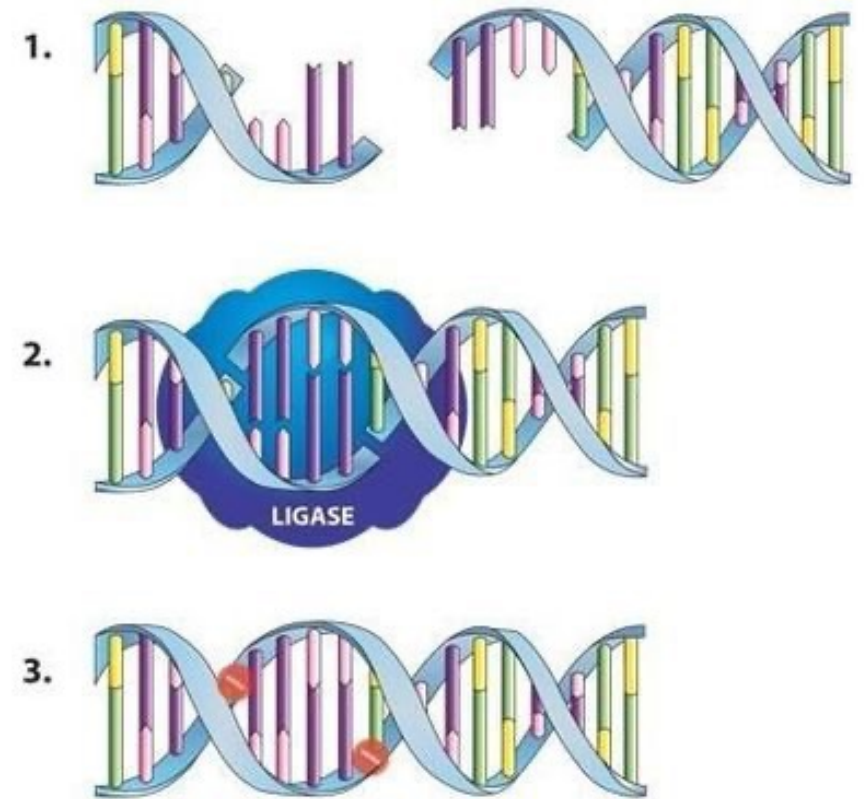


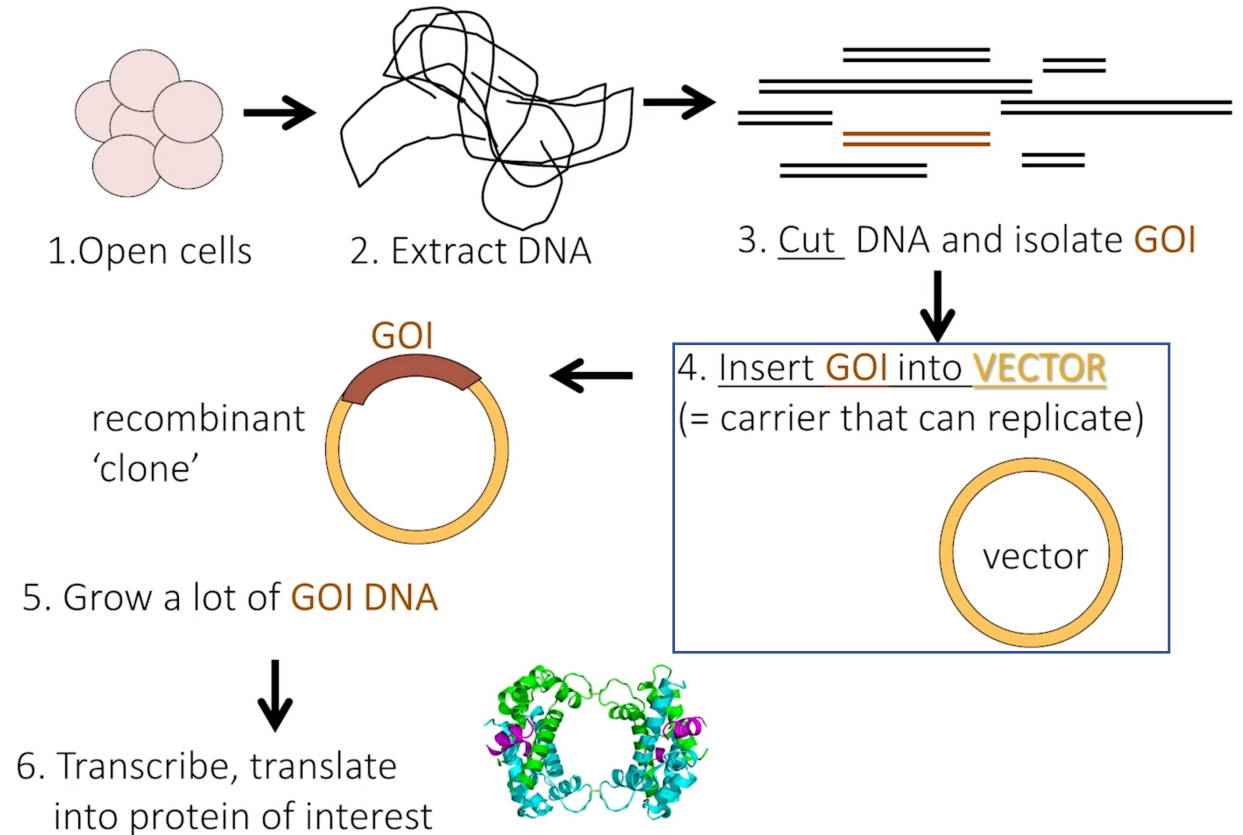
Lesson 18 – Genetic engineering: Vectors and ligation enzymes



