enzymes, have a unique location in that plasmid for your GOI insertion



Plasmids

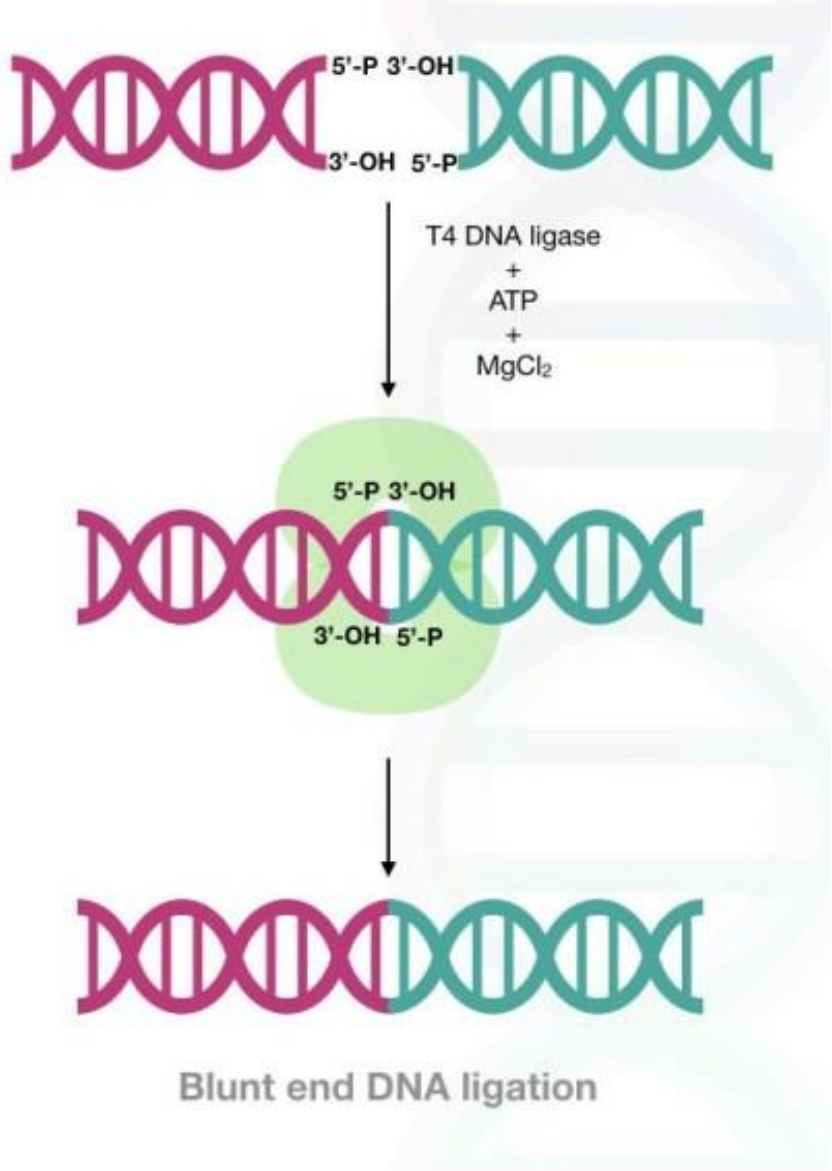
- Plasmids are non-essential self-replicating double-stranded DNA molecules → in essence, a plasmid is a *sort of rudimental DNA virus*
- The have an **ORI site** = **origin of (DNA) replication site***
  - ORI = DNA sequence that directs the host cell to initiate plasmid replication (i.e., DNA synthesis), thus enabling the plasmid to reproduce itself as it must survive within cells
- ORIs allow plasmids to replicate in bacterial host cells to a very high copy number (≧ 10^4 copies/cell)
  - Bacterial cultures can be easily expanded to billions of microorganisms
    - You can obtain large amounts (e.g., grams or even kilograms) of DNA carrying your GOI



