

# Restriction enzymes

# Discovery

- Typically bacteriophage have a single host they can infect, or a small number of related bacteria: the 'host range'
- In the 50's and 60's it was noticed that occasionally a virus could become active in a new strain but would no longer be effective in the original host
- This change in host range seemed to be associated with specific bases in the viral DNA being methylated: something that would have to be done by the host

# Methylases

- In the late 60's Werner Arber and Stewart Linn discovered that most bacteria contained a class of enzymes termed methylases. These could recognize a specific sequence of DNA and add a methyl group onto certain bases, most often an adenine or cytosine. The presence of these methyl groups on the viral DNA seemed to be the cause of their ability to grow in some strains of bacteria, but not others.

# Restriction enzymes

- It became clear that the ability to grow was because the methyl groups were protecting the viral DNA from an unusual type of enzyme.
- The bacteria contained an endonuclease that also recognized a specific base sequence (recognition site). DNA that was methylated was no longer recognized by the enzyme, and was safe from attack.
- Because the nuclease limited, or restricted, the number of bacteria that made up the host range; they became known as ‘host range restriction endonucleases’ which quickly became ‘restriction enzymes’

# Restriction/modification

- Obviously if a bacterium is going to have a restriction enzyme, it also needs the methylase (aka modification enzyme), otherwise it will destroy its own DNA
- There are two quite different types of restriction enzymes: Type I and Type II

# Type I

- These were the first to be discovered. Each consists of three subunits, coded separately
- Type I enzymes contain both a methylase and a nuclease. The binding is at a specific recognition site, which can be methylated and then the DNA loops back. The cut site may be as far as 1000 bases from the recognition site
- Each is used just once, then inactivated

# Type II

- These are the ones that are used in most experiments
- The nuclease and methylase are separate molecules, but recognize the same base sequence
- The nuclease will not attach if the site is methylated
- The cut site is within the recognition site: therefore a given enzyme will always cut in the same way, unlike Type I nucleases

# Naming restriction enzymes

- Usually the name is taken from the generic and specific names of the bacterium that makes the enzyme
- *Bacillus globigii*---Bgl
- *Moraxella bovis*---Mbo
- They are then numbered in order of discovery. So HpaI, HpaII *etc.*

# Recognition sites

- May be between 4 and 12 bases long. Enzymes are often termed 'four cutters', 'six cutters' etc. based on the length of the site
- Usually palindromic: reason for success. The enzyme attaches to BOTH strands and cuts them
- In some cases not every base is constant
- The cut site may be centered or off-center: The former produce what are termed 'blunt ends' and the enzymes are termed 'blunt cutters' Most produce a 'staggered cut' that leaves 'sticky ends'