Vectors and ligation enzymes

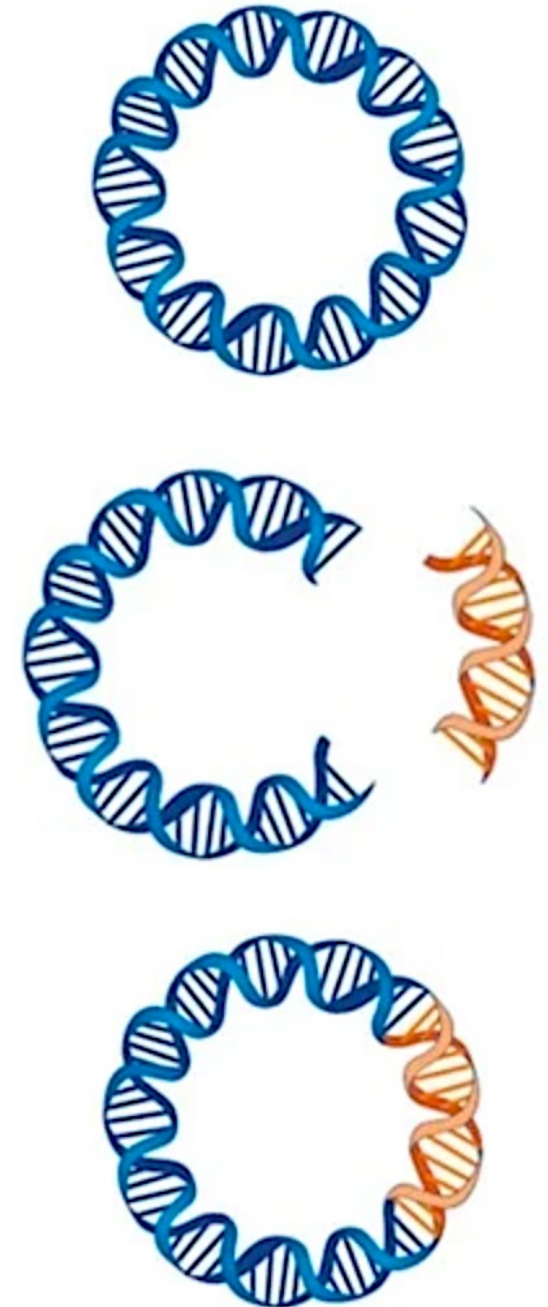
- How do you isolate your GOI?
 - Advanced topic
- How do you insert your GOI into a DNA carrier molecule (vector)
 - Allows to replicate (clone) your GOI
- Vectors and ligation enzymes (aka **ligases**)