*Our chrs also have ORI sites

DNA ligases

- GOI must be **pasted** (= covalently linked via phosphodiester bonds) into the plasmid
- This operation is performed by specific enzymes called **DNA ligases**
  - DNA ligases = enzymes that join DNA compatible (= matching) ends
- Any two blunt ends can easily ligate



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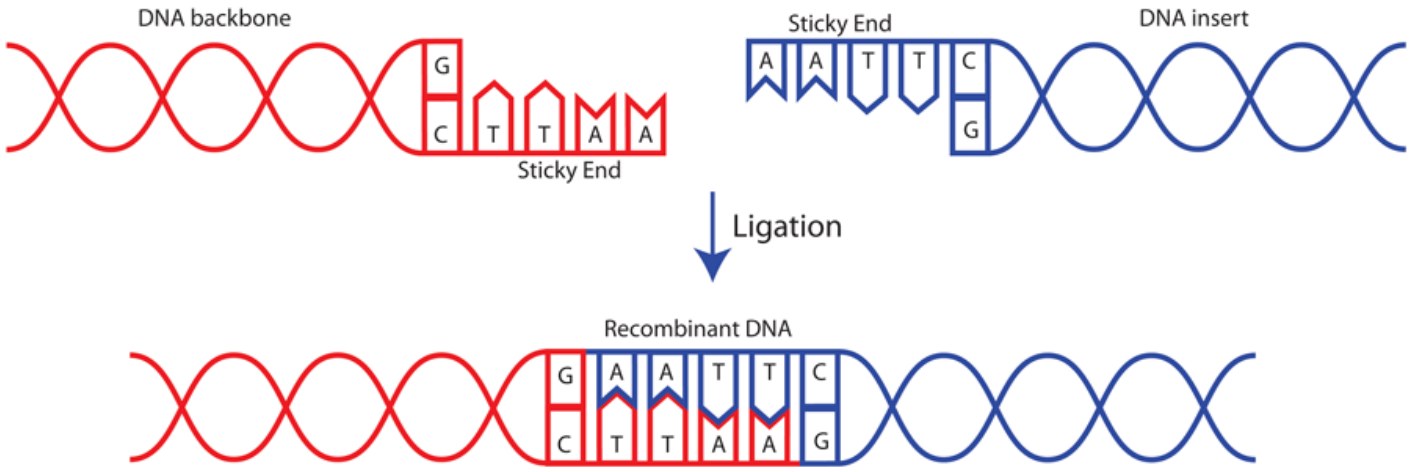

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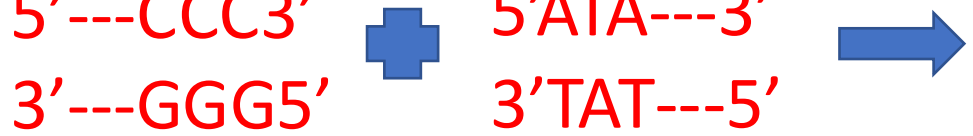
- After ligation you may or may not reform an endonuclease restriction site

Compatible ends - 1

- Any blunt ends can ligate



RECOMBINANT DNA
IN PLASMID

5’---CCCATA---3’
3’---GGGTAT---5’