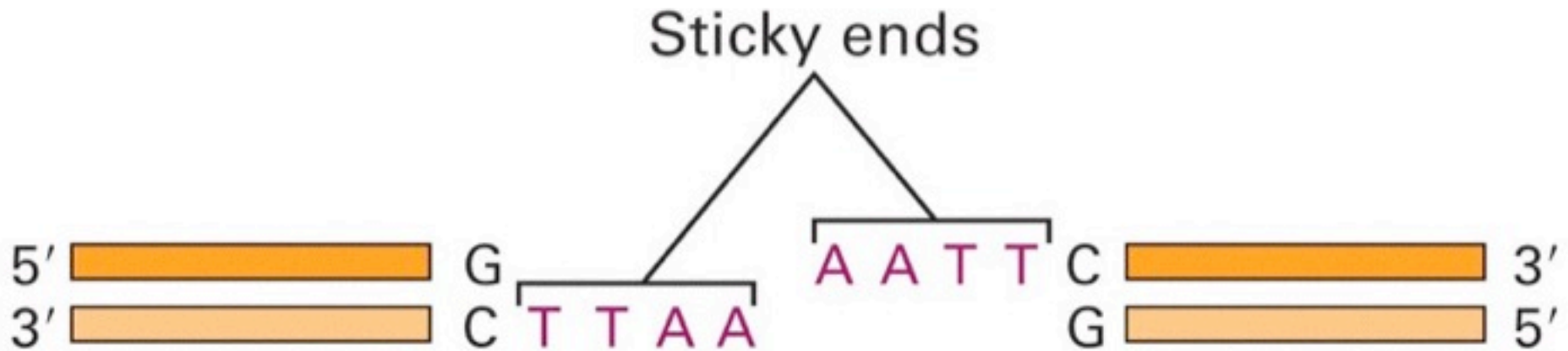
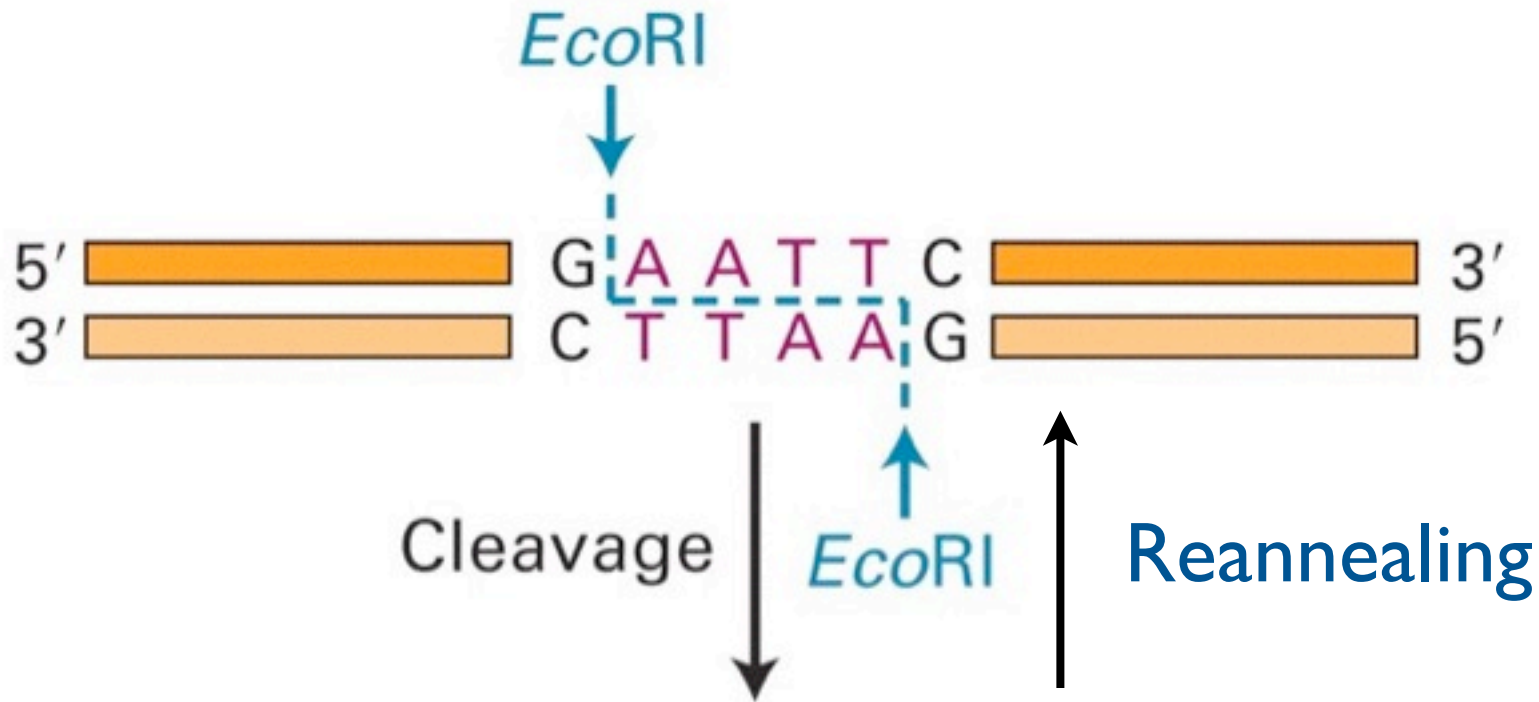
# Examples of sites

<b>HpaII</b>	C/CGG GGC/C	Four cutter, palindromic, sticky ends
<b>HpaI</b>	GTT/AAC CAA/TTG	Six cutter, palindromic, blunt ends
<b>SmaI</b>	CC/TNAGG GGANT/CC	Seven cutter, variable base, ,sticky ends
<b>DraIII</b>	RG/GNCCY YCCNG/GR	Seven cutter, very variable, sticky ends
<b>EcoRI</b>	GGC/GCC CCG/CGG	Six cutter, blunt end (look at next)
<b>NarI</b>	G/GCGCC CCGCG/G	Six cutter, sticky: isoschizomer of EcoRI