Cloning and using a gene of interest (GOI)



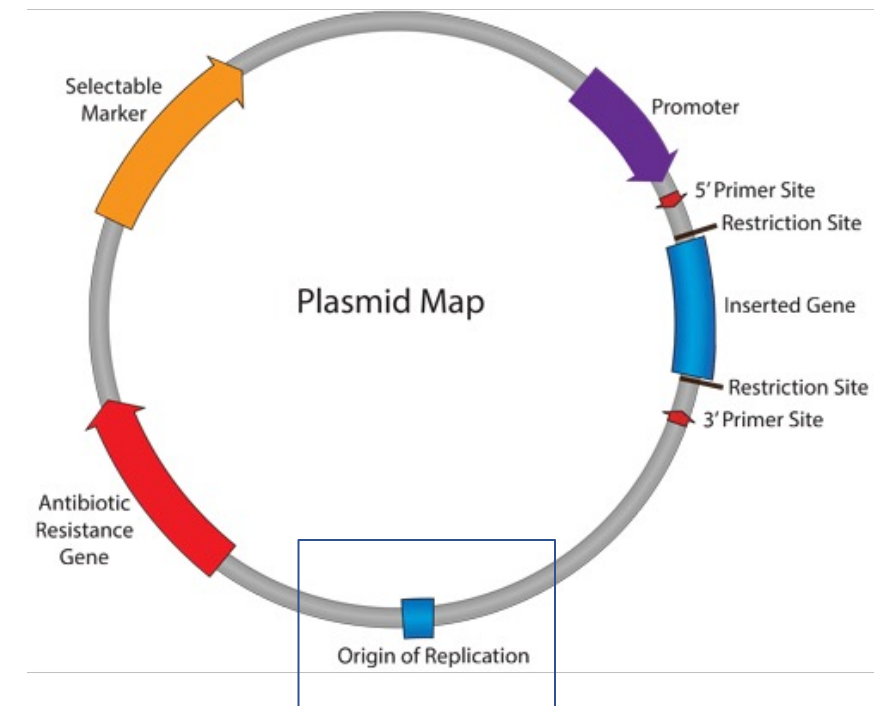
Vectors

- 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
 - Plasmids have a huge size distribution (*e.g.*, 2kb-200kb, kb = kilobases)
- Plasmids can only exist and replicate within a cell where they use 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