NOT A PALINDROME
NOT A RESTRICTION SITE

Compatible ends - 1

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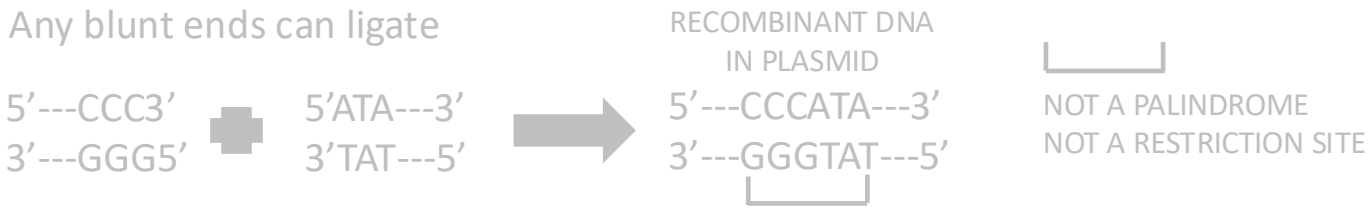
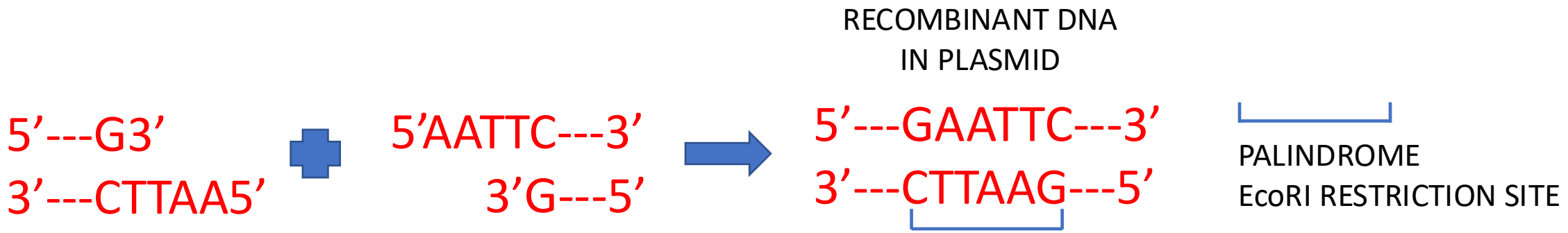


- Any complementary sticky ends can ligate



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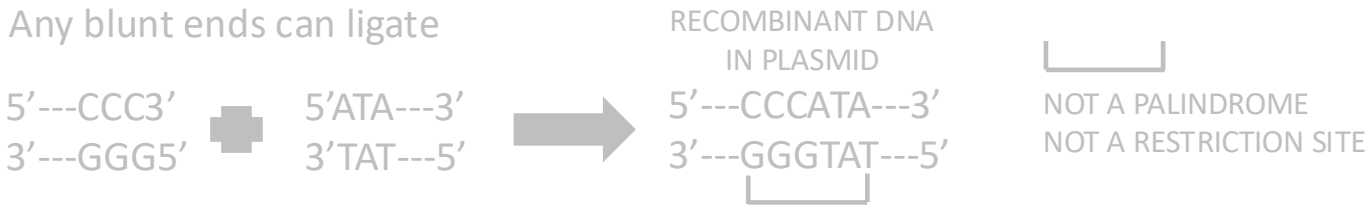

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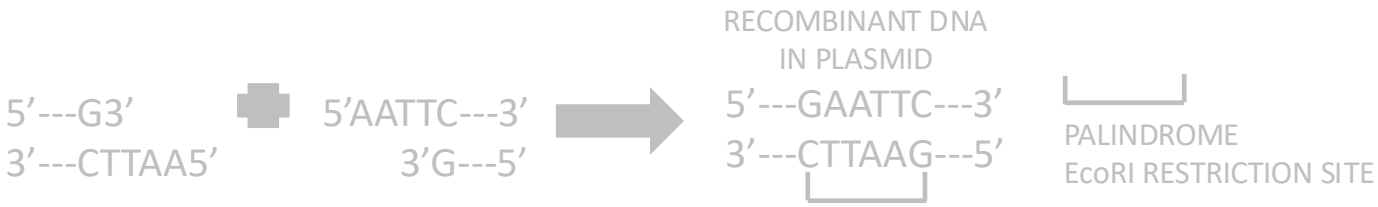
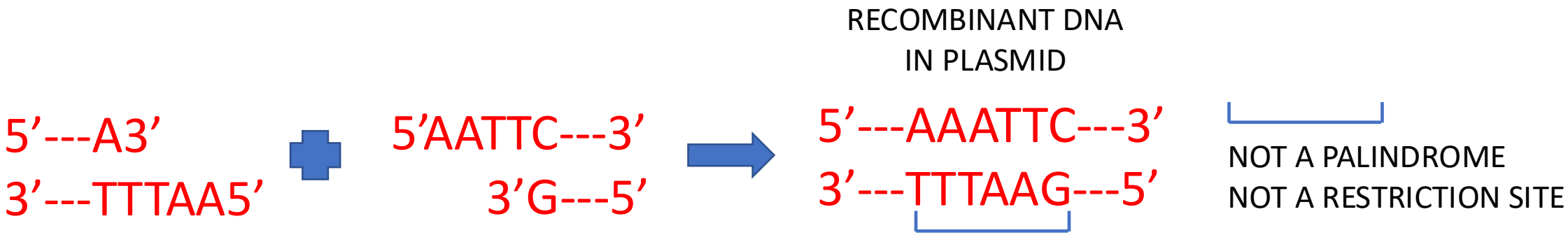