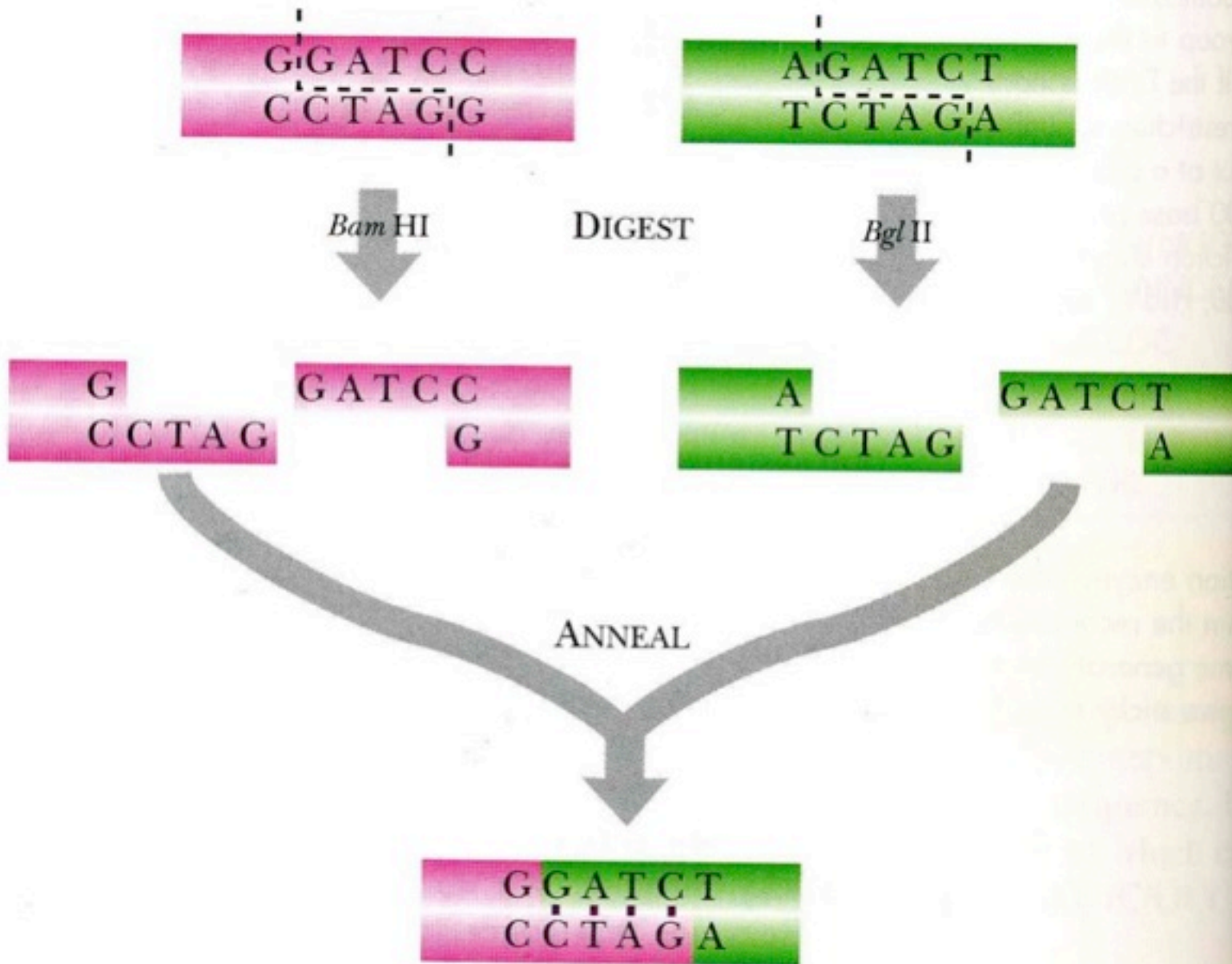
# Frequency of sites

- The longer a site is, the less likely it is to occur by chance. A site of four bp will show up (on average) only once in every  $4^4$  bp, i.e. 1/1024: a eight bp site only 1/1,048,576. So, a piece of DNA 10,000 bp long is likely to have several copies of the first, but probably does not contain a single example of the second