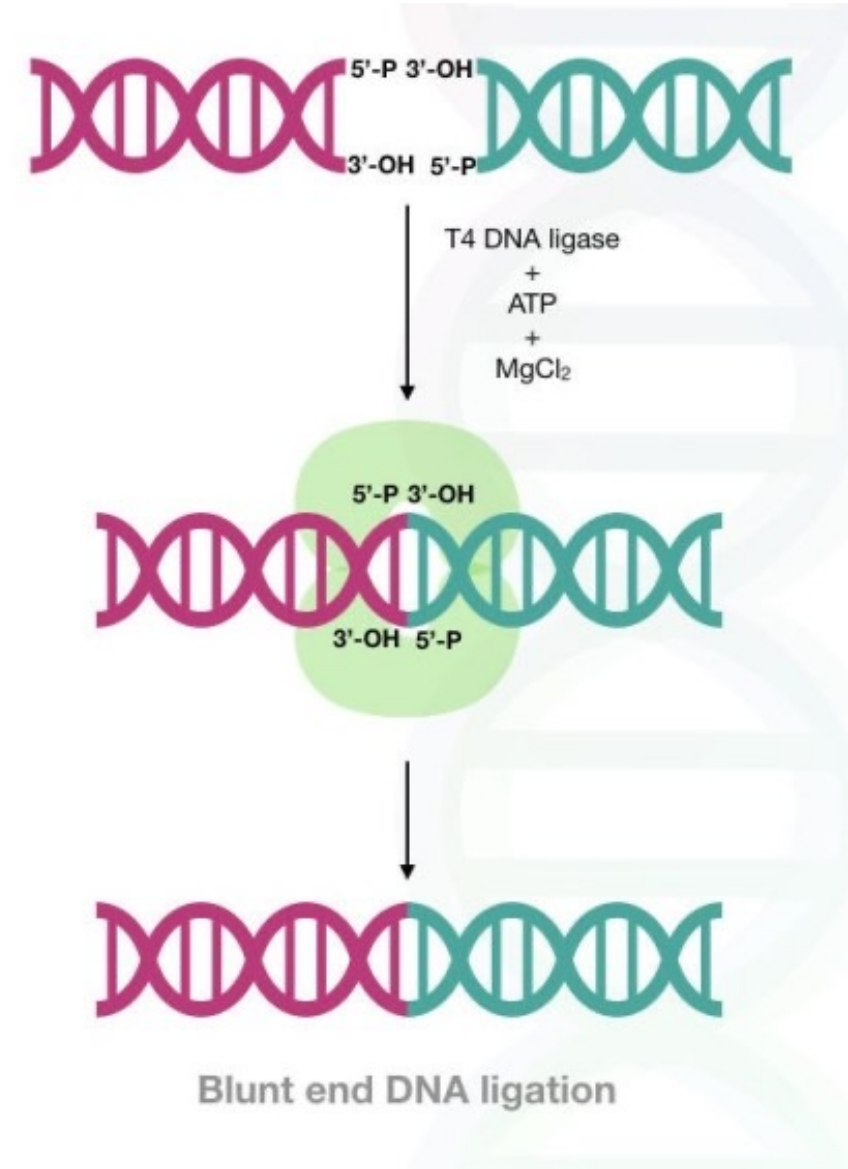
- Plasmids are non-essential self-replicating double-stranded DNA molecules → in essence, a plasmid is a *sort of rudimentary DNA virus*
- They have an **ORI site = origin of (DNA) replication site***
 - ORI = DNA sequence that directs the host cell to initiate plasmid replication (i.e., DNA synthesis), thus enabling the plasmid to reproduce itself as it must to survive within cells
- ORIs allow plasmids to replicate in bacterial host cells to a very high copy number ($\geq 10^4$ 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

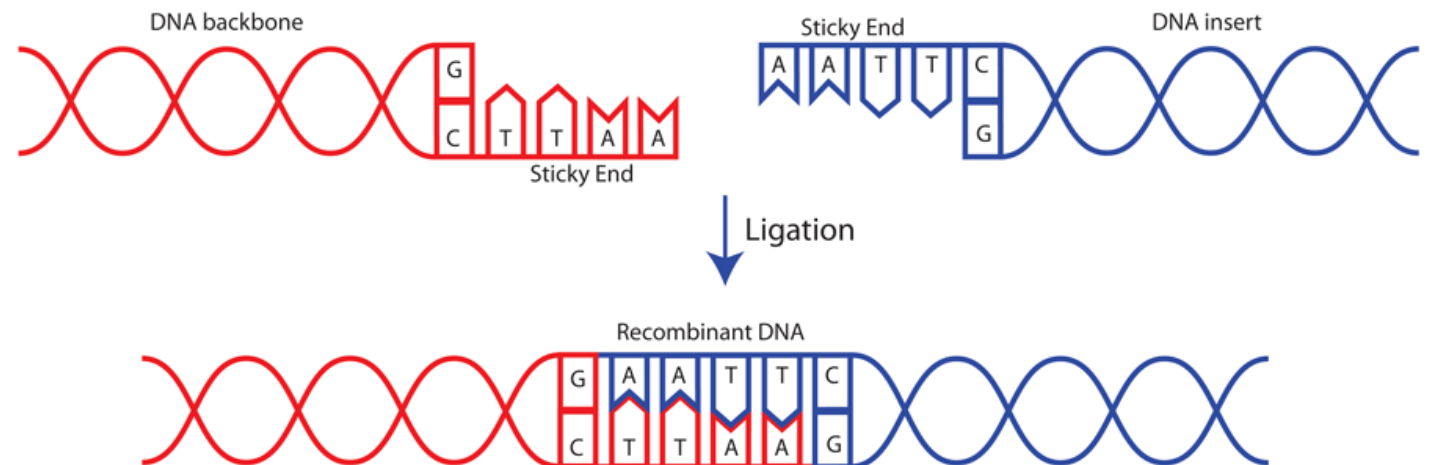
DNA ligases

- 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



DNA ligases

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- **Only complementary sticky ends can ligate**
(*i.e.*, base-pair)