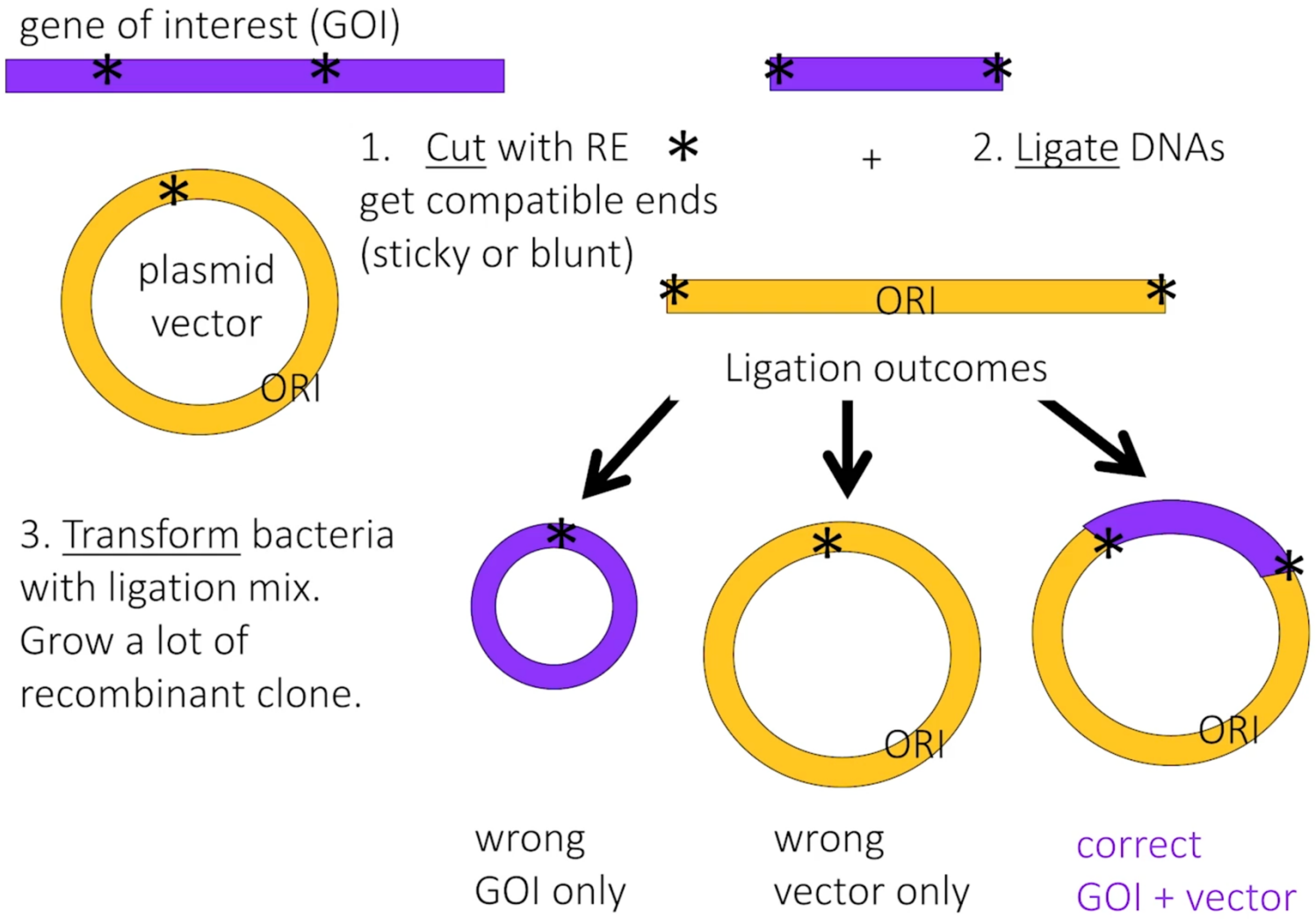


Steps to get your GOI into your vector

1. Prepare your vector by cutting the target restriction site using a specific RE (A)
2. Cut your GOI in the original DNA (possibly using the **same RE** employed in vector preparation) (B)
   - This ensures having matching ends on the vector and the GOI required for ligase action (exquisite example of genetic engineering skill)
3. Mix A and B and add the DNA ligase to seal the recombinant plasmid (C)