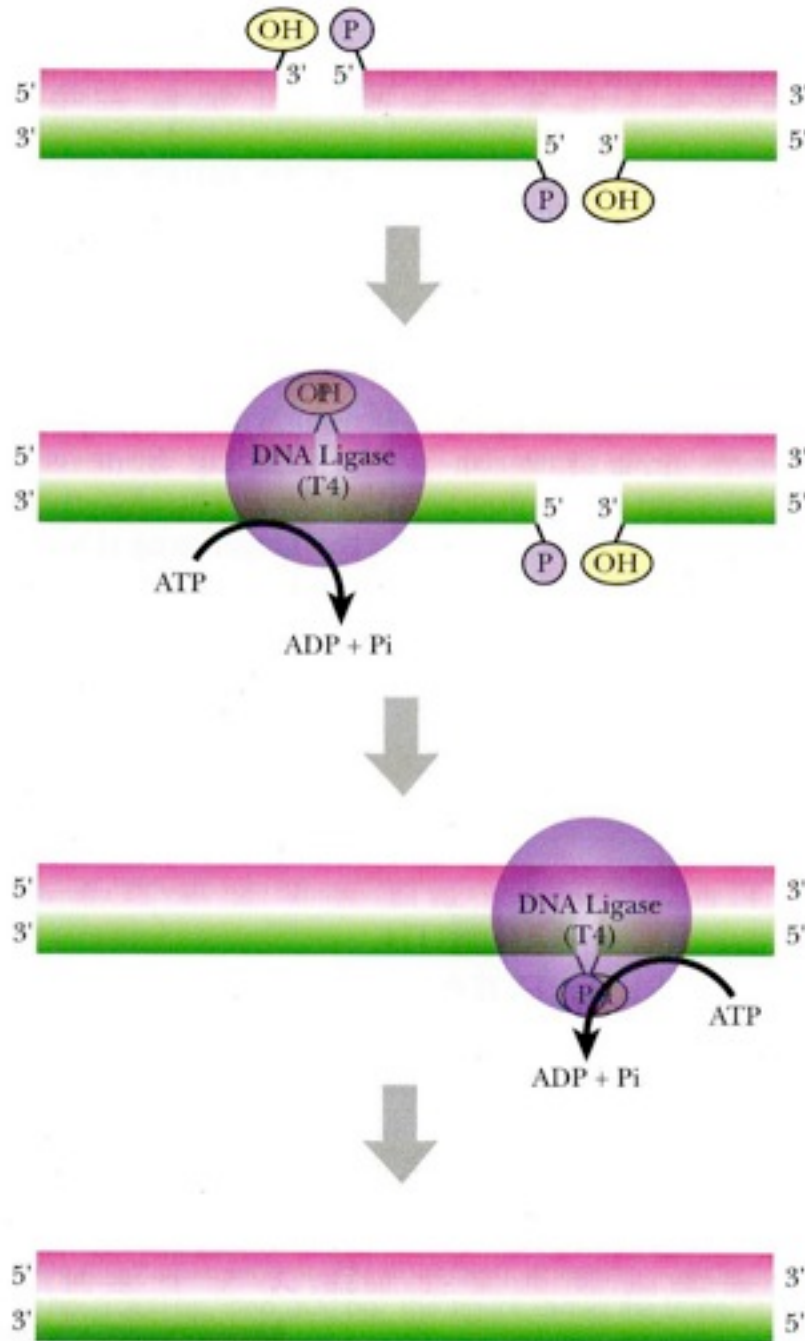
# Sticky ends



Note: as long as the same sticky ends are produced, they do not have to be made by the same enzyme



# Ligation



Ligation can be done even with blunt ends. It is a lot slower since there are fewer times where the molecules line up. However, ANY two blunt ends can be joined, which can be an advantage.