DNA ligases

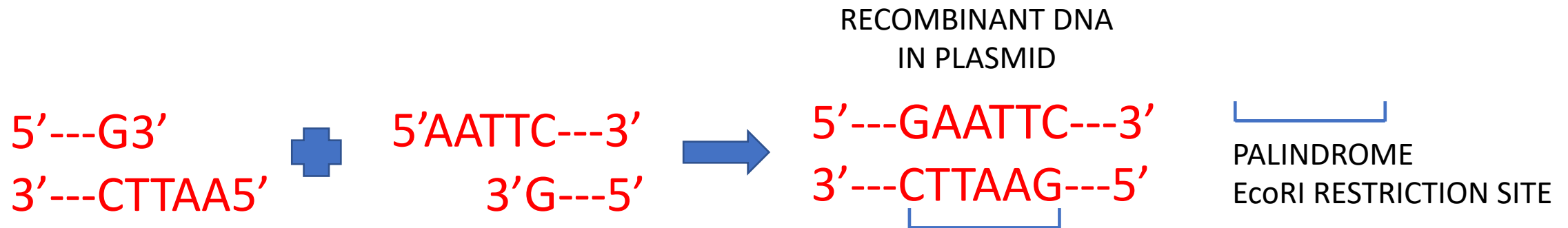
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- **After ligation you may or may not reform an endonuclease restriction site**

Compatible ends - 1

- Any blunt ends can ligate



- Any complementary sticky ends can ligate

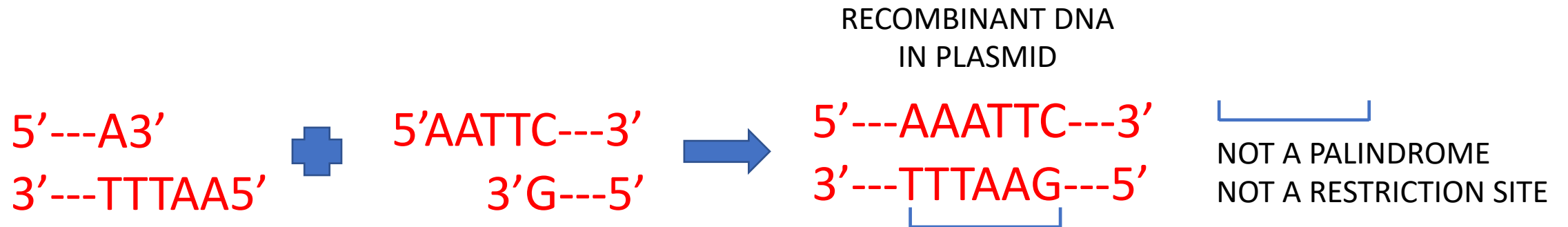
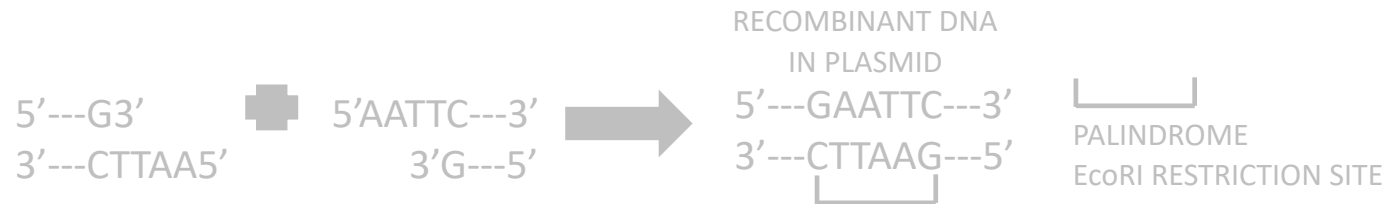