   - You do this on millions of molecules in one shot
4. Take C and insert (**transform**) into host bacteria
   - The same bacteria from where the original plasmids were derived

- **ATTENTION**: not all the bacteria will take up (transform) your C
  - Actually, most of them would not → you have to get rid of these "ineffective" bacteria and select only those who can work for you (more later)

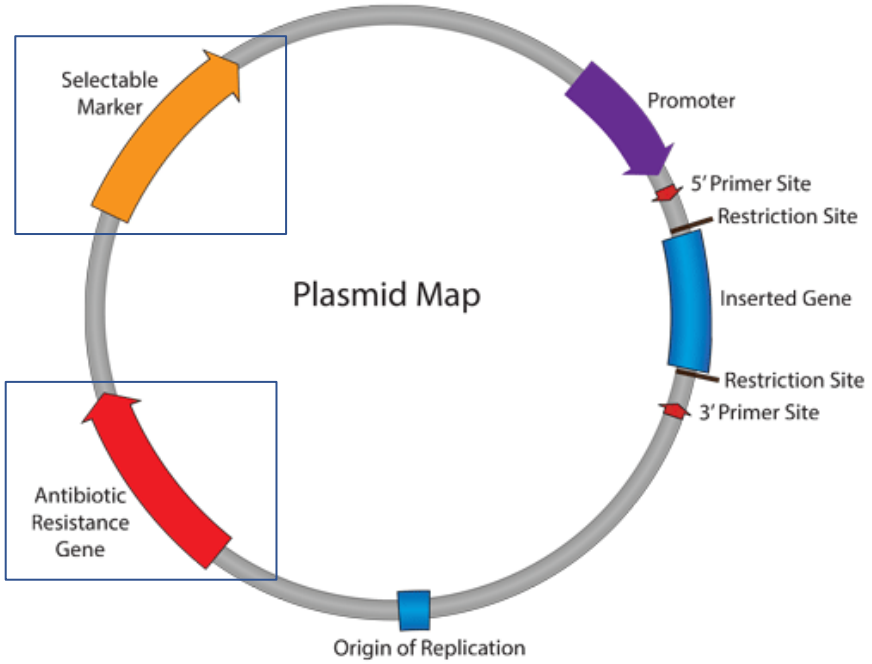
DNA cloning recap (simplified)