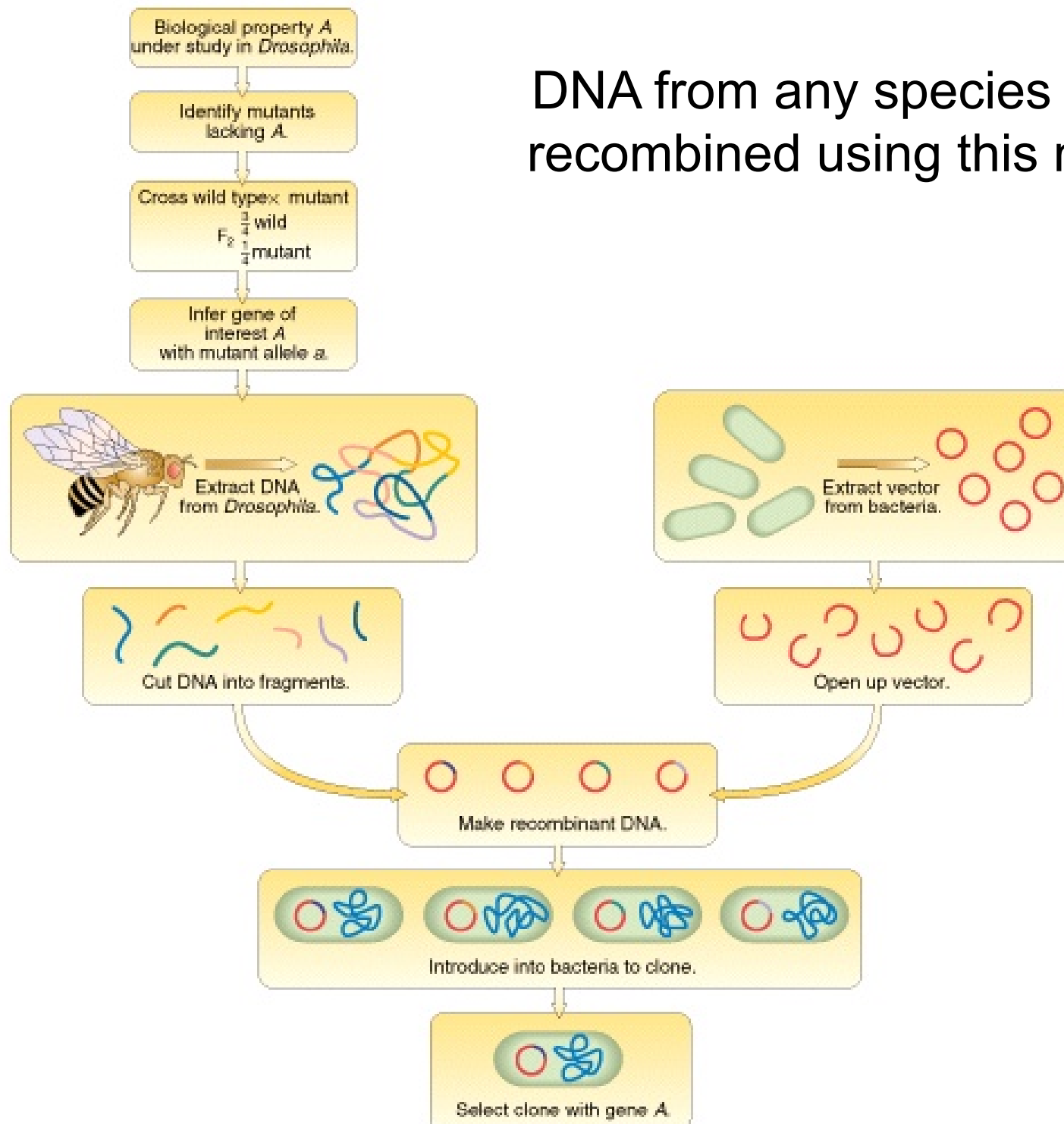
# Uses

- The two characteristics that make type II restriction enzymes so useful are
  - Most leave sticky ends
  - They always cut the DNA at the same location