Compatible ends - 1

- Any blunt ends can ligate



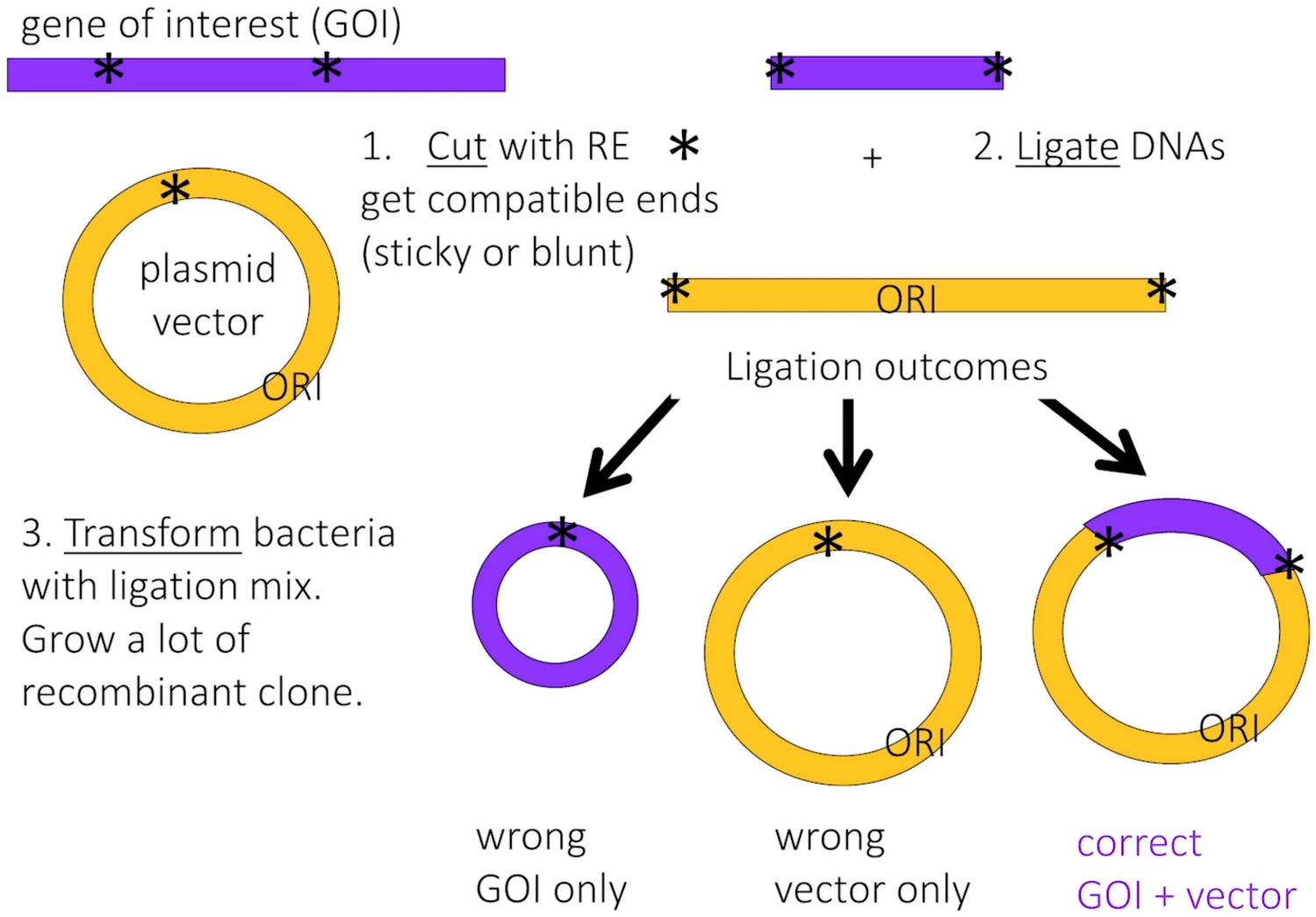
- Any complementary sticky ends can ligate