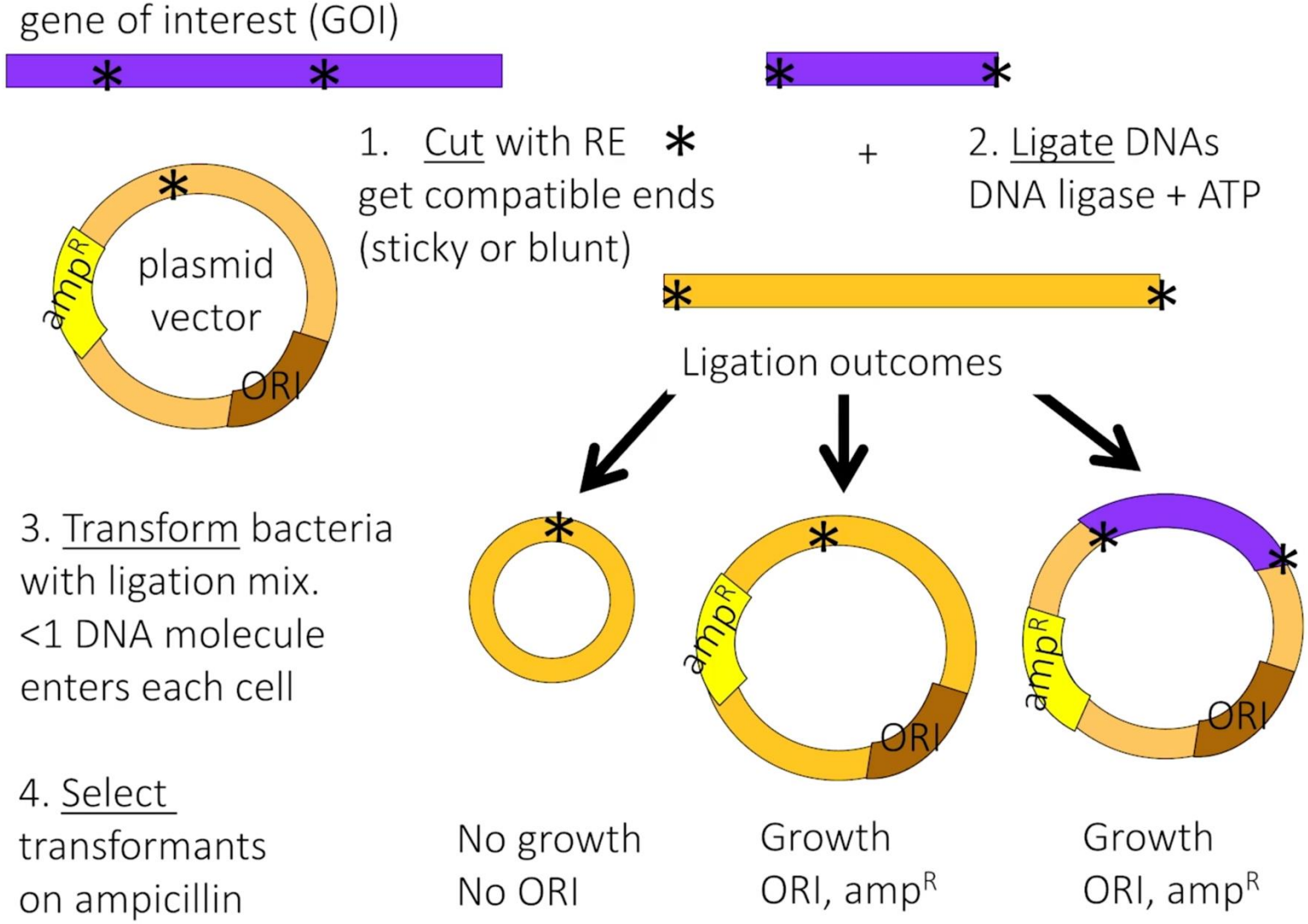
Plasmids - 2

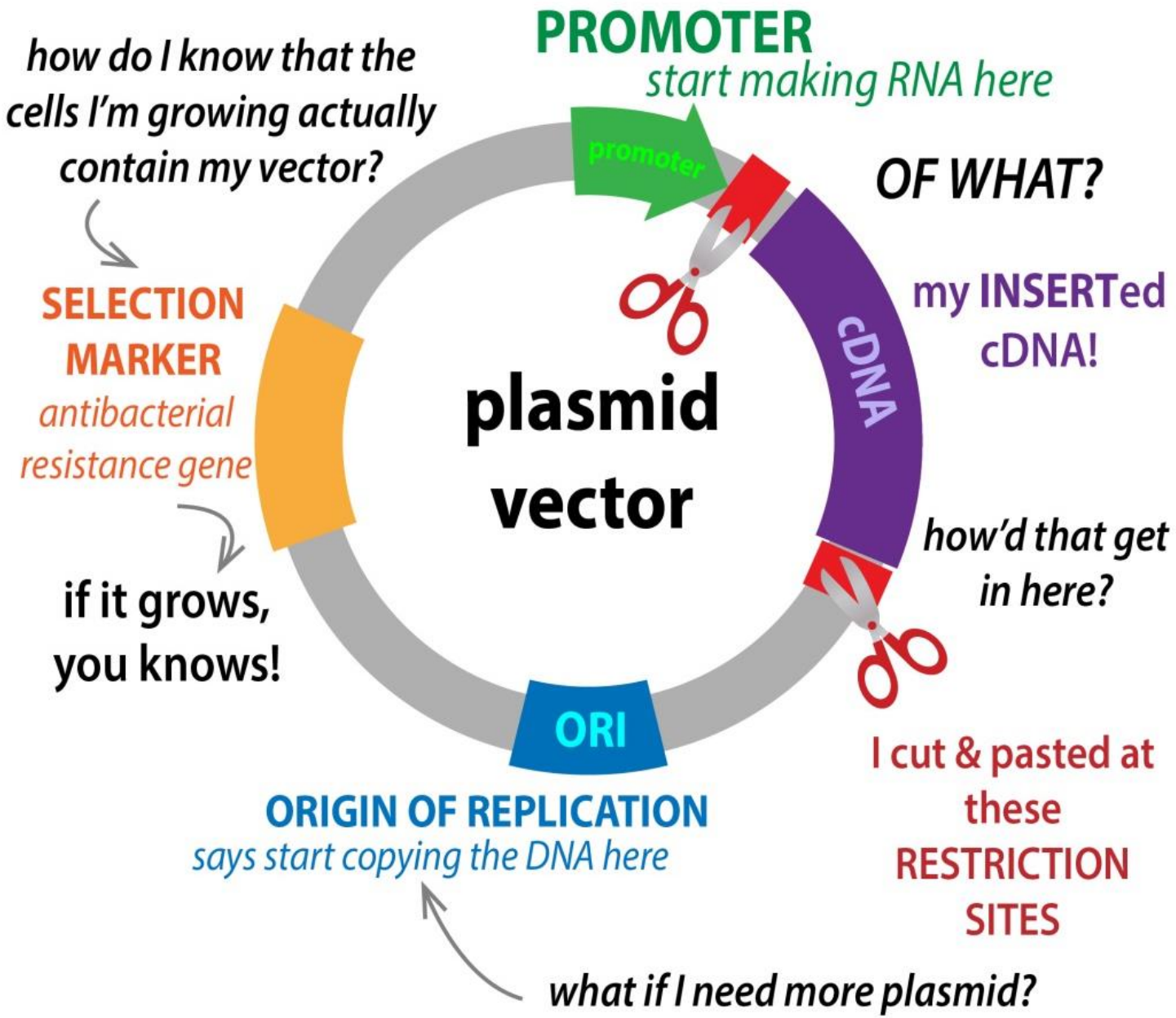
- How to get rid of these “ineffective” bacteria and select only those who carry the GOI-plasmid?
- You need to insert one or more **selectable marker(s)** in your plasmid
- Selectable markers = conditionally dominant genes that confer an ability to grow in the presence of applied selective agents that are normally toxic to host cells
  - Typically resistance to antibiotics
    - *e.g.*, Ampicillin
- They are usually inserted on the same plasmid carrying the GOI

5. Treat all transformed bacteria (effective and ineffective ones) with the specific antibiotic (*e.g.*, ampicillin)
   - Under this condition, only bacteria that contain plasmids with the ampicillin-resistant selectable marker can survive



DNA cloning recap 2 (simplified)




PROMOTER
start making RNA here
how do I know that the cells I'm growing actually contain my vector?
promoter
OF WHAT?
SELECTION MARKER
antibacterial resistance gene
plasmid vector
cDNA
my INSERTed cDNA!
how'd that get in here?
if it grows, you knows!
ORI
I cut & pasted at these RESTRICTION SITES
ORIGIN OF REPLICATION
says start copying the DNA here
what if I need more plasmid?