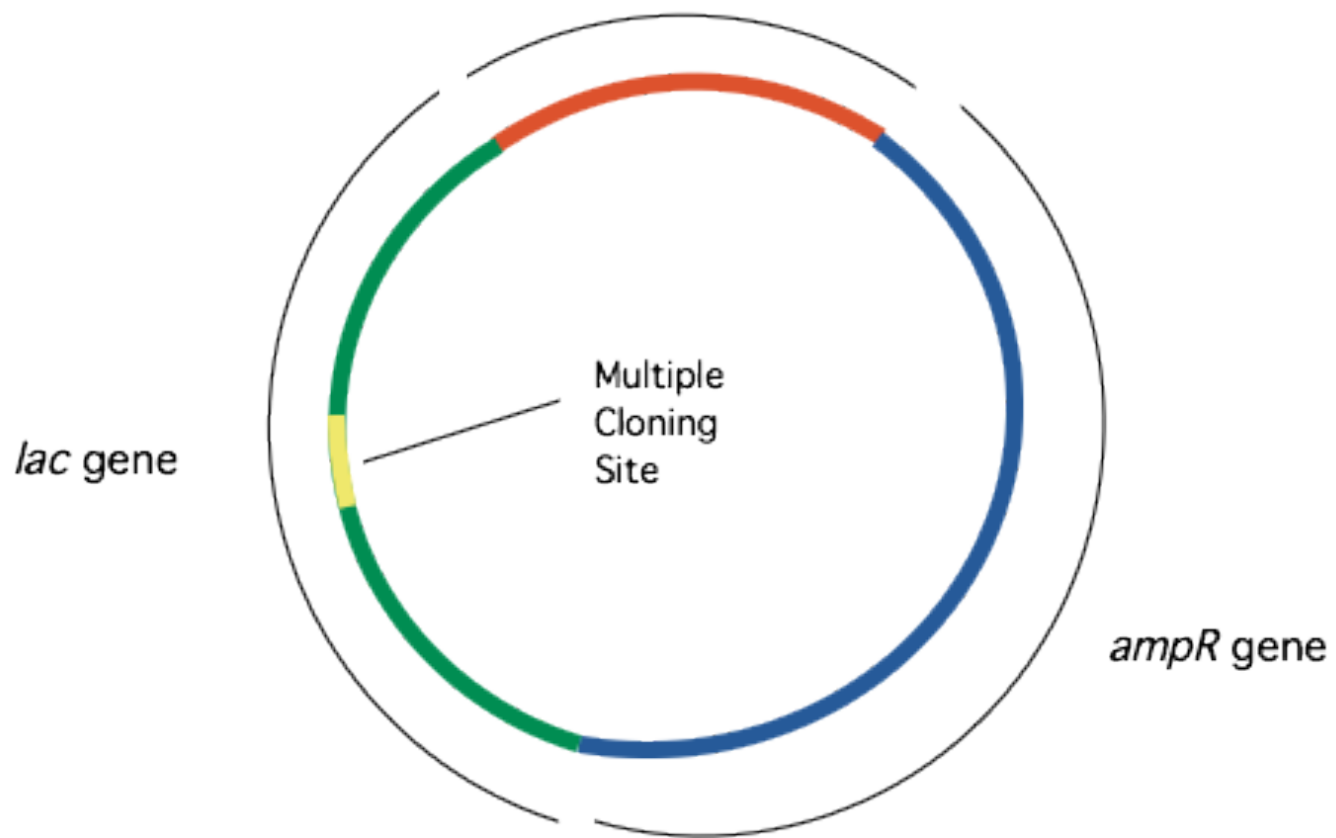
# Sticky ends

- Because any two complementary sticky ends will tend to stay attached for a period of time, it gives ligase a chance to heal the nicks in the backbone
- The result is that it becomes possible to link together any two pieces of DNA, regardless of their origin, to produce a piece of recombinant DNA