Steps to get your GOI into your vector

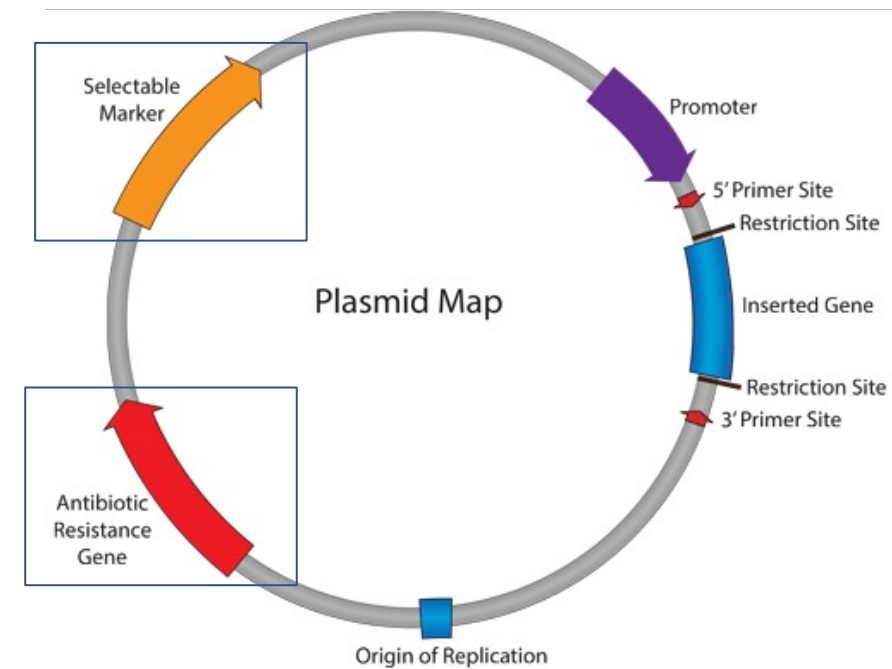
1. Prepare your vector by cutting the target restriction site using a specific RE (A)
2. Cut your GOI in the original DNA (possibly using the **same RE** employed in vector preparation) (B)
 - This ensures having matching ends on the vector and the GOI required for ligase action (exquisite example of 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)



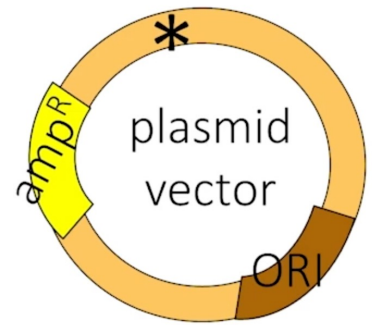
Plasmids - 2

- 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)

gene of interest (GOI)



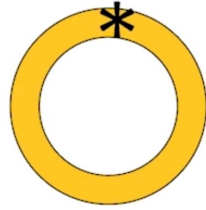
1. Cut with RE *
get compatible ends
(sticky or blunt)

2. Ligate DNAs
DNA ligase + ATP

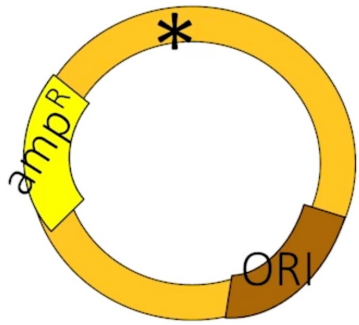


Ligation outcomes

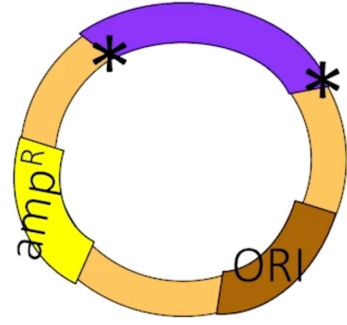
3. Transform bacteria
with ligation mix.
<1 DNA molecule
enters each cell



No growth
No ORI



Growth
ORI, amp^R



Growth
ORI, amp^R

4. Select
transformants
on ampicillin