DNA from any species can be recombined using this method

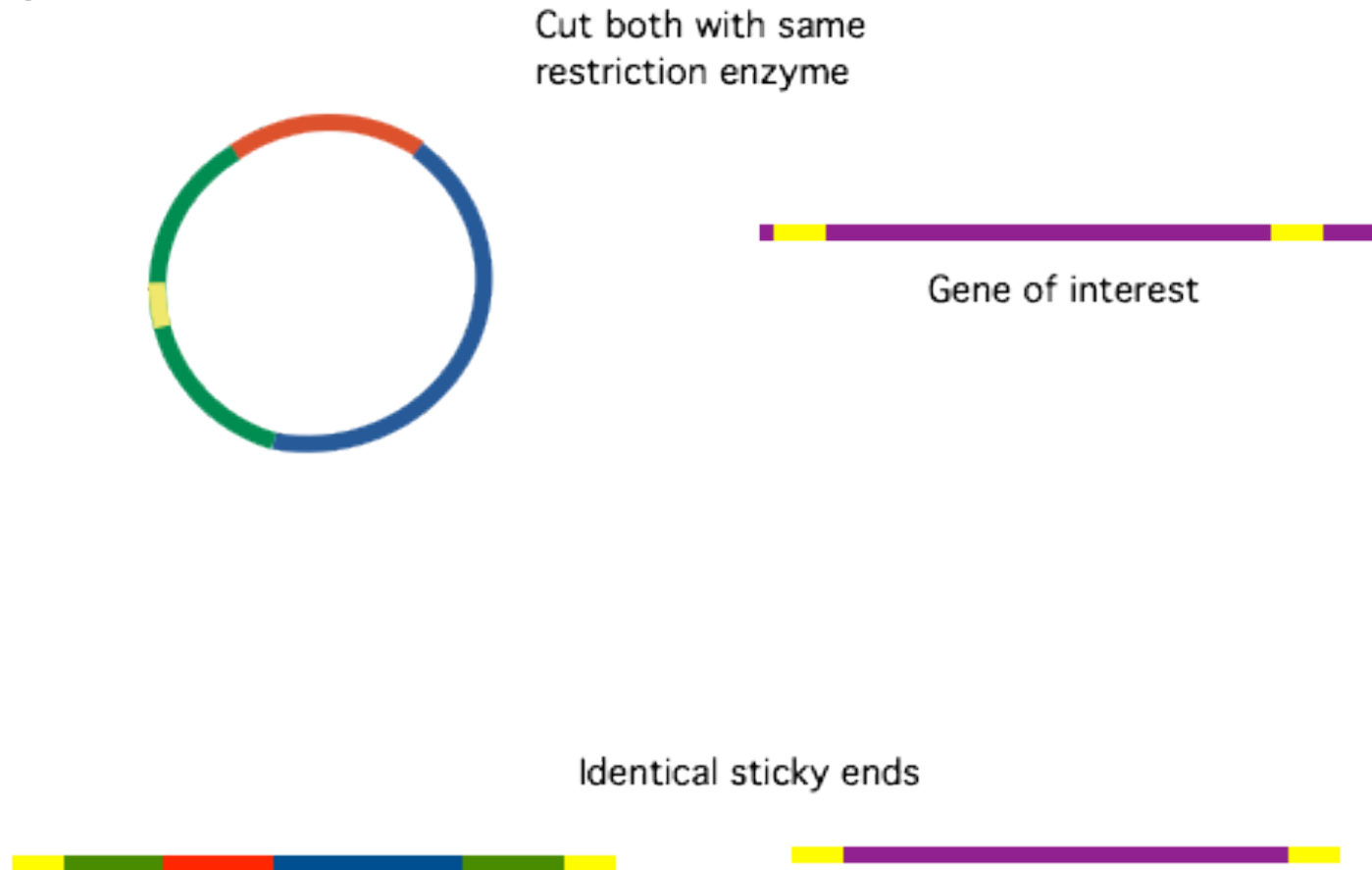


Origin of Replication



# Using a plasmid I

- Cut plasmid and foreign DNA with the same restriction enzyme, to create identical sticky ends.



# Using a plasmid II

- Mix the two pieces together and add ligase, to rejoin the ends

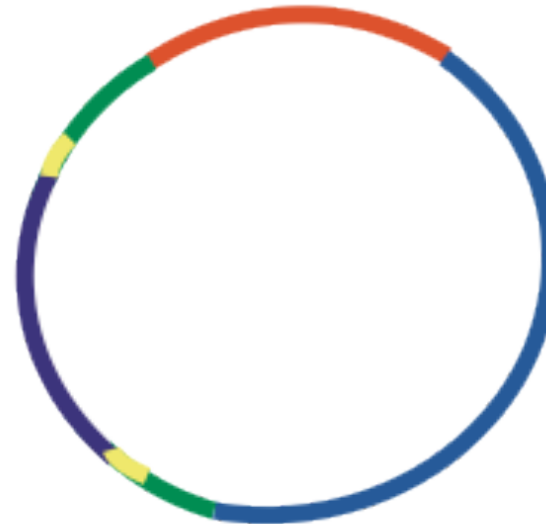
Mix and add ligase



Two outcomes

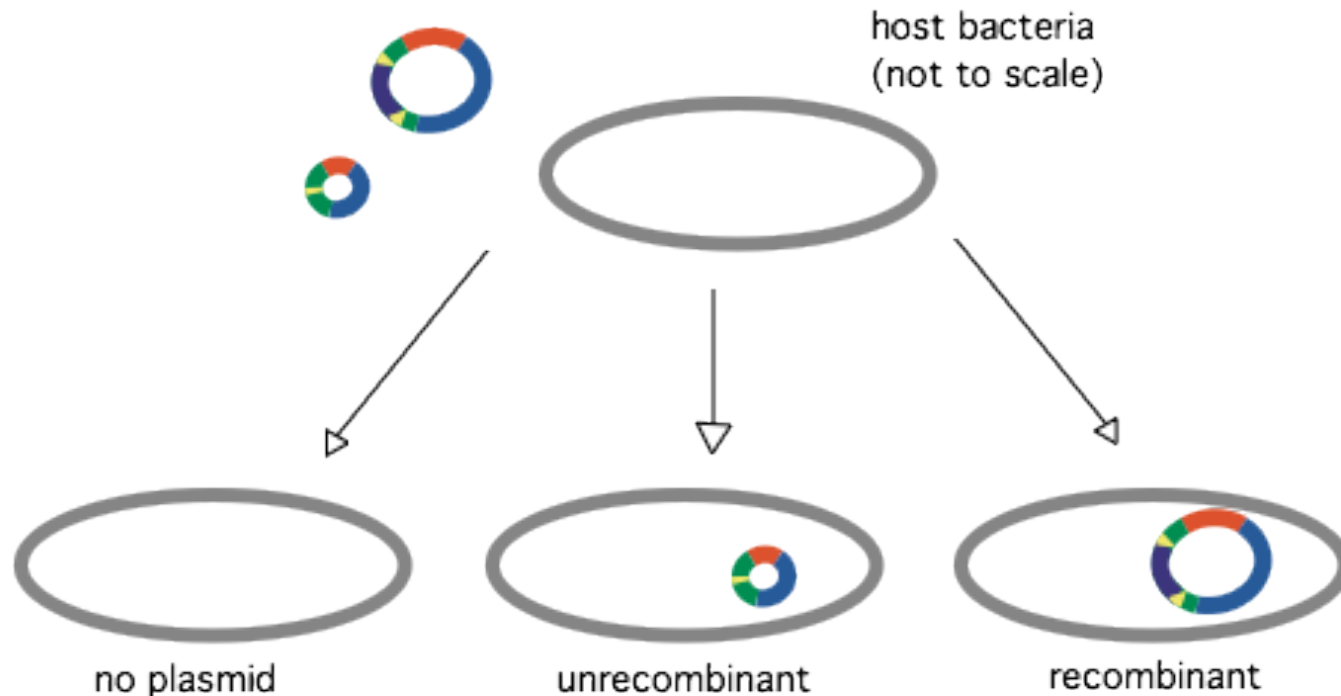


Plasmid reforms without  
foreign DNA



# Using a plasmid III

- Transform  $amp^S$ ,  $lac^-$  bacteria with the mix of plasmids. Most will not take up any plasmid at all (99.99%). Of those that do take up a plasmid, some will have the unrecombined plasmid and others will have the recombinant plasmid containing the gene we want. It is only the last type we are interested in



# Using a plasmid IV

- Plate onto amp plates and grow until colonies appear.



no plasmid



unrecombinant



recombinant

Effect of amp

Kills

None

None

Makes B-gal?

No, dead

Yes

No

Appearance

No colonies

Blue colonies

White colonies



The white colonies are the ones you want: these cells have the gene you are interested in.

# Problems with plasmids

- While this type of plasmid works well for introducing many genes into bacteria, they have drawbacks:
  - only small pieces of DNA can be transferred
  - They don't work in eukaryotes

# Other vectors

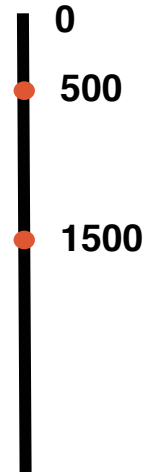
- Large pieces of DNA can be transferred using a virus, such as lambda.
- Species-specific plasmids can be used for some yeast and plants
- ‘Shuttle vectors’ contain origins of replication for both bacteria and yeast
- YACs allow very large pieces of DNA to be transferred

# Using restriction digests

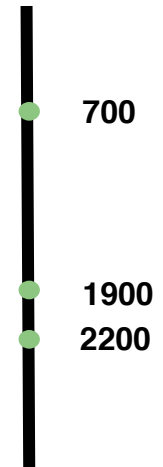
- When a DNA sample is treated with a restriction enzyme, the result is termed a 'restriction digest'. Depending on the time and conditions it may be partial or complete
- The key feature is that, given identical DNA molecules and using the same enzyme, you will always end up with exactly the same pieces of DNA. Once the enzyme cuts at all the restriction sites, it does no further damage.

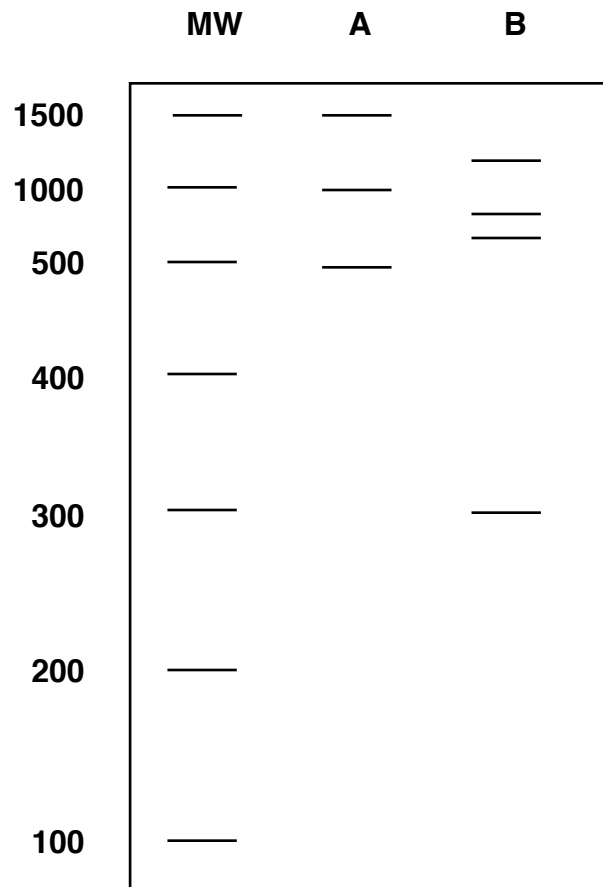
# Restriction fragments

- Suppose you have a DNA molecule that is 3000bp long that has two sites: one at 500bp and the other at 1500bp. When this is cut it will always produce three pieces of DNA, 500, 1000 and 1500 bp long.
- If these are run on a gel, they will produce three DNA bands, corresponding to the three fragments.



- The same piece of DNA cut with a different enzyme will produce different results.
- In this case there are four pieces: 300, 700, 800 and 1200 bp in length
- If both sets are run on a gel, the results will look like this:





Note: the total length of all the fragments should always add up to the same. If not there may be two fragments that are essentially the same size